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CONTENTS

L. Senesi, L. Farinelli, S. Manzotti, M. Fravisini and A. Gigante. Nerve growth factor administration on cultured human ligamentocytes: an in vitro pilot study	1
G. Vicenti, D. Bizzoca, M. Carrozzo, G. Solarino and B. Moretti. Multi-omics analysis of synovial fluid: a promising approach in the study of osteoarthritis	9
G. Vicenti, D. Bizzoca, I. Caruso, V. Nappi, G. Giancaspro, M. Carrozzo and B. Moretti. New insights into the treatment of non-healing diabetic foot ulcers	15
G. Vicenti, D. Bizzoca, V.S. Nappi, F. Moretti, M. Carrozzo, V. Belviso and B. Moretti. Biophysical stimulation of the knee with PEMFs: from bench to bedside	23
O. Galasso, M. De Gori, S. Cerbasi, F. Familiari, P. Recano, G. Balato, G. Gasparini and Massimo Mariconda. Tantalum monoblock cups in total hip arthroplasty: clinical results and outcome predictors	29
G. Cassiolas, M. Berni, G. Marchiori, M. Bontempi, L. Bragonzoni, G. Valente, A. Grassi, S. Zaffagnini, A. Visani and N. F. Lopomo. How cartilage status can be related to joint loads in anterior cruciate ligament reconstruction: a preliminary analysis including MRI T2 mapping and joint biomechanics	35
L. Fassina, N. Bloise, G. Montagna, L. Visai, M.E. Mognaschi, F. Benazzo and G. Magenes. Biomaterials and biophysical stimuli for bone regeneration	41
A. Ziranu, M. Lillo, M. Fantoni, N. Maffulli and G. Maccauro. Single dose Cefazolin is safe and effective for pre-operative prophylaxis in orthopaedic oncology	45
P. Di Benedetto, M.M. Buttironi and A. Causero. Biomarkers and infections in orthopedics: our experience and literature review	51
A. Poggetti, A. Del Chiaro, M. Nicastro, P. Parchi, N. Piolanti and M. Scaglione. A local anesthesia without tourniquet for distal fibula hardware removal after open reduction and internal fixation: the safe use of epinephrine in the foot. A randomized clinical study	57
S. Colangeli, P. Parchi, L. Andreani, G. Beltrami, G. Scoccianti, F. Sacchetti, M. Ceccoli, F. Totti, D.A. Campanacci and R. Capanna. Cryotherapy efficacy and safety as local therapy in surgical treatment of musculoskeletal tumours. A retrospective case series of 143 patients	65
R. Ghermandi, V. Pipola, S. Colangeli, P. Parchi, L. Andreani, R. Capanna and A. Gasbarrini. Polymethylmethacrylate-augmented fenestreted pedicle-screw fixation in low bone quality patients: a case series and literature review	71
I. Simonetti, V. Chianca, M. Zappia, M. Carfora, E. Jannelli and F. Di Pietto. A rare case of Hoffa's fat pad herniation in a young patient: dynamic ultrasound and MRI diagnosis	77
P. Lomoro, I. Simonetti, A. Nanni, R. Cassone, G. Vinci, V. Fichera, E. Jannelli and L. Sammarchi. Musculoskeletal hydatid disease: report of a rare case with primary and exclusive localization in right hemi-pelvis and femur	83
P. Sirtori, G.M. Peretti, M. Ulivi, R. Ciliberto, M.D. Lombardo, F. Verdoni, G. Michielon and L. Mangiavini. Comparison of early functional outcomes between two different surgical approaches for total hip arthroplasty	89
I. Tessaro, V.T. Nguyen, A. Di Giancamillo, M. Agnoletto, F. Verdoni, M. Domenicucci, R. Scurati, G.M. Peretti and L. Mangiavini. Animal models for cartilage repair	97
I. Tessaro, A. Di Giancamillo, E. Benasciutti, V.T. Nguyen, U. Polito, L. Mangiavini and G.M. Peretti. Characterization of different <i>in vitro</i> culture conditions to induce a fibro-chondrogenic differentiation of swine adipose-derived stem cells	105
F. Verdoni, D. Curci, F. Grasso, M. Agnoletto, R. Scurati, G.M. Peretti And L. Mangiavini. External fixation versus titanium endomedullary nail in the treatment of diaphyseal fractures of the lower limb in pediatric age: our experience	117
A. Perna, Luca Ricciardi, Giuseppe Barone, F. C. Tamburrelli, L. Proietti And Enrico Pola. Medical management of acute non-specific low back pain: comparison of different medical treatments, one center's retrospective analysis	121
O. Galasso, D. Castioni, M. Mercurio, M. Sticozzi, E. Porco, M. De Gori and G. Gasparini. Reinfusion-drains reduce blood transfusions in total joint arthroplasty	131
R. Vanelli, M. Ghiara, E. Jannelli, M.C. Rivellino, E. Ferranti Calderoni, M.R. Fiore, F. Benazzo and F. Cuzzocrea. Aggressive hemangioma starting from L5 body, treated with mass embolization and radiation therapy with protons: a case report	139
G. Pasta, R. Vanelli, E. Jannelli, A. Castelli, M. Mosconi, P.S. Preti, C. Ambaglio and F. Benazzo. Primary total knee replacement in hemophiliacs: experience of a single institution over fourteen years of surgical procedures	145
G. Cazzato, G. Masci, F. Liuzza, L. Capasso, M. Florio, C. Perisano, R. Vitiello, G. Ciolli and G. Maccauro. Secondary femur fracture following treatment with antegrade nailing: the state of the art	151
C. Perisano, T. Greco, R. Vitiello, G. Maccauro, F. Liuzza, F.C. Tamburelli and F. Forconi. Mueller-Weiss disease: review of the literature	157
F. Liuzza, L. Capasso, M. Florio, F. Mocini, G. Masci, G. Cazzato G. Ciolli, N. Silluzio and G. Maccauro. Transiliosacral fixation using the O-ARM 2® and STEALTH STATION S8® navigation system	163
C. Carulli, M. Innocenti, F. Lazzarini, M. Villano, G. Pasta, M.L. Brandi and M. Innocenti. The biomolecular interactions between endothelium and bone cells: an overview	173
S.M.P Rossi, M. Ghiara, A. Castelli, A. Ivone, E. Jannelli and F. Benazzo. How to obtain better results, choice of implant or surgical technique? A new surgical technique with an extramedullary referenced cutting guide and a blended alignment method	181
F. Cuzzocrea, E. Jannelli, A. Ivone, R. Vanelli, M. Ghiara, A. Fioruzzi, E. Brunetti and F. Benazzo. Hydatid cyst in the spine. The alliance between surgeon and infectiologist is winning: a case report	187
G.M. Peretti, M. Ulivi, L. De Girolamo, V. Meroni, M.D. Lombardo, F. Verdoni and L. Mangiavini. Evaluation of the use of autologous micro-fragmented adipose tissue in the treatment of knee osteoarthritis: preliminary results of a randomized controlled trial	193
T. Chompoosang, R. D'apolito, C. Lacagnina, G. Peretti and L. Zagra. Total hip arthroplasty in rapidly progressive osteoarthritis of the hip: clinical and radiological outcome of a consecutive series of patients	201
A. Castelli, S.M.P. Rossi, E. Jannelli, L. Rocca and F. Benazzo. Treatment of vancouver B1, C periprosthetic hip fractures with periprosthetic polyaxial locking plate system: A 3-year follow-up	209
G. Vadalà, F. Russo, M. Musumeci, A. Giacalone, R. Papalia and V. Denaro. Targeting VEGF-A in cartilage repair and regeneration: state of the art and perspectives	217

NERVE GROWTH FACTOR ADMINISTRATION ON CULTURED HUMAN LIGAMENTOCYTES: AN *IN VITRO* PILOT STUDY

L. SENESI¹, L. FARINELLI¹, S. MANZOTTI¹, M. FRAVISINI² and A. GIGANTE¹

¹*Clinical Orthopaedics, Department of Clinical and Molecular Sciences, Università Politecnica delle Marche, Ancona;* ²*Department of Orthopaedic, "Sol et Salus" Hospital, Torre Pedrera, Italy*

Nerve growth factor (NGF) is involved in several joint pathologies. It has been demonstrated that its concentration increases in synovial fluid and tissue from arthritis. However, its role in joint homeostasis and pathophysiology still remain to be clarified. This study analyzed the effect of 200 ng/ml on cultured human ligamentocytes by evaluating cell proliferation, cell phenotype and gene expression. The MTT test excluded an influence on cell viability at 7 and 14 days. Regarding cell phenotype, we observed that NGF might promote the synthesis of COL1A1. On the other hand, real time PCR showed that NGF did not influence gene expression of COL3A1, FGF-BETA, IGF1, MMP2, MMP3, MMP9 and MMP13. However, COL1A1 gene was significantly upregulated in treated cell at 14 days. Our results suggest that NGF may have an anabolic effect on ligament. Additional investigations are necessary to determine how NGF may influence ligamentocytes.

Nerve Growth Factor (NGF) is a dimeric protein made up of two identical, not covalently bound polypeptide chains linked by intrachain disulfide bonds. (1).

NGF is involved in nervous, endocrine and immune system function, but it is also involved in some inflammatory and rheumatic diseases. Previous studies demonstrated its ability to participate in pain initiation and neurogenic inflammation through stimulating of neuropeptide overexpression and activating immune cells (2).

To date, it has been widely established that NGF and its receptors are present in normal and over expressed in pathological articular cartilage (3) and synovium (4), but the findings about their role in joint pathophysiology are scant and conflicting (5). In addition, growing evidences indicate that blocking NGF signaling using anti NGF agents

(i.e. tanezumab) provide effective pain relief in osteoarthritic patients confirming the role of NGF-TrKA axis in pain response (6).

Even though, the impact of NGF and its signaling pathways seem to be significant in joint homeostasis, there is an inconsistent literature about the relationship between NGF and human ligaments. Nowadays, only studies on rats were found (7, 8). Nithya M et al. reported the ability of NGF to enhance wound healing through increasing the synthesis of collagen (7) whereas, Mammoto et al. reported the ability of NGF to improve the healing of injured medial collateral ligament by promoting reinnervation and angiogenesis (8).

For these reasons, the aim of the present in vitro study was to analyze the effect of NGF on the proliferation, phenotype and gene expression of human healthy ligamentocytes.

Key words: nerve growth factors, ligament, ligamentocytes, basic science

Corresponding author:

Dr Luca Farinelli,
Clinical Orthopaedics,
Department of Clinical and Molecular Sciences,
Università Politecnica delle Marche,
Via Tronto 10/A, 60126, Ancona (AN), Italy
e-mail: Farinelli.luca92@gmail.com

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MATERIALS AND METHODS

Ligamentocytes cultures

Healthy ligamentocytes were harvested from the anterior cruciate ligament (ACL) of knees of 5 subjects (2 males, 3 females) of similar age (65-75 years) that underwent total knee arthroplasty. Each of these ACLs was undamaged and appeared to be free of aggressive inflammation. All participants provided a written informed consent prior to the operation. The fragments of ligament collected during the surgery were not of any medical use. Ligaments cultures were subdivided in two groups: group A - untreated ligamentocytes used as controls; group B - cultures treated with 200 ng/ml of NGF (Sigma Aldrich, St. Louis, MO, USA); The medium and NGF were replaced twice weekly. The effects of NGF exposure were evaluated in ligamentocytes monolayers at 7 (T1) and 14 days (T2).

Cell viability (MTT assay)

For viability evaluation, cells were seeded in 48-well multi-plates (5000 cells/well). The 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-2H-tetrazolium bromide (MTT) test was performed at 7 and 14 days. The culture medium was replaced with a filtered 10% sterile solution of 5 mg/ml 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-2H-tetrazolium bromide (MTT) in DMEM without phenol red (both from Sigma Aldrich) and incubated at 37°C for 4 h. After removing the supernatant, the dark blue formazan crystals that had accumulated in cells were dissolved by adding in 1 ml of solvent (0.01 N HCl in absolute isopropanol) and quantified by spectrophotometry (BioPhotometer Plus, Eppendorf, Hamburg, Germany) at

550 nm. Data are the mean of 3 assays and are expressed as optical density (O.D.) for each cell group and time point.

Cell phenotype

Cells for immunohistochemistry were incubated with anti-MMP9, anti-MMP13 and anti-COL1A1 polyclonal antibody. The percentage of stained cells was assessed by computerized morphometric analysis using Leica Application Suite 4.1 (Leica Microsystem, Heerbrugg, Switzerland) by examining 5 random fields (10x) / culture at 14 days. Cells were seeded in chamber slides at a concentration of 5000 cells/ml. At 14 days they were fixed in 4% paraformaldehyde for 15 min. The reaction was detected using the Dako LSAB+ System-HRP kit according to the manufacturer's instructions (all reagents from Dako Cytomation, Carpinteria, CA, USA).

RNA Purification

Sub-confluent cells were harvested from 25 cm² flask after 7 and 14 days. Total RNA was extracted using Trizol® reagent (Life Technologies, Paisley, Scotland, UK) according to the manufacturer's instructions. RNA was resuspended in nuclease-free water and its concentration was determined by UV spectrophotometry at 260 nm using a NanoDrop 2000 (ThermoScientific, Waltham, MA, USA).

RNA purity was assessed by the A260:280 ratio (range, 1.7-1.9) and the A260:230 ratio (>1.6). Total RNA degradation was assessed by 1% agarose gel electrophoresis prior to RT-PCR. Gels were viewed after staining with GelRed™ (Biotium, Hayward, CA, USA) for 20 min. Total RNA (1 µg) was used in a reverse

Table I. Results of non-parametric analysis.

<i>Cell viability</i>	Mean	Standard Deviation
Group A (T1)	0.469866667	0.149852245
Group B (T1)	0.4852	0.160548702
Group A (T2)	1.099133333	0.386696793
Group B (T2)	1.210733333	0.472199181

transcription reaction using SuperScript®III First-Strand Synthesis SuperMix kit for qRT-PCR (Life Technologies, Carlsbad, CA, USA) according to the manufacturer's recommendations. The reaction was run in an MJ Mini ThermalCycler (Bio Rad Laboratories, Hercules, CA, USA).

Real-time PCR and gene expression analysis

The expression of 9 ligamentocytes marker genes - type I collagen (COL1A1), type III collagen (COL3A1), metalloprotease (MMP) 2 (MMP-2), MMP-3, MMP-9 and MMP-13, fibroblast growth factor (FGF), insulin-like growth factor-1 (IGF-1) - was determined by real-time PCR (RT-PCR) 7 and 14 days into culture in monolayers treated with NGF and in untreated chondrocytes. All experiments were performed in triplicate. cDNA was amplified by real-time PCR using Power SYBR® Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) and the specific assay designed for the genes investigated except in the case of COL1A1, whose cDNA was amplified using TaqMan universal PCR master mix (Applied Biosystems). SYBR® Green assay reactions were performed in a 20 µl volume using the ABI PRISM 7500 (Applied Biosystems). Each reaction contained 10 µl 2X Power SYBR® Green PCR Master Mix, 400 nM

concentration of each primer, and cDNA. TaqMan assay amplification were performed in a 20 µl volume using the ABI PRISM 7500. Each reaction contained 10 µl 2X Power SYBR® Green PCR Master Mix, 400 nM of each primer, 200 nM of the specific probe, and cDNA. All experiments included non-template controls to rule out reagent contamination.

Gene expression levels were normalized to the expression of the housekeeping gene RPL13 and are presented as fold changes relative to expression in control cells. Results are the mean of 6 observations. Quantification was done with the delta/delta calculation method using 7500 Software v2.0.5 (Applied Biosystems). Forward and reverse primers for RT-PCR were designed using Applied Biosystems Primer Express software.

Statistical analysis

A non-parametric approach was used, as variables were not normally distributed. Variables were summarized using median and interquartile range (1st-3rd quartiles). A ranked-based non-parametric analysis for longitudinal data was used to compare MTT viability data (at O.D. 550 nm) in the two cell groups at 7 and 14 days. The effect of treatment type and time and their interaction was evaluated; results are presented as a box plot. The Kruskal-

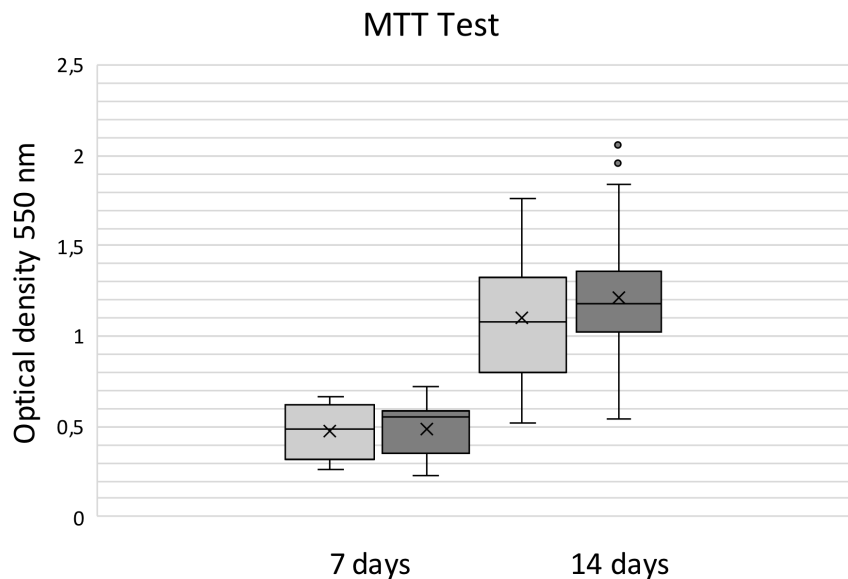


Fig 1. Light grey: group A; dark grey: group B. No significant differences in cell viability were observed in both groups at 7 and 14 days.

Wallis test was applied to compare histochemical staining (COL1A1, MMP-9) at 14 days. Statistical significance was set at a level of probability of 0.05. Changes in gene expression are given as fold change; >1.5 fold change expression for upregulation and <0.5 fold change for downregulation were considered significant ($p<0.05$).

RESULTS

Cell Viability (MTT assay)

The results of non-parametric analysis are reported in Table I and Fig. 1. No significant differences in cell viability were observed between two groups at T1 and T2. Significant differences were observed between T1 and T2 in both groups ($P<0.05$). Median reading at T1 were 0.486 (1st-3rd quartiles: 0.314-0.615) in group A; 0.554 (1st-3rd quartiles: 0.347-0.59) in group B. Median reading at T2 were 1.083 (1st-3rd quartiles: 0.803-1.328) in group A; 1.186 (1st-3rd quartiles: 1.021-1.363) in group B.

Histomorphometry

The median percent values of cells staining with COL1A1 were 5.16% in group A (1st-3rd quartiles:

3.65-6.32); 18.78% (1st-3rd quartiles: 16.375-21.41) in group B. The median percent values of cells staining with MMP9 were 12.762% in group A (1st-3rd quartiles: 11.07-13.745); 13.65% (1st-3rd quartiles: 12.871-14.767) in group B. The median percent values of cells staining with MMP13 were 14.068% in group A (1st-3rd quartiles: 12.61-16.04); 15.4% (1st-3rd quartiles: 13.365-17.85) in group B as reported in Fig. 2.

Gene expression

COL3A1, FGF-BETA, IGF1, MMP2, MMP3, MMP9 and MMP13 gene expression did not differ significantly in group A compared to group B. COL1A1 gene was significantly upregulated in group A only at 14 days. The gene expression data as fold change was reported in Fig. 3.

DISCUSSION

It has been well established that all tissues within the joint as cartilage, synovium and bone are characterized by expression of NGF and its receptors. Even though, our knowledge regards their role in

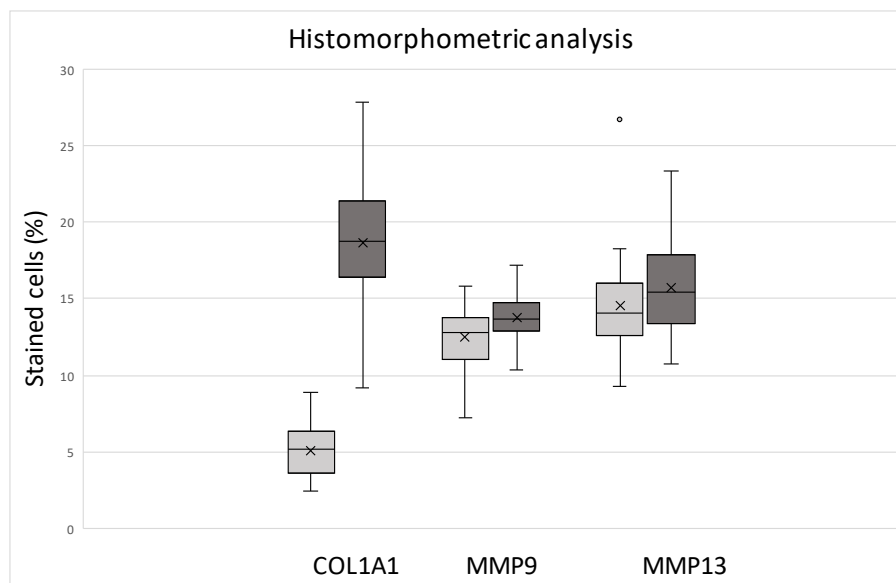


Fig. 2. Light grey: group A; dark grey: group B. It is worth mentioning that the median proportion of stained cells for COL1A1 was significantly lower in Group A than Group B ($P<0.05$).

pathophysiology of osteoarthritis (OA) is marginal and unclear, NGF has been detected over-expressed in synovial fluid of OA (9). In addition, several studies pointed out that chondrocytes and synovium could produce NGF as a reaction of various stimuli of damage, through IL-1 β and/or TGF- β signaling (10). Hence, it is reasonable to postulate that the ligaments of the central pivot of the knee might be subjected to the autocrine/paracrine effect of NGF. To the best of our knowledge, this is the first study describing the *in vitro* effect of NGF on proliferation, phenotype and gene expression of human ligamentocytes in primary cultures.

In regards to cells viability, MTT test disclosed that NGF did not influence cell proliferation; this

suggests that it should not act neither as growth factor nor toxin on ligamentocytes, but rather similar to a cytokine through the activation and/or inhibition of specific signaling pathways.

Results of the present study suggested that the administration of NGF should promote the synthesis of COL1A1 in ligamentocytes differentiation. Indeed, it was the only gene that seems to be upregulated by RT-PCR and immunohistochemical analysis compared to control (Fig. 4).

Taken together, our findings suggest that NGF may have an anabolic effect on ligamentocytes as observed by Mammoto et al. in rats (8). Even though these data required replication, we could assume that in case of acute knee injury, the NGF delivered by

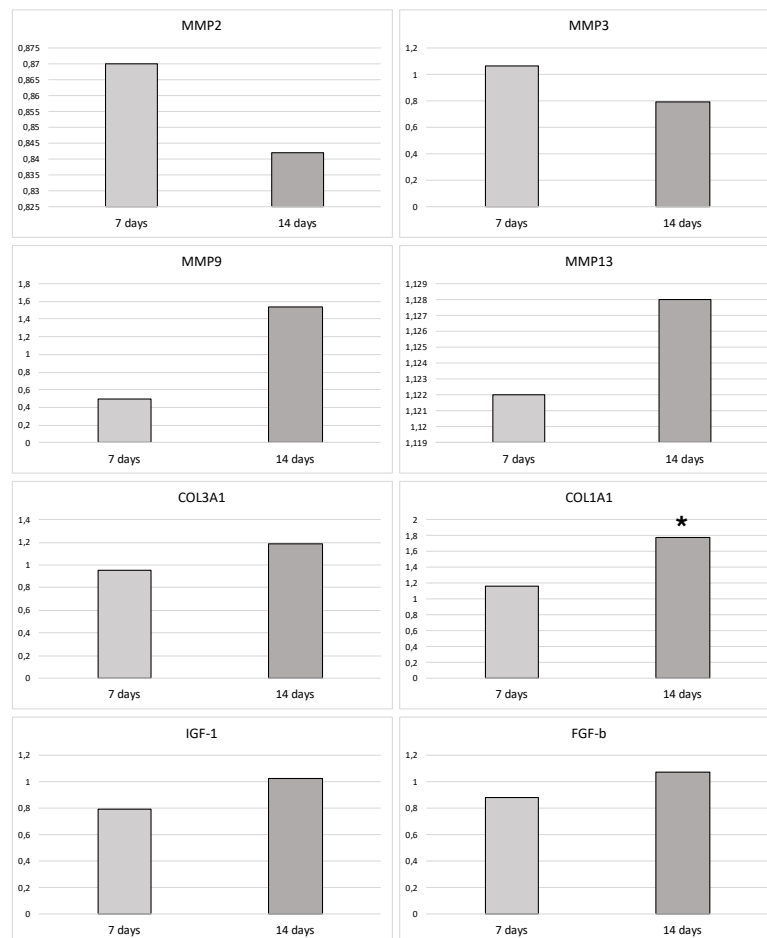


Fig. 3. Gene expression pattern: Mean gene expression values in NGF-treated samples from 5 healthy adult individuals normalized to control values. Changes in gene expression are given as fold change; >2 fold change expression for upregulation and <0.5 fold change for down regulation were considered significant ($p < 0.05$) (Marked with *).

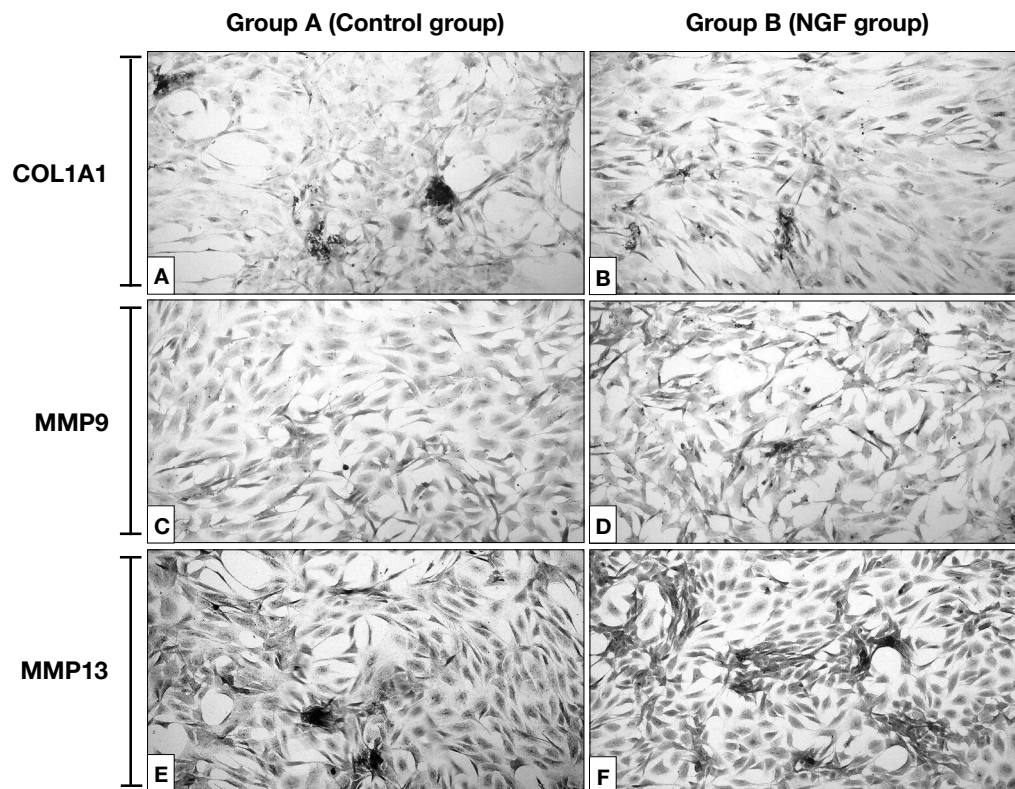


Fig 4. Immunohistochemical analysis. All photos were taken at 10X magnification. Cytoplasmatic staining of COL1A1, MMP9, MMP13 was detected after 14 days of culture. It is worth mentioning the difference regarding COL1A1 as highlighted in Fig. 2.

synovium in response of damage, might promote an anabolic response of the central pivot acting as a defense mechanism of the joint.

Among metalloproteases, several families of these zinc dependent enzymes participate actively to development, remodeling and subsequent pathological processes of the joints (11). Considering our results, NGF may have an uncertain effect on human ligamentocytes. In our cultures, these genes expression were not influenced in significative manner by NGF administration.

It has been demonstrated that MMP-13 is not expressed in healthy anterior cruciate ligament collected from total knee arthroplasty, whereas its expression is predominant in tendinopathies (12). Our results are controversial, because we showed that NGF administration seems to increase MMP-13 expression. On the other hand, our research pointed out that NGF seems to have an anabolic effect on ligamentocytes. For these reasons, we

hypothesize that mRNAs will not be translated in the corresponding enzyme under NGF stimuli or that there is a simultaneously production of inhibitors of MMP in favor of an ultimate anabolic effect.

Among the growth factor family, FGF- β and IGF-1 have proven to play a crucial role in the joint pathophysiology. The former regulates a broad spectrum of biological functions, including cellular proliferation, survival, migration, and differentiation. It was originally identified as a protein capable of promoting fibroblast proliferation. Indeed, FGF have been utilized for the regeneration of damaged tissues, including skin, tendon and ligament (13). The latter plays a crucial role in wound healing and connective tissue repair in rats (14) even though these data need to be confirmed in human. In the present study both gene expressions were not influenced by NGF, suggesting that the anabolic effect observed in vitro might not be due to IGF-1 and FGF- β signaling.

Additional analyses regarding gene expression

and protein synthesis are necessary to clarify the behavior of ligamentocytes under NGF stimulation, as considering our data, we could only hypothesize that this anabolic response occurs *in vivo*.

REFERENCES

1. Aloe L. Rita Levi-Montalcini and the discovery of NGF, the first nerve cell growth factor. Arch Ital Biol 2011; 149:175-81.
2. Lindsay RM, Harmer AJ. Nerve growth factor regulates expression of neuropeptides genes in adult sensory neurons. Nature 1989; 337:362-4.
3. Gigante A, Bevilacqua C, Pagnotta A, Manzotti S, Tosca A, Greco F. Expression of NGF, TrkA and p75 in human cartilage. Eur J Histochem 2003; 47:339-44.
4. Takano S, Uchida K, Inoue G, Miyagi M, Aikawa J, Iwase D, Iwabuchi K, Matsumoto T et al. Nerve growth factor regulation and production by macrophages in osteoarthritic synovium. Clin Exp Immunol 2017; 190:235-43.
5. Gigante A, Senesi L, Manzotti S, Palmieri A, Skrami E, Sollazzo V. Effect of nerve growth factor on cultured human chondrocytes. J Biol Regul Homeost Agents 2016; 30:1-6.
6. Schnitzer TJ, Ekman EF, Spierings EL, Greenberg HS, Smith MD, Brown MT, West CR, Verburg KM. Efficacy and safety of tanezumab monotherapy or combined with non-steroidal anti-inflammatory drugs in the treatment of knee or hip osteoarthritis pain. Ann Rheum Dis 2015; 1202-11.
7. Nithya M, Suguna L, Rose C. The effect of nerve growth factor on the early responses during the process of wound healing. Biochim Biophys Acta 2003; 1620:25-31.
8. Mammoto T, Seerattan RA, Paulson KD, Leonard CA, Bray RC, Salo PT. Nerve Growth factor improves ligament healing. J Orthop Res 2008; 26:957-64.
9. Iannone F, De Bari C, Dell'Accio F, Covelli M, Patella V, Lo Bianco G, Lapadula G. Increased expression of nerve growth factor (NGF) and high affinity NGF receptor (p140 TrkA) in human osteoarthritic chondrocytes. Rheumatology (Oxford) 2002; 41:14.
10. Pecchi E, Priam S, Gosset M, Pigenet A, Sudre L, Laiguillon MC, Berenbaum F, Houard X. Induction of nerve growth factor expression and release by mechanical and inflammatory stimuli in chondrocytes: possible involvement in osteoarthritis pain. Arthritis Res Ther 2014; 16:16.
11. Aigner T, Zien A, Gehrsitz A, Gebhard PM, McKenna L. Anabolic and catabolic gene expression pattern analysis in normal versus osteoarthritic cartilage using complementary DNA-array technology. Arthritis Rheum 2001; 44:2777-89.
12. Foos MJ, Hickox JR, Mansour PG, Slaughterbeck JR, Hardy DM. Expression of matrix metalloprotease and tissue inhibitor of metalloprotease genes in human anterior cruciate ligament. J Orthop Res 2001; 19:642-9.
13. Yun YR, Won JE, Jeon E, et al. Fibroblast Growth Factors: Biology, Function, and Application for Tissue Regeneration. Journal of Tissue Engineering 2010; 2010: 218142.
14. Provenzano PP, Alejandro-Osorio AL, Grorud KW, Martinez DA, Vailas AC, Grindeland RE, Vanderby R Jr. Systemic administration of IGF-I enhances healing in collagenous extracellular matrices: evaluation of loaded and unloaded ligaments. BMC Physiology 2007; 7:2.

MULTI-OMICS ANALYSIS OF SYNOVIAL FLUID: A PROMISING APPROACH IN THE STUDY OF OSTEOARTHRITIS

G. VICENTI, D. BIZZOCA, M. CARROZZO, G. SOLARINO and B. MORETTI

1Department of Neuroscience and Organs of Sense, Orthopaedics Section, University of Bari Aldo Moro, Bari, Italy

Osteoarthritis (OA), affecting 250 million individuals worldwide, is a significant social health problem. Therefore, the search for synovial fluid (SF) biomarkers that could anticipate the diagnosis of OA is gaining increasing importance in orthopaedics. This review summarizes the recent progresses performed in the multi-omics approach to OA, mainly focusing on proteome and metabolome analysis of SF. Proteomics of the SF has shown the up-regulation of several components of the classic complement pathway in OA samples, including C1, C2, C3, C4A, C4B, C5 and C4 C4BPA, thus depicting that complement is involved in the pathogenesis of OA. Moreover, proteomics has displayed that some pro-inflammatory cytokines, namely IL-6, IL-8 and IL-18, have a role in OA. The metabolomic profiling of the SF in OA has identified some metabolites as potential biomarkers of OA and has shown the existence of metabolically different OA subgroups. However, further studies with larger samples sizes and matched-control groups are needed to identify SF biomarkers that could be useful in the diagnosis, treatment and follow-up of OA.

Osteoarthritis (OA) is the most prevalent degenerative joint disease and a leading cause of pain and disability in elderly people. The Osteoarthritis Research Society International (OARSI) defines OA as “a disorder involving movable joints characterized by cell stress and extracellular matrix degradation initiated by micro- and macro-injury that activates maladaptive repair responses including pro-inflammatory pathways of innate immunity”(1).

OA, affecting 250 million individual worldwide, mainly over-65 adults, represents a significant social health problem (2).

Even if several genetic and environmental risk factors promote the development of OA, i.e. age, gender, ethnicity, body mass index (BMI), physical activity and muscle weakness, the exact pathogenesis of OA is still unclear (3). The anatomopathological

features of OA include cartilage degeneration, intra-articular synovial inflammation and subchondral osteosclerosis (4).

Currently, the diagnosis of OA is based on physical examination, while the progression of the joint degenerative changes could be assessed with imaging techniques only (3, 4).

Osteoarthritis causes articular pain, joint stiffness and loss of function, thus progressively affecting the working ability and the social life of the patient (5). Nonetheless, the symptoms onset occurs after that the irreversible joint changes have developed (2). Therefore, arthroplasty still is the most successful procedure for the treatment of OA, with a reported high patient's satisfaction rate (6).

Thus, the search for synovial fluid (SF) biomarkers that could anticipate the diagnosis of OA is gaining

Key-words: osteoarthritis, synovial fluid, omics sciences, metabolomics, proteomics, genomics

Corresponding Author

Dr Giovanni Vicenti, MD, PhD,
Department of Basic Medical Sciences,
Neuroscience and Sense Organs, Orthopaedic Clinic, School of Medicine,
University of Bari “Aldo Moro”
AOU Consorziiale Policlinico Piazza Giulio Cesare 11, 70100 Bari, Italy
Tel.: +39 0805593402
e-mail: dott.gvicenti@gmail.com

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increasing importance in orthopaedics (2). This review summarizes the recent progresses performed in the multi-omics approach to OA, mainly focusing on proteome and metabolome analysis of SF, to depict the future perspectives in the diagnosis and treatment of OA.

Synovial fluid physiology and composition

Diarthrodial joints are lined with a tissue called synovium that produces the joint fluid, a viscous, mucinous substance that lubricates joints (7). It is also called synovial fluid (SF) because of its resemblance to egg white. SF is an ultrafiltrate of plasma, containing the same plasmatic levels of glucose and uric acid. The SF protein concentration, however, is about one third of the plasmatic one (8).

Plasma constituents that enter joint fluid must cross a double-barrier membrane. First, the endothelial lining of the capillaries is crossed, followed by movement through a matrix that surrounds synovial cells (9). This ultrafiltrate is finally combined with the hyaluronate synthesized by the synovium (9).

The SF provides nutrition and lubrication to the articular cartilage in minute amounts. It accumulates in a pathologic joint, and reflects the ongoing process of the joint disorder (10).

Proteomics of synovial fluid in osteoarthritis

The proteome is the cell's specific protein complement in a defined physiological context at a specific point in time (11). Proteome analysis or "proteomics" is the large-scale protein-based systematic analysis of the proteome, or a defined sub-proteome, from a cell, tissue, or entire organism (11). A relatively new discipline that has been growing rapidly in the last two decades (12).

Proteomics of the SF has shown the upregulation of several components of the classic complement pathway in OA samples, including C1, C2, C3, C4A, C4B, C5 and C4 Binding Protein Alpha (C4BPA), thus depicting that complement is involved in the pathogenesis of OA (10-14).

Corigliano et al, in a clinical study analysing the SF obtained from 25 arthritic knees at different stages, have recently reported that the expression levels of two Complement C3 peptide fragments, i.e. C3f and

C3f Des-Arg, decrease with the progression of OA degrees severity (15). These authors consequently suggest that C3f could be a useful tool for the staging of knee OA (15).

Wang et al, in an interesting *in vivo* study performed on mice genetically deficient in C5, C6, or CD59a, have shown that complement, and specifically the membrane attack complex (MAC)-mediated arm of complement, is critical to the development of arthritis.

Moreover, these authors have shown in chondrocytes of mice genetically deficient for C5, the expression of inflammatory and degradative molecules was lower than in C5-sufficient mice (14).

Based on this finding, Wang et al have hypothesized that increased SF levels of some complement proteins should cause the MAC formation, with subsequent chondrocytes apoptosis and inflammatory response (14).

It should be noted that these data still have a limited clinical usefulness, since it is reported that the complement inhibition causes an increased risk of infection, mainly caused by *Neisseria* (13). Eculizumab, an anti-C5f human monoclonal antibody FDA-approved for the treatment of Paroxysmal Nocturnal Hemoglobinuria (PNH) could be, however, a promising tool in the treatment of OA (13, 16, 17).

Proteomics studies have also suggested that some pro-inflammatory cytokines, namely IL-6, IL-8 and IL-18, could have a central role in the pathogenesis of OA (18-21).

Interestingly, Livshits et al have evaluated the circulating levels of IL-6 in 908 women at recruitment and at 5-, 8- and 15-years follow-up (22). All the patients underwent also an X-ray of the knee at baseline and at 10- and 15-years follow-up; the X-rays findings were finally used to classify in stages, according to Kellgren and Lawrence, the severity of knee OA in the recruited patients (22). These authors reported that circulating levels of IL-6 are significantly higher in women that develop OA, concluding IL-6 could be a potential therapeutic target in the treatment of knee OA (22).

Wang et al, in a controlled clinical-radiographic study comparing 33 patients with primary knee OA

at different stages with 15 health subjects, have observed that plasma, synovial fluid and articular cartilage IL-18 levels were significantly increased in OA patients, and these elevated levels were positively correlated with radiographic OA severity (23).

Metabolomics of synovial fluid in osteoarthritis

Metabolomics is a rapidly emerging “omics” science, which involves the study of the small molecules (i.e. molecular weight < 10 kDa) produced by active and living cells at a specific time point, during their life cycles (7). Consequently, metabolomics provides a snapshot of a biological system status, showing how it changes in response to stress, environmental changes and diseases (7, 24).

The basis for metabolomics is that a specific pathological condition may alter the concentration of a specific groups of metabolites, which can then be targeted as a biomarker of that disease (25).

In the field of osteoarthritis research, several studies have investigated the metabolomic profiling of the synovial fluid (SF), reporting interesting findings (26-30).

Carlson et al, in a recent pilot study, performed the global metabolite profiling of 13 synovial fluid samples - i.e. osteoarthritis (n=5), rheumatoid arthritis (n=3) and healthy subjects *post-mortem* samples (n=5) - using high-performance liquid chromatography (HPLC) and mass spectroscopy (MS) (26). These authors found 35 metabolites as potential biomarkers of OA, including phosphatidylcholine, lysophosphatidylcholine, ceramides, myristate derivatives, and carnitine derivatives (26).

Zheng et al had previously analysed with HPLC-MS the SF obtained from the knee of 35 subjects, 25 patients affected by OA and 10 control subjects (i.e. patients undergoing high amputation) (27). These authors, through multi-variate data analysis, identified six metabolites strongly associated with OA: glutamine, 1,5-anhydroglucitol, gluconic lactone, tyramine, threonine and 8-aminocaproic acid (28).

Mickiewicz et al, in a controlled study, have analysed the SF obtained from 55 arthritic knees and 13 control knees (*post-mortem* sample) (29).

These authors observed fructose and citrate levels were increased in SF samples collected from OA knee joints, whereas the concentrations of O-acetylcarnitine, N-phenylacetyl glycine, methionine, ethanol, creatine, malate, ethanolamine, 3-hydroxybutyrate and hexanoylcarnitine were decreased in OA samples compared to the controls (29).

Kim et al, on the other hand, in the differential analysis of the SF obtained from eight patients with early knee OA (Kellgren-Lawrence stages 1-2), compared with seven patients with late knee OA (stages 3-4 according to Kellgren-Lawrence), have identified 28 critical metabolites for discriminating between the early and late knee OA, with a significantly different concentration in the two groups (28). These metabolites, mainly related to fatty acid metabolism, glycerolipid metabolism, and the tricarboxylic acid cycle, included malate, ethanolamine, squalene, glycerol, myristic acid, oleic acid, lanosterol, heptadecanoic acid, and capric acid (28).

Zhang et al evaluated the SF metabolomic profile of 80 patients with hip and knee osteoarthritis, undergoing total hip or knee replacement surgery, and showed that OA consists of three metabolically distinct subgroups (30). The existence of metabolically different OA subgroups, that are likely due to the differences in carnitine, lipid and collagen metabolism, will help to understand the pathogenesis and develop targeted therapies for OA (30).

DISCUSSION

Multi-omics analysis of the synovial fluid is a promising tool in the study of OA. However, further studies with larger samples sizes and matched-control groups are needed to identify SF biomarkers that could be useful in the diagnosis, treatment and follow-up of OA.

REFERENCES

1. Mobasheri A, Bay-Jensen A-C, van Spil WE, Larkin J, Levesque MC. Osteoarthritis Year in Review 2016: biomarkers (biochemical markers). Osteoarthritis Cartil

- 2017; 25(2):199-208.
2. Hunter DJ, Nevitt M, Losina E, Kraus V. Biomarkers for osteoarthritis: Current position and steps towards further validation. *Best Pract Res Clin Rheumatol* 2014; 28(1):61-71.
 3. Goldring MB, Goldring SR. Osteoarthritis. *J Cell Physiol* 2007; 213(3):626-34.
 4. Blanco FJ, Ruiz-Romero C. Metabolomic characterization of metabolic phenotypes in OA. *Nat Rev Rheumatol* 2012; 8(3):130-2.
 5. Cross M, Smith E, Hoy D, et al. The global burden of hip and knee osteoarthritis: estimates from the Global Burden of Disease 2010 study. *Ann Rheum Dis* 2014; 73(7):1323-30.
 6. Hepinstall MS, Rutledge JR, Bornstein LJ, Mazumdar M, Westrich GH. Factors that impact expectations before total knee arthroplasty. *J Arthroplasty* 2011; 26(6):870-6.
 7. Peng B, Li H, Peng X-X. Functional metabolomics: from biomarker discovery to metabolome reprogramming. *Protein Cell*. 2015; 6(9):628-37.
 8. Hui AY, McCarty WJ, Masuda K, Firestein GS, Sah RL. A systems biology approach to synovial joint lubrication in health, injury, and disease. *Wiley Interdiscip Rev Syst Biol Med* 2012; 4(1):15-37.
 9. Tamer TM. Hyaluronan and synovial joint: function, distribution and healing. *Interdiscip Toxicol* 2013; 6(3):111-125.
 10. Wang Q, Rozelle AL, Lepus CM, et al. Identification of a central role for complement in osteoarthritis. *Nat Med* 2011; 17(12):1674-9.
 11. Balakrishnan L, Nirujogi R, Ahmad S, et al. Proteomic analysis of human osteoarthritis synovial fluid. *Clin Proteomics* 2014; 11(1):6.
 12. Balakrishnan L, Bhattacharjee M, Ahmad S, et al. Differential proteomic analysis of synovial fluid from rheumatoid arthritis and osteoarthritis patients. *Clin Proteomics* 2014; 11(1):1.
 13. Mahendran SM, Oikonomopoulou K, Diamandis EP, Chandran V. Synovial fluid proteomics in the pursuit of arthritis mediators: An evolving field of novel biomarker discovery. *Crit Rev Clin Lab Sci* 2017; 54(7-8):495-505.
 14. Wang Q, Rozelle AL, Lepus CM, et al. Identification of a central role for complement in osteoarthritis. *Nat Med* 2011; 17(12):1674-9.
 15. Corigliano A, Preianò M, Terracciano R, Savino R, De Gori M, Galasso O, Gasparini G. C3f is a potential tool for the staging of osteoarthritis. *J Biol Regul Homeost Agents* 2017; 31(4S1):29-35.
 16. Hillmen P, Young NS, Schubert J, et al. The Complement Inhibitor Eculizumab in Paroxysmal Nocturnal Hemoglobinuria. *N Engl J Med* 2006; 355(12):1233-43.
 17. Brodsky RA, Young NS, Antonioli E, et al. Multicenter phase 3 study of the complement inhibitor eculizumab for the treatment of patients with paroxysmal nocturnal hemoglobinuria. *Blood* 2008; 111(4):1840-7.
 18. Wanner JP, Subbaiah R, Skomorovska-Prokvolit Y, et al. Proteomic profiling and functional characterization of early and late shoulder osteoarthritis. *Arthritis Res Ther*. 2013; 15(6):R180.
 19. Tsuchida AI, Beekhuizen M, Rutgers M, et al. Interleukin-6 is elevated in synovial fluid of patients with focal cartilage defects and stimulates cartilage matrix production in an in vitro regeneration model. *Arthritis Res Ther* 2012; 14(6):R262.
 20. Kokebie R, Aggarwal R, Lidder S, Hakimiyan AA, Rueger DC, Block JA, Chubinskaya S. The role of synovial fluid markers of catabolism and anabolism in osteoarthritis, rheumatoid arthritis and asymptomatic organ donors. *Arthritis Res Ther*. 2011; 13(2):R50.
 21. Kaneko S, Satoh T, Chiba J, Ju C, Inoue K, Kagawa J. Interleukin-6 and interleukin-8 levels in serum and synovial fluid of patients with osteoarthritis. *Cytokines Cell Mol Ther* 2000; 6(2):71-9.
 22. Livshits G, Zhai G, Hart DJ, et al. Interleukin-6 is a significant predictor of radiographic knee osteoarthritis: The Chingford study. *Arthritis Rheum* 2009; 60(7):2037-45.
 23. Wang Y, Xu D, Long L, Deng X, Tao R, Huang G. Correlation between plasma, synovial fluid and articular cartilage Interleukin-18 with radiographic severity in 33 patients with osteoarthritis of the knee. *Clin Exp Med* 2014; 14(3):297-304.
 24. Patel S, Ahmed S. Emerging field of metabolomics: big promise for cancer biomarker identification and drug discovery. *J Pharm Biomed Anal* 2015; 107:63-74.
 25. Priori R, Scrivo R, Brandt J, et al. Metabolomics in rheumatic diseases: The potential of an emerging

- methodology for improved patient diagnosis, prognosis, and treatment efficacy. *Autoimmun Rev* 2013; 12(10):1022-30.
26. Carlson AK, Rawle RA, Adams E, Greenwood MC, Bothner B, June RK. Application of global metabolomic profiling of synovial fluid for osteoarthritis biomarkers. *Biochem Biophys Res Commun* 2018; 499(2):182-8.
27. Zheng K, Shen N, Chen H, et al. Global and targeted metabolomics of synovial fluid discovers special osteoarthritis metabolites. *J Orthop Res* 2017; 35(9):1973-81.
28. Kim S, Hwang J, Kim J, Ahn JK, Cha H-S, Kim KH. Metabolite profiles of synovial fluid change with the radiographic severity of knee osteoarthritis. *Jt Bone Spine* 2017; 84(5):605-10.
29. Mickiewicz B, Kelly JJ, Ludwig TE, et al. Metabolic analysis of knee synovial fluid as a potential diagnostic approach for osteoarthritis. *J Orthop Res* 2015; 33(11):1631-38.
30. Zhang W, Likhodii S, Zhang Y, et al. Classification of osteoarthritis phenotypes by metabolomics analysis. *BMJ Open* 2014; 4(11):e006286. doi:10.1136/bmjopen-2014-006286.

NEW INSIGHTS INTO THE TREATMENT OF NON-HEALING DIABETIC FOOT ULCERS

G. VICENTI¹, D. BIZZOCA¹, I. CARUSO², V. NAPPI¹, G. GIANCASPRO¹,
M. CARROZZO¹ and B. MORETTI¹

¹*Department of Neuroscience and Organs of Sense, Orthopaedics Section, University of Bari Aldo Moro, Bari, Italy;* ²*Department of Emergency and Organ Transplantation, Section of Internal Medicine, Endocrinology, Andrology and Metabolic Diseases, University of Bari Aldo Moro, Bari, Italy*

Diabetic foot ulcers (DFUs) are one of the most serious and devastating complication of diabetes mellitus, affecting about 15% of diabetic patients. This review describes the innovative treatment options currently available in the treatment of non-healing DFUs. The use of Platelet-Rich-Plasma (PRP) is a safe and valid approach in the treatment of DFUs. However, the methods used to obtain and prepare autologous PRP vary between the studies, thus further evidences are eagerly awaited. Adipose tissue-derived mesenchymal stem cells (ADSCs) are a promising tool in the treatment of DFUs, but additional largescale and long-term follow-up clinical trials are needed. Bone marrow mesenchymal stem cells (BM-MSCs) transplantation, on the other hand, revealed effective in reducing incidents and improving the quality of life of patients with amputations. Autologous Peripheral Blood Mononuclear Cells (A-PBMNCs) showed a good efficacy in the treatment of diabetic patients with CLI, but further RCTs are awaited to best investigate this new therapeutic approach. Photobiomodulation (PBM) therapy revealed effective in the treatment of DFUs in two RCTs, but a standardization of therapeutic protocols as well as level-I studies are needed.

Diabetes mellitus (DM) is a major public health problem worldwide (1). According to the International Diabetes Federation (IDF), the global prevalence of DM in 2011 was 366 million, but it is estimated that in 2030 DM (1) will affect 552 million of people.

Diabetic Foot Ulcers (DFUs) are one of its most serious and devastating complications, affecting about 15% of diabetic patients, especially older patients with poor glycaemic control, critical limb ischemia (CLI) and history of chronic lower extremity wounds (2).

DFUs management involves a multidisciplinary approach that includes wound debridement, management of any infection, advanced wound dressings, revascularization procedures when

indicated and off-loading (3).

Nonetheless, it is reported that 10-15% of DFUs remain active after traditional treatment, and 5-24% of them will finally lead to limb amputation within a period of 6-18 months after first evaluation (4). An impaired healing process often occurs in patients with: poor glycaemic control; concomitant limb ischemia; concomitant soft tissue infections; foot deformities (2-4).

Consequently, the research for new therapeutic approaches that could improve DFUs healing is gaining increasing importance. This review describes the innovative treatment options currently available in the treatment of non-healing DFUs, mainly focusing on Platelet-Rich-Plasma (PRP),

Key words: diabetic foot ulcers, critical limb ischemia, mesenchymal stem cells, platelet-rich-plasma, autologous-peripheral blood mononuclear cells, photobiomodulation therapy, low-level light therapy

Corresponding Author:

Dr Giovanni Vicenti, MD, PhD,
Department of Basic Medical Sciences, Neuroscience and Sense Organs,
Orthopaedic Clinic, School of Medicine, University of Bari "Aldo Moro"
AOU Consorziiale Policlinico, Piazza Giulio Cesare 11, 70100 Bari, Italy
Tel.: +39 0805593402
e-mail: dott.gvicenti@gmail.com

Mesenchymal Stem Cells (MSCs), Autologous Peripheral Blood Mononuclear Cells (A-PBMNCs) and photobiomodulation (PBM) therapy.

Platelet-rich plasma

Platelets are physiologically involved in tissue regeneration through the liberation of several substances such as growth factors (PDGF, EGF, FGF, VEGF, TGF-Beta), cytokines (5, 6) and fibrinogen, which is engaged in the constitution of a scaffold on which the cells can implant, thrive and achieve skin closure (7).

In diabetic patients, the wound healing process is affected by several factors, i.e. decreased growth factors (GFs) synthesis, impaired neo-angiogenesis, reduced macrophages function and extracellular matrix imbalances (8). Therefore, the application of GFs, isolated or all together in platelet rich plasma (PRP), has been proposed as a catalyst for diabetic ulcers closure.

It has been shown that the amount of GFs in the granulation tissue of refractory DFUs significantly increases since the third day from PRP gel application (9).

Interestingly, PRP growth factors are enclosed in exosomes and exert their effects only at the fusion with fibroblasts and endothelial cells cytomembrane. Guo et al. demonstrated that PRP-derived exosomes have a central role in promoting neo-angiogenesis and fibroblast proliferation, through the modulation of RhoA/YAP signaling (10).

Furthermore, diabetic wounds are usually burdened by polymicrobial infections sustained by multi-drug resistant bacteria. The *in vitro* study by Çetinkaya et al. suggested that the use of PRP is not contraindicated in the treatment of infected wounds, since it exerts a leukocyte-independent antimicrobial effect on common germs such as methicillin resistant *S. aureus*, *K. pneumoniae* and *S. aeruginosa* (11). Consequently, PRP treated wounds are less prone to infections (12).

The methods used to obtain and prepare autologous PRP vary between the studies and the available evidences are inconclusive regarding which is the best procedure (9). Usually, a venous blood sample is drawn from the patient and centrifuged to separate the platelets from the other blood cells and

concentrate them to at least 1 million/microliter.

GFs liberation is achieved by adding calcium to the sample or by freezing it, in order to physically induce the lysis of the platelets (9). Then, autologous PRP is injected around and into the bed of the wound or applied to it as a gel (9).

A recent and thorough review by Martinez-Zapata et al, including 10 Randomized Controlled Trials (RCTs), suggested that autologous PRP might be beneficial in diabetic foot ulcer healing (RR 1.22, 95% CI 1.01 to 1.49), but several imprecisions and lack of statistical power of the examined trials make this evidence of very low quality (9). Results from RCTs conducted on venous ulcers or ulcers of mixed etiology failed to clarify the role of PRP on complete wound healing (9). Data regarding the adverse reaction profile are also unclear, but seem to be similar between treated and control group (9).

More recent studies (12-14) assert PRP safety and efficacy in achieving diabetic foot ulcers complete closure, but due to their lack in methodological rigor, further evidences are eagerly awaited.

Mesenchymal stem cells

The use of Mesenchymal stem cells (MSCs) is a new attractive treatment option to promote chronic wound, venous and diabetic foot ulcers healing, due to the ability of these cells to differentiate into multiple cells types such as neurons, muscle cells, endothelial cells, adipocytes, osteocytes and chondrocytes (15).

There are different types of mesenchymal stromal cells, autologous or allogeneic, which can be isolated from peripheral blood, bone marrow, umbilical cord, placenta, brain, gingiva, oral mucosa, adipose tissue and amniotic fluid (16).

MSCs can be applied locally to augment wound healing, or with intramuscular and subcutaneously injections, sometimes in association with autologous platelet gels to improve wound healing efficiency (17).

Among the different types of MSCs, adipose tissue-derived mesenchymal stem cells (ADSCs) and bone marrow mesenchymal stem cells (BM-MSCs) have been mainly used in the treatment of non-healing DFUs (21-33).

Adipose tissue is an abundant and easy accessible

source of MSCs, which could be obtained with traditional extraction methods, without altering their activity (18).

Preclinical studies (18-20) have pointed out ADSCs accelerate wound healing thanks to their differentiating, regenerating and angiogenic properties.

Lee et al, in a pilot study on 15 CLI patients (only 3 diabetic patients were recruited), injected ADSCs into the muscles of the lower extremities and reported clinical improvement, in terms of increased ulcer healing rate, advanced walking distance, and the formation of vascular collateral networks in the 3 diabetic patients (21).

In 2014, the first phase-I trial evaluating the feasibility and safety of intramuscular injections of autologous ADSCs in 60 patients with nonrevascularizable CLI, revealed significant improvement in leg pain, ulcer size and pain-free walking distance at a 2-year follow-up (22).

Vicenti et al, in a pilot study on 30 patients with non-healing DFUs, have recently shown that stromal vascular fraction (SVF) enriched lipoinjection is more effective than autologous lipotransfer in reducing the wounds size and the healing time (23).

The results of ADSCs in the treatment of DFUs are promising and no major adverse effect has been reported; however, additional largescale and long-term follow-up clinical trials are needed to determine the long-term safety of this therapeutic approach (24).

To date, six clinical trials involving ADSCs therapies for DFUs are in progress but their results are not available (25).

Bone marrow is a common source of MSCs, even if they are obtained through a highly invasive procedure (26).

Some RCTs (27-29) have investigated the efficacy of intramuscular injection of BM-MSCs in diabetic patients with CLI depicting a significant improvement of amputation rate, Ankle-Brachial Index (ABI), DFUs healing rate and lower limb pain in treated patients.

A recent meta-analysis (30) has concluded that autologous BM-MSCs transplantation is a safe and effective approach that could improve the quality of life of patients with amputations.

Autologous peripheral blood mononuclear cells

Autologous Peripheral Blood Mononuclear Cells (A-PBMNCs), i.e. monocyte/macrophage and lymphocyte circulating populations, are a promising source for cell therapy, since they keep the same differentiating and regenerating properties as the bone marrow mononuclear cells (BMMNCs), but they could be easily isolated from peripheral blood (31).

A-PBMNCs, especially circulating monocytes CD14+, are able to differentiate into different cytotypes, according to the tissue in which they are infiltrated (32). In this differentiation process, both soluble factors released by CD14+ cells and the connection with fibronectin or collagen play an important role (32).

Moreover, A-PBMNCs enhance the tissue repair process through paracrine mechanisms, thanks to the release of several cytokines, including VEGF, FGF2, MCP-1 and angiopoietin, and the activation of a cross-talk with muscle cells and satellite stem cells, thus resulting in an amplified release of angiogenic cytokines (31, 33).

A-PBMNCs act at different stages of the wound healing process, by promoting vasculogenesis, collagen deposit, extracellular matrix remodelling, endothelial progenitor cell recruitment, re-epithelization and neoangiogenesis (31-34).

The great plasticity of CD14+ cells depends on their marked sensitivity to small changes in the tissue microenvironment: circulating monocytes could acquire two different phenotypes, known as M1 (classically activated macrophages) and M2 (alternatively activated macrophages), thanks to the macrophages polarization process (35, 36).

M1 macrophages are predominant in the initial stage of the inflammatory process, when they are triggered by bacterial or viral molecules and inflammatory cytokines; this phenotype aims to provide host protection against microbes in this phase (35, 36). In the resolution stage of an inflammatory process, on the other hand, macrophages are polarized in direction of M2 phenotype by anti-inflammatory cytokines, in order to promote angiogenesis and tissue remodelling (36).

Furthermore, it is reported that M2 macrophages reduce neuropathic pain through the production of

opioid peptides, thus they could also improve the pain management in diabetic patients (37).

Despite the high number of reports (38-45) that have investigated the efficacy of A-PBMNCs in the treatment of critical limb ischemia (CLI) and non-healing chronic DFUs, to date there is a lack of studies with high statistical power: only one RCT (43) and one systematic review (46) have been published.

Huang et al, in a RCT on 28 diabetic patients, have assessed the efficacy of autologous transplantation of granulocyte colony-stimulating factor (G-CSF)-mobilized PBMNCs in the treatment of CLI (Fontaine class III or IV) of diabetic patients (43). These authors reported that, the main manifestations, including lower limb pain and DFUs, as well as the laser Doppler blood perfusion of lower limbs and Mean ankle-brachial pressure index, significantly improved in the treated group, at 3-month's follow-up, in absence of adverse effects (43).

In recent years, however, a new device (Pall Celeris System) which allows to isolate MNCs from peripheral blood thanks to blood gravity filtration, without using G-CSF, has been developed (44-45).

Spaltro et al have recently assessed *in vitro* and *in vivo*, in a hind limb ischemia mouse model, the angiogenic potency of PBMNCs isolated from healthy donors with Pall Celeris system (44). *In vitro*, these authors observed that PBMNCs secrete a panel of angiogenic factors and migrate in response to vascular endothelial growth factor and stromal-derived factor-1 stimuli (44). *In vivo*, moreover, PBMNCs induced neovascularization by increasing capillary, arteriole and regenerative fibre numbers, with human cells detected in murine tissue up to 7 days after ischemia (44).

De Angelis et al, in a prospective non-randomized clinical trial, compared 43 patients, suffering for CLI (Fontaine class IV) and concomitant non-healing DFUs, treated with A-PBMNC with a historically matched control group (45). Patients were implanted with 12mL of A-PBMNC, collected by selective filtration from 120mL of peripheral blood with Pall Celeris system, in the ischemic area of the limbs; A-PBMNC treatment was repeated for a maximum of three times (45). These authors reported the local injection of A-PBMNC has striking early and long-

term effects, together with a favourable safety profile, significantly decreasing the risk of amputation (45).

The only systematic review on this topic, based on only two RCTs, was published in 2014 by Moazzami et al (46), who concluded that there is insufficient evidence to support the local transplantation of autologous MNCs for CLI. On the base of these findings, further Level-I studies are needed to best investigate this new therapeutic approach.

Photobiomodulation therapy

Photobiomodulation (PBM) or Low-Level Light Therapy (LLLT) is a new attractive alternative therapy to enhance wound healing (47). This therapeutic approach involves the tissues exposure to either coherent light sources (lasers) or non-coherent light sources, consisting of filtered lamps or light-emitting diodes (LED), in order to modulate their biological activity: herein the term photobiomodulation (PBM) (48).

The photons, however, to influence a living biological system such as the skin, must be absorbed by tissue photo-acceptors (49). In this transduction process, mitochondria seem to play a central role, since Cytochrome c oxidase (Cox), the terminal enzyme of the electron transport chain, is considered the primary photo-acceptor for the red-Near Infrared (NIR) range in mammalian cells (50).

The absorption of photons by Cox induces, by fastening electron transfer reactions, an increased ATP synthesis and proton gradient, thus enhancing the activity of the Na⁺/H⁺ and Ca²⁺/Na⁺ antiporters, and of all the ATP driven carriers for ions, such as Na⁺/K⁺ ATPase and Ca²⁺ pumps⁵¹. This process finally leads to increased intracellular levels of two important second messengers, i.e. cAMP and Ca²⁺ that influence, on their turn, several biological activities.

Moreover, it is reported that PBM therapy may alter the cell redox potential thus affecting the activity of the redox-dependent transcription factors (51).

Based on these findings, the beneficial effects of PBM on wound healing might be attributed to anti-inflammatory signalling, cell proliferation, protein synthesis, and decreased bacterial infection (52).

In vivo studies on diabetic mouse models, showed that PBM at 633 nm improves the ulcers

healing process by an average of about 10% (53). Furthermore, it is reported that the application of PBM at 635 nm three-times daily, at power densities of 1, 5 and 15 mW/cm², reduces the wounds polymorphonuclear leucocytes infiltration, in normal and diabetic mice (54).

Landau et al (55), in a double-blind RCT on 16 patients with DFUs or chronic leg ulcers, illuminated the treatment group wounds with 180 mW/cm² broadband light (400-800nm) twice a day for 4 min/session. At 12 weeks follow-up, all the wounds were closed in 9 out of 10 patients (90%) from the treatment group; the wound size reduction in the treatment group versus the placebo group was 89% and 54%, respectively.

Franguez et al (56), in a larger sample double blind RCT on 40 diabetic patients and 39 non-diabetic patients with chronic wounds, compared the effects of PBM performed with LED at 625, 660, 850 nm with 2.4 J/cm² power three-times weekly for 8 weeks (treated group) with PBM performed with broadband (580-900 nm) light and a power density of 0.72 J/cm² (control group). The authors observed a significant increase in blood flow in treated diabetic and non-diabetic patients ($p=0.040$ and $p=0.033$); Falanga wound bed score evaluation showed a significant improvement in both treated groups as compared to the control group.

Although PBM therapy is a promising approach in the treatment of non-healing DFUs, a standardization of therapeutic protocols as well as further level-I studies are still needed.

DISCUSSION

Non-healing DFUs can be currently managed with new interesting therapeutic approaches. However, before introducing these innovative treatments in clinical practice further multicentric level-I studies are needed to create standard protocols and definitively assess their efficacy and safety.

REFERENCES

1. James PT, Leach R, Kalamara E, Shayeghi M. The Worldwide Obesity Epidemic. *Obes Res* 2001; 9(S4):228S-33S.
2. Lefrancois T, Mehta K, Sullivan V, Lin S, Glazebrook M. Evidence based review of literature on detriments to healing of diabetic foot ulcers. *Foot Ankle Surg* 2016; 23(4):215-24.
3. Armstrong DG, Boulton AJM, Bus SA. Diabetic foot ulcers and their recurrence. *N Engl J Med* 2017; 376(24):2367-75.
4. Alexiadou K, Doupis J. Management of diabetic foot ulcers. *Diabetes Ther* 2012.
5. Gonchar I V., Lipunov AR, Afanasov IM, Larina V, Faller AP, Kibardin A V. Platelet rich plasma and growth factors cocktails for diabetic foot ulcers treatment: State of art developments and future prospects. *Diabetes Metab Syndr Clin Res Rev* 2018; 12(2):189-94.
6. Anitua E, Andia I, Ardanza B, Nurden P, Nurden AT. Autologous platelets as a source of proteins for healing and tissue regeneration. *Thromb Haemost* 2004; 91(1):4-15.
7. Munirah S, Samsudin OC, Chen HC, Salmah SHS, Aminuddin BS, Ruszymah BHI. Articular cartilage restoration in load-bearing osteochondral defects by implantation of autologous chondrocyte-fibrin constructs: an experimental study in sheep. *J Bone Joint Surg Br* 2007; 89(8):1099-109.
8. Brem H, Tomic-Canic M. Cellular and molecular basis of wound healing in diabetes. *J Clin Invest* 2007; 117(5):1219-22.
9. Mj M, Aj M, Solà I, et al. Autologous platelet-rich plasma for treating chronic wounds (Review) *Cochrane Database Syst Rev* 2016;
10. Guo SC, Tao SC, Yin WJ, Qi X, Yuan T, Zhang CQ. Exosomes derived from platelet-rich plasma promote the re-epithelization of chronic cutaneous wounds via activation of YAP in a diabetic rat model. *Theranostics* 2017; 7(1):81-96.
11. Çetinkaya RA, Yenilmez E, Petrone P, et al. Platelet-rich plasma as an additional therapeutic option for infected wounds with multi-drug resistant bacteria: in vitro antibacterial activity study. *Eur J Trauma Emerg Surg* 2018; 0(0):1-11.
12. Babaei V, Afradi H, Gohardani HZ, Nasser F, Azarafza M, Teimourian S. Management of chronic diabetic foot ulcers using platelet-rich plasma. *J Wound Care* 2017; 26(12):784-7.

13. Suthar M, Gupta S, Bukhari S, Ponemone V. Treatment of chronic non-healing ulcers using autologous platelet rich plasma: a case series. *J Biomed Sci* 2017; 24(1):1-10.
14. Ahmed M, Reffat SA, Hassan A, Eskander F. Platelet-rich plasma for the treatment of clean diabetic foot ulcers. *Ann Vasc Surg* 2017; 38:206-11.
15. Burgos-alonso N, Lobato I, Hern I, Sebastian KS. Adjuvant biological therapies in chronic leg ulcers. *Int J Mol Sci* 2017; 18(12).
16. Tondreau T, Lagneaux L, Dejeneffe M, et al. Isolation of BM mesenchymal stem cells by plastic adhesion or negative selection: phenotype, proliferation kinetics and differentiation potential. *Cytotherapy* 2004; 6(4):372-9.
17. Argôlo Neto NM, Del Carlo RJ, Monteiro BS, et al. Role of autologous mesenchymal stem cells associated with platelet-rich plasma on healing of cutaneous wounds in diabetic mice. *Clin Exp Dermatol* 2012; 37(5):544-53.
18. Skardal A, Mack D, Kapetanovic E, et al. Bioprinted amniotic fluid-derived stem cells accelerate healing of large skin wounds. *Stem Cells Transl Med* 2012; 1(11):792-802.
19. Nambu M, Kishimoto S, Nakamura S, et al. Accelerated wound healing in healing-impaired db/db mice by autologous adipose tissue-derived stromal cells combined with atelocollagen matrix. *Ann Plast Surg* 2009; 62(3):317-21.
20. Nie C, Yang D, Xu J, Si Z, Jin X, Zhang J. Locally administered adipose-derived stem cells accelerate wound healing through differentiation and vasculogenesis. *Cell Transplant* 2011; 20(2):205-16.
21. Lee HWHC, An SG, Lee HWHC, et al. Safety and effect of adipose tissue-derived stem cell implantation in patients with critical limb ischemia. *Circ J* 2012; 76(7):1750-60.
22. Bura A, Planat-Benard V, Bourin P, et al. Phase I trial: the use of autologous cultured adipose-derived stroma/stem cells to treat patients with non-revascularizable critical limb ischemia. *Cytotherapy* 2014; 16(2):245-57.
23. Vicenti G, Solarino G, Pesce V, et al. Autologous lipotransfer versus stromal vascular fraction enriched lipoinjection for diabetic foot wounds healing: A pilot study. *J Biol Regul Homeost Agents* 2017; 31(4suppl1):141-6.
24. Meng F, Zhou D, Li W. Adipose-derived stem cells as a potential weapon for diabetic foot ulcers. *Int J Clin Exp Med* 2017; 10(12):15967-73.
25. Search of: adipose stem cells diabetes - List Results - ClinicalTrials.gov. <https://clinicaltrials.gov/ct2/s?cond=adipose+stem+cells+diabetes&term=&cntry=&state=&city=&dist=>. Accessed August 31, 2018.
26. Zhang Y, Deng H, Tang Z. Efficacy of cellular therapy for diabetic foot ulcer: a meta-analysis of randomized controlled clinical trials. *Cell Transplant* 2017; 26(12):1931-9.
27. Iafrati MD, Hallett JW, Geils G, et al. Early results and lessons learned from a multicenter, randomized, double-blind trial of bone marrow aspirate concentrate in critical limb ischemia. *J Vasc Surg* 2011; 54(6):1650-8.
28. Lu D, Chen B, Liang Z, et al. Comparison of bone marrow mesenchymal stem cells with bone marrow-derived mononuclear cells for treatment of diabetic critical limb ischemia and foot ulcer: A double-blind, randomized, controlled trial. *Diabetes Res Clin Pract* 2011; 92(1):26-36.
29. Powell RJ, Comerota AJ, Berceli SA, et al. Interim analysis results from the RESTORE-CLI, a randomized, double-blind multicenter phase II trial comparing expanded autologous bone marrow-derived tissue repair cells and placebo in patients with critical limb ischemia. *J Vasc Surg* 2011; 54(4):1032-41.
30. Sun X, Ying J, Wang Y, et al. Meta-analysis on autologous stem cell transplantation in the treatment of limb ischemic. *Int J Clin Exp Med* 2015; 8(6):8740-8.
31. Tateno K, Minamino T, Toko H, et al. Critical roles of muscle-secreted angiogenic factors in therapeutic neovascularization. *Circ Res* 2006; 98(9):1194-202.
32. Seta N, Kuwana M. Human circulating monocytes as multipotential progenitors. *Keio J Med* 2007 56(2):41-7.
33. Heil M, Eitenmüller I, Schmitz-Rixen T, Schaper W. Arteriogenesis versus angiogenesis: Similarities and differences. *J Cell Mol Med* 2006; 10(1):45-55.
34. Willenborg S, Eming SA. Macrophages - sensors and effectors coordinating skin damage and repair. *J Dtsch Dermatol Ges* 2014; 12(3):214-21, 214-23.
35. Schwartz M. "Tissue-repairing" blood-derived macrophages are essential for healing of the injured spinal cord: From skin-activated macrophages to infiltrating blood-derived cells? *Brain Behav Immun*

- 2010; 24(7):1054-7.
36. Delavary BM, van der Veer WM, van Egmond M, Niessen FB, Beelen RHJ. Macrophages in skin injury and repair. *Immunobiology* 2011; 216(7):753-62.
 37. Pannell M, Labuz D, Celik MO, et al. Adoptive transfer of M2 macrophages reduces neuropathic pain via opioid peptides. *J Neuroinflammation* 2016; 13(1):262.
 38. Hoshino J, Ubara Y, Hara S, et al. Quality of life improvement and long-term effects of peripheral blood mononuclear cell transplantation for severe arteriosclerosis obliterans in diabetic patients on dialysis. *Circ J* 2007; 71(8):1193-8.
 39. Moriya J, Minamino T, Tateno K, et al. Long-term outcome of therapeutic neovascularization using peripheral blood mononuclear cells for limb ischemia. *Circ Cardiovasc Interv* 2009; 2(3):245-54.
 40. Capiod J-C, Tournois C, Vitry F, et al. Characterization and comparison of bone marrow and peripheral blood mononuclear cells used for cellular therapy in critical leg ischaemia: towards a new cellular product. *Vox Sang* 2009; 96(3):256-65.
 41. Matoba S, Tatsumi T, Murohara T, et al. Long-term clinical outcome after intramuscular implantation of bone marrow mononuclear cells (Therapeutic Angiogenesis by Cell Transplantation [TACT] trial) in patients with chronic limb ischemia. *Am Heart J* 2008; 156(5):1010-8.
 42. Zafarghandi MR, Ravari H, Aghdami N, et al. Safety and efficacy of granulocyte colony-stimulating factor administration following autologous intramuscular implantation of bone marrow mononuclear cells: A randomized controlled trial in patients with advanced lower limb ischemia. *Cytotherapy* 2010; 12(6):783-91.
 43. Huang P, Li S, Han M, Xiao Z, Yang R, Han ZC. Autologous transplantation of granulocyte colony-stimulating factor-mobilized peripheral blood mononuclear cells improves critical limb ischemia in diabetes. *Diabetes Care* 2005; 28(9):2155-60.
 44. Spaltro G, Straino S, Gambini E, et al. Characterization of the Pall Celeris system as a point-of-care device for therapeutic angiogenesis. *Cytotherapy* 2015; 17(9):1302-13.
 45. De Angelis B, Gentile P, Orlandi F, et al. Limb rescue: a new autologous-peripheral blood mononuclear cells technology in critical limb ischemia and chronic ulcers. *Tissue Eng Part C Methods* 2015; 21(5):423-35.
 46. Moazzami K, Moazzami B, Roohi A, Nedjat S, Dolmatova E. Local intramuscular transplantation of autologous mononuclear cells for critical lower limb ischaemia. *Cochrane Database Syst Rev* 2014; 19(12):CD008347.
 47. Avci P, Gupta A, Sadasivam M, et al. Low-level laser (light) therapy (LLLT) in skin: stimulating, healing, restoring. *Semin Cutan Med Surg* 2013; 32(1):41-52.
 48. Kim HP. Lightening up light therapy: Activation of retrograde signaling pathway by photobiomodulation. *Biomol Ther* 2014; 22(6):491-6.
 49. Sutherland JC. Biological effects of polychromatic light. *Photochem Photobiol* 2002; 76(2):164-70.
 50. Karu TI, Kolyakov SF. Exact action spectra for cellular responses relevant to phototherapy. *Photomed Laser Surg* 2005; 23(4):355-61.
 51. Farivar S, Malekshahabi T, Shiari R. Biological effects of low level laser therapy. *J Lasers Med Sci* 2014; 5(2):58-62.
 52. De Freitas LF, Hamblin MR. Proposed Mechanisms of Photobiomodulation or Low-Level Light Therapy. *IEEE J Sel Top Quantum Electron* 2016; 22(3).
 53. Adamskaya N, Dungal P, Mittermayr R, et al. Light therapy by blue LED improves wound healing in an excision model in rats. *Injury* 2011; 42(9):917-21.
 54. Gupta A, Dai T, Hamblin MR. Effect of red and near-infrared wavelengths on low-level laser (light) therapy-induced healing of partial-thickness dermal abrasion in mice. *Lasers Med Sci* 2014; 29(1):257-65.
 55. Landau Z, Migdal M, Lipovsky A, Lubart R. Visible Light-Induced Healing of Diabetic or Venous Foot Ulcers: A Placebo-Controlled Double-Blind Study. *Photomed Laser Surg* 2011; 29(6):399-404.
 56. Frangez I, Cankar K, Ban Frangez H, Smrke DM. The effect of LED on blood microcirculation during chronic wound healing in diabetic and non-diabetic patients-a prospective, double-blind randomized study. *Lasers Med Sci* 2017; 32(4):887-94.

BIOPHYSICAL STIMULATION OF THE KNEE WITH PEMFs: FROM BENCH TO BEDSIDE

G. VICENTI¹, D. BIZZOCA¹, V.S. NAPPI¹, F. MORETTI², M. CARROZZO¹,
V. BELVISO¹ and B. MORETTI¹

¹*Department of Neuroscience and Organs of Sense, Orthopaedics Section, University of Bari Aldo Moro, Bari, Italy;* ²*National Center For Chemicals, Cosmetic Products And Consumer Protection, National Institute of Health, Rome, Italy*

Clinical biophysics investigates the relationship between non-ionizing physical energy and the human body. Although several types of electrical stimulation devices have received US FDA approval for orthopaedic application, the use of Pulsed Electromagnetic Field (PEMFs) play a central role in joint biophysics. This narrative review aims to summarize the current evidences on the efficacy of PEMF-therapy in the treatment of knee articular diseases. Preclinical studies have assessed the effects of PEMFs on chondrocytes, synoviocytes, articular cartilage explants and animal models, showing positive effects of PEMF-therapy on cells proliferation, extracellular matrix (ECM) production, chondrocytes apoptosis and inflammatory cytokines down-regulation. Currently, PEMF-therapy is a valid option in the conservative management of several knee articular diseases, including early OA, patellofemoral pain syndrome and SONK. PEMFs could be also used as an adjunct after an arthroscopic knee procedure or TKA implantation, in order to control the joint post-operative inflammatory state.

Biophysics is the study of biological systems and biological processes using physics-based methods or based on physical principles (1). Clinical biophysics has recently evolved as a medical branch that investigates the relationship between non-ionizing physical energy and the human body (2).

A physical stimulus triggers a biological response by regulating specific intracellular pathways, thus acting as a drug (3). The efficacy of an electrical stimulus, however, depends on the specific features of its physical wave, i.e. frequency and amplitude- and on the exposure time, since only a specific type of wave could activate specific cellular targets (3).

Consequently, the concept of a new soft-pharmacology has been recently developed in clinical biophysics and, for each type of biophysical stimulation; a dose-response relationship has been

described (3).

In the field of Orthopaedics and Traumatology, clinical biophysics focuses on both traumatic, orthopaedic and joint diseases, thus three different subspecialties could be depicted, namely orthopaedic biophysics, traumatic orthopaedic biophysics and joint biophysics (4).

Several types of electrical stimulation devices have received US FDA approval for orthopaedic application, namely inductive coupling, such as Pulsed Electromagnetic Fields (PEMFs) and Combined Magnetic Fields (CMFs), Capacitive Coupling Electric Fields (CCEFs) and Low-Intensity Pulsed Ultrasound (LIPUS) (5).

In joint biophysics, the use of PEMF-therapy plays a central role, since several preclinical and clinical studies have reported positive effects of PEMFs on

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Corresponding Author

Dr Giovanni Vicenti, MD, PhD,
Department of Basic Medical Sciences,
Neuroscience and Sense Organs, Orthopaedic Clinic,
School of Medicine, University of Bari "Aldo Moro", AOU
Consortiale Policlinico, Piazza Giulio Cesare 11, 70100 Bari, Italy
Tel.: +39 0805593402
e-mail: dott.gvicenti@gmail.com

articular cartilage, subchondral bone and synovia (7-37). This narrative review aims to summarize the current evidence on the efficacy of PEMF-therapy in the treatment of knee articular diseases.

Mechanism of action

An electromagnetic field is a physical phenomenon resulting from the combination of an electric field and a magnetic field. The electromagnetic fields employed in clinical biophysics have a frequency below 100 Hz and the magnetic flux density in the range between 0.1mT and 30mT (6).

The PEMF device acts by producing a magnetic field that, in its turn, induces currents to flow in nearby tissues (3). These induced currents mimic the natural electrical activities created within bones during movements, thus triggering a cascade of activities, from the cell membrane to the nucleus and on to the gene level, where specific changes take place (7).

PEMFs mechanism of action, deeply studied in MC3T3-E1 cell line, involves the activation of the ryanodine-sensitive calcium channels in the sarcoplasmic reticulum². The resulting increased cytosolic calcium concentration triggers, in its turn, the calcium-calmodulin pathway, thus causing the upregulation of the genes of the TGF-beta/BMP superfamily (8).

In human chondrocytes (T/C-28a cells), PEMFs also acts by activating and upregulating the adenosine receptors, mainly the A2A and A3 subtypes, thus leading to an anti-inflammatory effect, thanks to the activation of the NF-kB pathway (9,10). This is an interesting biologic effect that could explicate the efficacy of PEMFs in reducing articular inflammation.

Preclinical evidence in joint biophysics

Several preclinical studies have assessed the *in vitro* effects of PEMFs on chondrocytes, synoviocytes, and articular cartilage explants, showing positive effects of PEMF-therapy on cells proliferation, extracellular matrix (ECM) production, chondrocytes apoptosis and inflammatory cytokines down-regulation (11).

The effects of PEMFs on cell proliferation and cartilage ECM component synthesis, including

proteoglycans (PGs) and collagen type II, have been shown in different experimental models (11–17). Moreover, Ongaro et al have recently shown, on human cartilage explants, that PEMFs increase PGs synthesis of the same magnitude as that induced by the IGF-1, one of the most efficient cartilage anabolic growth factor (11,18).

IL-1b is a pro-inflammatory cytokine that promotes the cartilage ECM degradation in healthy and osteoarthritic-joint-derived cells (11). It is reported that PEMFs inhibit the negative effect of the cytokine IL-1b on the production of ECM components in cartilage explants, thus potentially preventing the cartilage ECM degradation (18-21).

Furthermore, it is known that synoviocytes contribute to the homeostasis of the joint environment, by secreting a wide range of pro-inflammatory mediators, such as PGE₂ (22).

Studies on synoviocytes have shown that PEMFs significantly inhibit the release of PGE₂ and the proinflammatory cytokines IL-6 and IL-8, while stimulating the release of IL-10, an anti-inflammatory cytokine (23, 24).

Finally, *in vivo* preclinical studies have reported positive effects of PEMFs on subchondral and epiphyseal bone deposition (26), articular cartilage protection (27) and integration of autologous osteochondral grafts (28) and collagenous scaffold with bone marrow concentrate (29).

From bench to bedside: the realization of a PEMF device

Based on these preclinical data, a PEMF device was finally developed (I-One®, Igea, Carpi, Italy). The magnetic field of this device is generated by a pair of circular coils of copper wire, placed opposite each other. The coils are powered by the generator system, that produces the input voltage of pulse.

The pulse duration of the signal is 1.3 ms and the repetition rate is 75 Hz, yielding a duty cycle of 1/10. The intensity peak of the magnetic field is 1.5 mT and the induced electric field, as detected with a standard coil probe (50 turns, 0.5 cm internal diameter of the coil probe, 0.2 copper diameter), is 0.07 mV/cm.

As previous stated, the exposure time for PEMF-

therapy effectiveness, consequently I-ONE® device should be used 4-6 h per day, for at least 30 to 60 days (30-37). The efficacy of this device, that could be used both in the conservative management of joint diseases both as an adjunct treatment after a surgical procedure, was then confirmed by different clinical trials (30-37).

PEMFS in the conservative management of joint diseases

Based on these preclinical findings, PEMFs could be successfully used in the conservative management of several knee articular pathologies, including early knee osteoarthritis (OA), patellofemoral pain syndrome, and spontaneous osteonecrosis of the knee (SONK).

Gobbi et al, in a perspective level IV study enrolling 22 patients with early symptomatic knee osteoarthritis and functional knee limitations, assessed the efficacy of PEMFs, performed 4 h per day for a total of 45 days, on clinical outcomes (30).

At 1-year follow-up, a significant improvement of pain, range of knee movement and activity level was depicted (30). At the 2-year follow-up, however, an impairment of the clinical scores was recorded, probably due to the reduction of PEMFs activity (24, 27, 31). Based on these findings, they proposed that PEMF-therapy should be annual repeated.

Iammarone et al, in a randomized controlled trial on 33 patients, demonstrated the superiority of PEMF-therapy, performed 4 h per day for 6 weeks, in combination with a home exercise program (HEP) compared with HEP alone, in the management of patellofemoral pain syndrome at 6 and 12 months follow-up (32).

Marcheggiani Muccioli et al, in a level IV study on 28 patients with Koshino stage I spontaneous osteonecrosis of the knee (SONK), have assessed the clinical and MRI effectiveness of PEMF-therapy, performed 6 h daily for 90 days, in the treatment of SONK (33). At the 6-month follow-up, a clinical improvement and a reduction of the SONK area was detected on MRI. At the 24-month follow-up, 86% of patients were preserved from knee prosthetic surgery (33).

PEMFS as an adjunct treatment after a surgical procedure

Arthroscopic surgical procedures could represent an inflammatory stimulus for the joint environment, since they might alter the physiological balance between cartilage, subchondral bone and synovia. Thus, biophysical stimulation with PEMFs could be a useful approach to fasten restoration of the physiological articular environment.

Zorzi et al, in a prospective randomized and double-blind study, evaluated the effect of PEMFs on chondroprotection in a cohort of 31 patients undergoing arthroscopic chondral abrasion and/or perforation and/or radiofrequency for knee osteoarthritis (34). The patients were randomized into two groups: a control group (magnetic field at 0.05 mT) and an active group (magnetic field of 1.5 mT). All the patients began PEMFs treatment within 5 days after the surgical treatment and were instructed to use PEMFs for 90 days, 6 h per day (34). The authors reported a better clinical outcome and a lower NSAIDs assumption at 90-days follow-up (34). At the 3-year follow-up, the number of patients who completely recovered was higher in the active group compared to the control group ($p < 0.05$) (34).

Benazzo et al, in a prospective randomized and double-blind study, evaluated the effectiveness of biophysical stimulation in reducing the articular cartilage inflammation in 29 patients undergoing arthroscopic reconstruction of the anterior cruciate ligament (ACL), with use of quadruple hamstrings semitendinosus and gracilis technique (35).

The patients were randomized to active or placebo treatments; active device generated a magnetic field of 1.5 mT at 75 Hz. All the patients were instructed to use the PEMF device for 4 h per day for 60 days and were recalled at 30, 60, 180 days and 2 years follow-up (35).

A faster clinical recovery was observed in treated patients at 6-months follow-up. At the 2-year follow-up, however, no statistically significant differences between the two groups were found. Based on these data, the authors conclude that the use of PEMFs should always be considered after ACL reconstruction, particularly in professional athletes, to shorten the recovery time, to limit joint

inflammatory reaction and its catabolic effects on articular cartilage and, ultimately, for joint preservation (35).

Moretti et al performed a prospective randomized controlled study on 30 patients undergoing TKA, to assess whether PEMFs can be used to limit the pain and enhance patient recovery after TKA, at 12-months follow-up (36). Patients were randomized into experimental or a control group; patients in the experimental group were instructed to use PEMF device 4 hours per day for 60 days (36). These authors reported an early functional recovery in treated group (36). Similar data were also reported by Adravanti et al, in a randomised controlled trial on 33 patients undergoing TKA (37).

All these data confirm the essential role of PEMFs in reducing post-operative joint inflammation, thus justifying their use, as an adjunct, after a surgical procedure.

CONCLUSION

PEMF-therapy is a valid option in the conservative management of several knee articular diseases, including early OA, patellofemoral pain syndrome and SONK. PEMFs could be also used as an adjunct after an arthroscopic knee procedure or TKA implantation, to better control the post-operative inflammatory state.

REFERENCES

1. Zhou H-X. Q&A: What is biophysics? *BMC Biol.* 2011; 9:13.
2. Aaron RK, Ciombor DM, Wang S, Simon B. Clinical biophysics: the promotion of skeletal repair by physical forces. *Ann N Y Acad Sci* 2006; 1068(1):513-31.
3. Massari L, Benazzo F, Moretti B. Impiego clinico della stimolazione elettrica in ortopedia e traumatologia. *Gaggi* 2017; 43:110-6.
4. Rossini M, Viapiana O, Gatti D, de Terlizzi F, Adami S. Capacitively coupled electric field for pain relief in patients with vertebral fractures and chronic pain. *Clin Orthop Relat Res.* 2010;4 68(3):735-40.
5. Piazzolla A, Solarino G, Bizzoca D, et al. Capacitive coupling electric fields in the treatment of vertebral compression fractures. *J Biol Regul Homeost Agents* 2015; 29(3).
6. Pasek J, Pasek T, Sieroń-Stołtny K, Cieślak G, Sieroń A. Electromagnetic fields in medicine – the state of art. *Electromagn Biol Med* 2015; 35(2):1-6.
7. Bassett CA. Bioelectromagnetics in the service of medicine: acceptance speech on the occasion of receiving the d'Arsonval Medal. *Bioelectromagnetics* 1992; 13(1):7-17.
8. Aaron RK, Boyan BD, Ciombor DM, Schwartz Z, Simon BJ. Stimulation of growth factor synthesis by electric and electromagnetic fields. *Clin Orthop Relat Res* 2004; (419):30-7.
9. Vincenzi F, Targa M, Corciulo C, et al. Pulsed electromagnetic fields increased the anti-inflammatory effect of A₂A and A₃ adenosine receptors in human T/C-28a2 chondrocytes and hFOB 1.19 osteoblasts. *PLoS One* 2013; 8(5):e65561.
10. Varani K, Vincenzi F, Ravani A, et al. Adenosine receptors as a biological pathway for the anti-inflammatory and beneficial effects of low frequency low energy pulsed electromagnetic fields. *Mediators Inflamm* 2017;2017:1-11.
11. Fini M, Pagani S, Giavaresi G, et al. Functional Tissue Engineering in Articular Cartilage Repair: Is There a Role for Electromagnetic Biophysical Stimulation? *Tissue Eng Part B Rev* 2013;19(4):353-67.
12. Sakai A, Suzuki K, Nakamura T, Norimura T, Tsuchiya T. Effects of pulsing electromagnetic fields on cultured cartilage cells. *Int Orthop* 1991; 15(4):341-6.
13. Pezzetti F, De Mattei M, Caruso A, et al. Effects of pulsed electromagnetic fields on human chondrocytes: an in vitro study. *Calcif Tissue Int* 1999; 65(5):396-401.
14. De Mattei M, Caruso A, Pezzetti F, et al. Effects of pulsed electromagnetic fields on human articular chondrocyte proliferation. *Connect Tissue Res* 2001; 42(4):269-79.
15. De Mattei M, Fini M, Setti S, et al. Proteoglycan synthesis in bovine articular cartilage explants exposed to different low-frequency low-energy pulsed electromagnetic fields. *Osteoarthr Cartil*

- 2007; 15(2):163-8.
16. Chang S-H, Hsiao Y-W, Lin H-Y. Low-frequency electromagnetic field exposure accelerates chondrocytic phenotype expression on chitosan substrate. *Orthopaedics* 2011; 34(1):20.
 17. Chang C-H, Loo S-T, Liu H-L, Fang H-W, Lin H-Y. Can low frequency electromagnetic field help cartilage tissue engineering? *J Biomed Mater Res A* 2010; 92(3):843-51.
 18. Ongaro A, Pellati A, Masieri FF, et al. Chondroprotective effects of pulsed electromagnetic fields on human cartilage explants. *Bioelectromagnetics* 2011; 32(7):543-51.
 19. Fioravanti A, Nerucci F, Collodel G, Markoll R, Marcolongo R. Biochemical and morphological study of human articular chondrocytes cultivated in the presence of pulsed signal therapy. *Ann Rheum Dis* 2002; 61(11):1032-3.
 20. De Mattei M, Pasello M, Pellati A, et al. Effects of electromagnetic fields on proteoglycan metabolism of bovine articular cartilage explants. *Connect Tissue Res* 2003; 44(3-4):154-9.
 21. Bobacz K, Graninger WB, Amoyo L, Smolen JS. Effect of pulsed electromagnetic fields on proteoglycan biosynthesis of articular cartilage is age dependent. *Ann Rheum Dis* 2006; 65(7):949-51.
 22. Martel-Pelletier J, Pelletier JP. Is osteoarthritis a disease involving only cartilage or other articular tissues? *Eklek Hastalik Cerrahisi* 2010; 21(1):2-14.
 23. Ongaro A, Varani K, Masieri FF, et al. Electromagnetic fields (EMFs) and adenosine receptors modulate prostaglandin E 2 and cytokine release in human osteoarthritic synovial fibroblasts. *J Cell Physiol* 2012; 227(6):2461-9.
 24. De Mattei M, Varani K, Masieri FF, et al. Adenosine analogs and electromagnetic fields inhibit prostaglandin E2 release in bovine synovial fibroblasts. *Osteoarthr Cartil* 2009; 17(2):252-62.
 25. Stolfi S, Skovránek M, Stolfi P, Rosocha J, Vasko G, Sabo J. Effects of static magnetic field and pulsed electromagnetic field on viability of human chondrocytes in vitro. *Physiol Res* 2007; 56(Suppl1):S45-9.
 26. Fini M, Torricelli P, Giavaresi G, et al. Effect of pulsed electromagnetic field stimulation on knee cartilage, subchondral and epyphiseal trabecular bone of aged Dunkin Hartley guinea pigs. *Biomed Pharmacother* 2008; 62(10):709-15.
 27. Fini M, Torricelli P, Giavaresi G, et al. Effect of pulsed electromagnetic field stimulation on knee cartilage, subchondral and epyphiseal trabecular bone of aged Dunkin Hartley guinea pigs. *Biomed Pharmacother* 2008; 62(10):709-15.
 28. Benazzo F, Cadossi M, Cavani F, et al. Cartilage repair with osteochondral autografts in sheep: Effect of biophysical stimulation with pulsed electromagnetic fields. *J Orthop Res* 2008; 26(5):631-42.
 29. Veronesi F, Cadossi M, Giavaresi G, et al. Pulsed electromagnetic fields combined with a collagenous scaffold and bone marrow concentrate enhance osteochondral regeneration: an in vivo study. *BMC Musculoskelet Disord*. 2015; 16:233.
 30. Gobbi A, Lad D, Petrera M, Karnatzikos G. Symptomatic early osteoarthritis of the knee treated with pulsed electromagnetic fields: two-year follow-up. *Cartilage*. 2014; 5(2):78-85.
 31. Cadossi R, Setti S. Low-frequency pulsed electromagnetic fields in orthopedic practice: bone and cartilage repair 2011.
 32. Servodio Iammarrone C, Cadossi M, Sambri A, Grosso E, Corrado B, Servodio Iammarrone F. Is there a role of pulsed electromagnetic fields in management of patellofemoral pain syndrome? Randomized controlled study at one year follow-up. *Bioelectromagnetics* 2016; 37(2):81-88.
 33. Marcheggiani Muccioli GM, Grassi A, Setti S, et al. Conservative treatment of spontaneous osteonecrosis of the knee in the early stage: pulsed electromagnetic fields therapy. *Eur J Radiol* 2013; 82(3):530-7.
 34. Zorzi C, Dall'Oca C, Cadossi R, Setti S. Effects of pulsed electromagnetic fields on patients' recovery after arthroscopic surgery: Prospective, randomized and double-blind study. *Knee Surgery, Sport Traumatol Arthrosc* 2007; 15(7):830-4.
 35. Benazzo F, Zanon G, Pederzini L, et al. Effects of biophysical stimulation in patients undergoing arthroscopic reconstruction of anterior cruciate ligament: Prospective, randomized and double blind study. *Knee Surg Sports Traumatol Arthrosc* 2008; 16(6):595-601.

36. Moretti B, Notarnicola A, Moretti L, et al. I-ONE therapy in patients undergoing total knee arthroplasty: A prospective, randomized and controlled study. *BMC Musculoskelet Disord* 2012; 13(1):1.
37. Adravanti P, Nicoletti S, Setti S, Ampollini A, De Girolamo L. Effect of pulsed electromagnetic field therapy in patients undergoing total knee arthroplasty: A randomised controlled trial. *Int Orthop* 2014; 38(2):397-403.

TANTALUM MONOBLOCK CUPS IN TOTAL HIP ARTHROPLASTY: CLINICAL RESULTS AND OUTCOME PREDICTORS

O. GALASSO¹, M. DE GORI², S. CERBASI³, F. FAMILIARI⁴, P. RECANO³, G. BALATO³,
G. GASPARINI¹ and MASSIMO MARICONDA³

¹Department of Orthopaedic and Trauma Surgery, "Magna Graecia" University, Catanzaro, Italy;

²Department of Orthopaedic and Trauma Surgery, "S. Francesco di Paola" Hospital, Paola, Italy; ³Department of Orthopaedic and Trauma Surgery, "Federico II" University, Naples, Italy;

⁴Department of Orthopaedic and Trauma Surgery, "Villa del Sole" Clinic, Catanzaro, Italy

This study aims to report the minimum 2-year follow-up results of the tantalum monoblock cup in primary THA and to identify possible outcome predictors. Eighty-eight porous tantalum monoblock acetabular cup in primary THA were reviewed. The Harris Hip Score (HHS) and the Short Form-36 Health Survey (SF-36) were used for the evaluation of outcomes. Radiographic evaluation included acetabular component orientation, presence of bone gaps, radiolucent lines, new bone formation and heterotopic ossifications. After a mean follow-up of 55.4±19.5 months, no component revision was noted. The HHS improved from 43.6±14.6 to 88.3±8.4 ($P<0.001$). The mean physical domain of the SF-36 did not significantly differ from that of age-matched, healthy subjects ($P=0.072$); the mean mental component of the SF-36 was significantly higher than that of age-matched, healthy subjects ($P<0.001$). Negative determinants of postoperative HHS (total adjusted $R^2=0.328$) using tantalum monoblock cups were age at surgery ($R^2=0.164$, $P<0.001$), female sex ($R^2=0.103$, $P<0.001$), and acetabular inclination ($R^2=0.084$, $P=0.003$).

Due to excellent rates of bone ingrowth and low rates of loosening, resulting in improved longevity, press-fit, cementless fixation has gained momentum during the past decades as the preferred method for acetabular reconstruction in total hip arthroplasty (THA) (1).

Faced with the increasing demand for joint arthroplasty surgery, intense efforts have been made to further improve fixation, ingrowth and survivorship, especially in younger and active patients in which disappointing failure rates can be still detected. In this light, cementless monoblock acetabular cups have been introduced as an alternative to cementless modular and cemented all-polyethylene components to increase the survival rate of primary THA. As the polyethylene liner is compression-molded into the

metallic shell, micromotions and wears at the interface are eliminated, thus decreasing the polyethylene wear rate, osteolysis, and aseptic loosening (2). The porous metallic shell has a high friction coefficient that enhances the primary mechanical stability of the acetabular component reducing micromotions at the bone-implant interface, and osteoconductive properties of the metal shell may help bone ingrowth of the component and therefore determine its secondary stability (3). The low modulus of elasticity produces a more physiologic load transfer with diminished stress shielding (4).

Excellent results of the monoblock tantalum cups have been demonstrated up to 15 years postoperatively (5), but there is still a lack of information dealing with possible determinants of clinical and radiographic

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Corresponding author:

Marco De Gori, MD,

Department of Orthopaedic and Trauma Surgery,

"S. Francesco di Paola" Hospital,

Via Promintesta, 1,

87027, Paola (CS), Italy

e-mail: marco.degori@aspcs.gov.it

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outcomes. This study aims to report the minimum 2-year follow-up clinical and radiographic results of the tantalum monoblock acetabular cup during primary THA, and to determine which variables behave as outcome predictors.

MATERIALS AND METHODS

Study population

The study protocol was approved by the local ethical committee and the research was carried out according to the Helsinki declaration. Informed consent was obtained from all individual participants included in the study.

From January 2010 to December 2012, patients undergoing THA were eligible for the use of a porous tantalum monoblock cup if there was sufficient periacetabular bone stock for peripheral rim fixation on preoperative X-rays. Contraindications were acetabular segmental or rim non-supportive defects, and severe acetabular dysplasia (6). Criteria to be included in this retrospective study were (1) primary THA and (2) a minimum follow-up of 24 months. In the study period, 130 patients underwent THA with a porous tantalum monoblock cup, and 95 of them fulfilled the inclusion criteria. Eighteen patients were lost to follow-up, leaving 78 (82.1%) patients available for the study. There were 46 (60%) males and 32 (40%) females, with a mean body mass index of $29.1 \pm 4.3 \text{ kg/m}^2$, and a mean age of 61.4 ± 12.7 years at surgery.

Ten patients underwent bilateral THA; thus, data were available for 88 cases. Osteoarthritis was the primary underlying diagnosis in 49 cases. Other diagnoses were: osteonecrosis of the femoral head in 16 cases; developmental hip dysplasia in 10 cases (grade I in 8 cases and grade II in 2 cases according to Crowe); Perthes' disease in 4 cases; rheumatoid arthritis in 3 cases; sequelae of epiphysiolysis in 2 cases; psoriatic arthritis, post-traumatic arthritis, sclerodermic arthritis and femoral neck fracture in 1 case each.

Surgical procedure

Patients were operated by two hip surgeons (OG and MM). All operations were performed under general anesthesia in lateral decubitus position. A posterolateral surgical approach was used in all cases.

The acetabulum was prepared with hemispherical reamers, and porous tantalum monoblock cups (Trabecular Metal Monoblock Acetabular Component System, Zimmer Inc., Warsaw, IN, USA) were implanted. As the cups had a hemi-ellipsoid geometry, the diameter of the final reamer matched the polar diameter of the acetabular component and was 2 mm less than the equatorial diameter. A line-to-line reaming technique was used: in details, a 44-mm cup was used in 3 cases, a 46-mm in 10 cases, a 48-mm in 20 cases, a 50-mm in 25 cases, a 52-mm in 18 cases, a 54-mm in 8 cases, a 56-mm in 3 cases and a 60-mm in 1 case. Cup version and inclination angles were chosen using a mechanical alignment guide. A 28-mm head was used in 8 cases, a 32-mm head in 35 cases and a 36-mm head in 45 cases; heads were made of either cobalt-chromium alloy (47 cases) or ceramic (41 cases). Head size was adapted to cup diameter and ceramic heads were used in patients ≤ 60 -years-of-age. No bone grafting was used in any case. The femur was then prepared and reamed and a CLS Spotorno stem was used in 57 cases, a VerSys stem in 24 cases, a Müller stem in 4 cases, and a Wagner stem (for all, Zimmer Inc., Warsaw, IN, USA) in 3 cases.

Immediate active and passive motion and full weight bearing were allowed as tolerated postoperatively. Hospital discharge occurred when the patient was able to walk independently with crutches, and physiotherapy was continued for 2 months. Starting one day preoperatively, all patients received a 6-week pharmacological subcutaneous antithromboembolic therapy with low-molecular-weight heparin; patients also received a 24-hour pharmacological venous antibiotic prophylaxis with wide spectrum β -lactams or glycopeptides.

Clinical evaluation

The Harris Hip Score (HHS) (7) and the Short Form-36 Health Survey (SF-36) questionnaire (8) were the instruments chosen for the evaluation of patients. The HHS is a widely used specific outcome measure for hip studies to assess pain and functional status. Sum scores are fitted in a 0 - 100 scale, with high values indicating less pain or better physical functioning. The HHS was assessed both preoperatively and at the last follow-up. The percent

HHS recovery rate (RR) between preoperative and follow-up was calculated as follows: [(postoperative value - preoperative value) / postoperative value] %. Information about comorbidity was gathered preoperatively using the Cumulative Illness Rating Scale (9). Pain, ability to work and postoperative satisfaction were also evaluated using an analog centesimal scale.

Radiographic evaluation

A radiographic assessment including a standing anteroposterior view of the pelvis was performed in all patients after surgery and at the last follow-up. Acetabular component inclination was determined as the angle between the greater diameter of cup face and a line tangent to the base of both Köhler teardrops (5). Anteversion of the acetabular cup was empirically determined using the ratio between short axis and long axis of the ellipse; cups oriented within an anteversion of $15^{\circ} \pm 10^{\circ}$ and an inclination angle of $40^{\circ} \pm 10^{\circ}$ were considered appropriate (10). Areas of absence of contact between the surface of the cup and the bone immediately after surgery were defined as gaps, in contrast with radiolucent lines that developed over the follow-up interval at the bone-implant interface (11). The presence of gaps, radiolucent lines and new bone growth on the interface were assessed according to the zones described by DeLee and Charnley (12). The presence of heterotopic ossifications was also recorded and assessed according to the Brooker classification system (13). A component was considered to have migrated if change in position between the immediate postoperative and the follow-up evaluation was greater than 3 mm and/or 8° (14). Radiograms were independently evaluated by 2 observers, and evaluations were repeated twice on 2 separate days; a consensus decision was reached in a final common readout. Correlation coefficients for interobserver and intraobserver reliability of repeated measures were >0.8 in all cases.

Statistical analysis

Descriptive statistics were used for continuous variables, and proportions for categorical variables. The Kolmogorov-Smirnov test was preliminarily used to assess the normality of data distribution. The Student's *t*-test was used to assess the significance of

differences. Univariate and multiple forward stepwise linear regression analysis were used to assess the association of independent variables with the HSS at follow-up, the RR of HSS, and the level of satisfaction with surgery. Only variables that showed a trend toward an association with the outcome of interest (i.e., $P < 0.1$) in the univariate analysis were included in the models of multiple regression analysis. In these models, the total adjusted R^2 for the model and changes in R^2 for the independent contribution of single predictors were calculated to assess the total variance in the outcome variable accounted for by the whole model and the single explanatory variables, respectively. In the case of collinearity between explanatory variables, only the best-fitting models (e.g., those including the collinear explanatory variable accounting for the most relevant variation in the variance of the outcomes) were adopted. In details, the explanatory variables included in the analyses were sex, age at surgery, body mass index, Cumulative Illness Rating Scale, acetabular inclination, acetabular version, bone gaps, radiolucent lines, new bone formation, and heterotopic ossifications. The preoperative and postoperative values of HHS, SF-36's PCS and SF-36's MCS were also checked as possible determinants of the level of satisfaction with surgery at follow-up.

To calculate the post hoc power ($1 - \beta$ error probability, 2-tailed) achieved by our statistical tests, we considered the sample size, the observed effect size, and the α value of 0.05. A post hoc power $>80\%$ was considered adequate to fully accept the results. IBM SPSS Statistics 21.0.0.1 software (IBM Corp, Armonk, NY, USA) and G*Power 3.1 software (Institut für Experimentelle Psychologie, Heinrich Heine Universität, Dusseldorf, Germany) were used for the database construction and the statistical analysis. A 2-tailed $P < 0.05$ was considered significant.

RESULTS

Clinical results

After a mean follow-up of 55.4 ± 19.5 (range, 24-102) months, the HHS improved from 43.6 ± 14.6 (range, 1 - 90.8) to 88.3 ± 8.4 (range, 68 - 100) ($P < 0.001$, 100% power), with a mean 51% recovery rate. The pain assessed on the centesimal analogue

scale decreased from 75 ± 20.5 preoperatively to 9.8 ± 14.7 at follow-up ($P < 0.001$, 100% power), whereas the ability to work increased from 46.4 ± 23.9 to 80 ± 18.6 ($P < 0.001$, 100% power). The mean PCS was 44 ± 6.7 , and this value did not significantly differ from that of age-matched, healthy subjects ($P = 0.072$, 81% power); the mean MCS was 59.8 ± 11.8 , and this value was significantly higher than that of age-matched, healthy subjects ($P < 0.001$, 100% power). The level of satisfaction at follow-up was 90 ± 14.4 . No major complications (i.e., periprosthetic joint infections, dislocations, loosening, and deep venous thrombosis / pulmonary embolism) were observed.

Radiographic results

No loosening or migration of components was observed at the last follow-up. There were 60 (68.2%) acetabular cups oriented in correct version and 28 (31.8%) in anteversion. The mean acetabular inclination was $41.7^\circ \pm 8.1$ (range 22° - 67°). Bone gaps were detected in 2 (2.2%) cases (1 in zone I and 1 in zone III) and appeared completely filled by newly formed bone at the last follow-up. Radiolucent lines occurred in 7 (8%) cases: 4 in zone II, 2 in zones I and III, and 1 in zones I, II, and III. New bone formation was observed in 17 (19.3%) cases: 13 in zone I, 3 in zones I and III, and 1 in zone III. Heterotopic ossifications were observed in 10 (11.4%) cases and there were 8 grade I and 2 grade II ossifications.

Regression analysis

Stepwise regression analysis revealed that the most important negative determinants of postoperative HHS (total adjusted $R^2 = 0.328$) were age at surgery ($R^2 = 0.164$, $P < 0.001$), female sex ($R^2 = 0.103$, $P < 0.001$), and acetabular inclination ($R^2 = 0.084$, $P = 0.003$). The postoperative HHS ($R^2 = 0.227$, $P < 0.001$) and MCS ($R^2 = 0.099$, $P = 0.001$) were the most important positive predictors of postoperative satisfaction (total adjusted $R^2 = 0.325$).

The presence of bone gaps, radiolucent lines, new bone formation, or heterotopic ossifications was not found to be predictive of any outcomes at follow-up.

DISCUSSION

Periprosthetic osteolysis and aseptic loosening

remain leading causes of THA failure (15) and improvements in techniques and designs of acetabular components have significantly decreased the rate of failures (16). The primary mechanical stability of tantalum monoblock acetabular cup due to its tribologic features has been advocated to improve THA stability and longevity. Potential disadvantages using such components include the difficulty to insert them in a proper way due to rough surface and dished contour, the difficulty for an inexperienced surgeon to evaluate the presence of bottoming out effect (16), and concerns about the component revision in case of infection (17) due to the high bone ingrowth and impossibility of a liner exchange.

Our results demonstrated that the use of monoblock tantalum acetabular cup in THA achieves a significant improvement of the HHS. This is a valid and reliable scale and is often used as a reference gold standard for assessing the construct validity of other hip outcome measures (18). In the current study, the HHS improved from 43.6 preoperatively to 88.3 five years after surgery that is a higher than thresholds of 16 - 18 points and 40 points respectively for minimal and moderate improvement previously reported for the HHS (19). These data are in full agreement with those of previous studies with a similar follow-up (11, 16); interestingly, De Martino et al. (5) detected a HSS improvement from 47 preoperatively to 94 at a minimum 15-year follow-up, thus assessing the effective durability of such reconstructions.

Researchers and clinicians increasingly are recognizing the importance of assessing quality of life outcomes when evaluating the efficacy of surgical procedures (20, 21). We noted that patients who had undergone THA with a monoblock tantalum cup had a similar mid-term quality of life with respect to age-matched healthy controls in terms of both physical and mental function. To the best of our knowledge, only one study by Ayers et al. (22) previously used the SF-36 in the evaluation of monoblock tantalum acetabular cups and detected a significant improvement in the sole PCS, while the MCS did not change up to 5 years postoperatively.

One randomized controlled trial (23) demonstrated that long-term results of tantalum cups are superior in

comparison with those of titanium monoblock cups in primary THA, with no revisions occurring for cup loosening 12 years postoperatively. Accordingly, in our series no component loosening was observed at the last follow-up. This finding is in agreement with most of the current available literature on tantalum monoblock cups (16, 22, 23, 24). Detection of bone gaps at the immediate postoperative radiographs may represent a concern of THAs with tantalum monoblock acetabular components. Indeed, these implants may prevent surgeon from assessing the complete seating of cup during implantation (2).

The occurrence of bone gaps in the current study is lower than that of previously published series (5, 22, 23). The frequency of radiolucent lines in our series concurs with the data by Wegrzyn et al. (23) and Gruen et al. (24) showing that despite bone gaps rate in tantalum cups was significantly higher than that in titanium cups, a lower rate of radiolucent lines has to be expected at the final follow-up due to the excellent osteointegration. Notably, our regression analysis indicates that neither the presence of bone gaps nor radiolucent lines significantly affect postoperative outcomes; however, the sample size impedes us to draw firm conclusions. We demonstrated that better clinical outcomes can be expected in males and younger patients, confirming, at least in part, previously published results (25). As a further outcome predictor, excessive acetabular inclination was associated with poor results. It has been previously demonstrated that proper acetabular orientation within a so-called safe zone in both version and inclination plays a pivotal role in determining outcomes following THA (10).

Main limitations of this study include the small sample size and lack of a control group with a different cup. The short duration of follow-up also prevented us to draw definite conclusions on the implant longevity and survivorship. As a further limitation, the hips were evaluated with plain radiographs that typically underestimate the true incidence of osteolysis. Limits arising from the retrospective design should be finally considered; we tried to partially overcome this issue by a post hoc validation of our statistical tests using a strict power analysis.

In conclusion, the use of tantalum monoblock acetabular cups in primary THA is followed by significant clinical improvement from 24 to 102 months after surgery. Patients show quality of life that is comparable to healthy subjects. Male sex, younger age at surgery and proper acetabular inclination behave as determinants of clinical outcomes.

REFERENCES

1. Della Valle CJ, Mesko NW, Quigley L, Rosenberg AG, Jacobs JJ, Galante JO. Primary total hip arthroplasty with a porous-coated acetabular component. A concise follow-up, at a minimum of twenty years, of previous reports. *J Bone Joint Surg Am* 2009; 91:1130-5.
2. Sculco TP. The acetabular component: an elliptical monoblock alternative. *Orthopedics* 1998; 21:973-4.
3. Bobyn JD, Stackpool GJ, Hacking SA, Tanzer M, Krygier JJ. Characteristics of bone ingrowth and interface mechanics of a new porous tantalum biomaterial. *J Bone Joint Surg Br* 1999; 81:907-14.
4. De Martino I, D'Apolito R, Sculco PK, Poultsides LA, Gasparini G. Total knee arthroplasty using cementless porous tantalum monoblock tibial component: a minimum 10-year follow-up. *J Arthroplasty* 2016; 31:2193-8.
5. De Martino I, De Santis V, Sculco PK, D'Apolito R, Poultsides LA, Gasparini G. Long-term clinical and radiographic outcomes of porous tantalum monoblock acetabular component in primary hip arthroplasty: a minimum of 15-year follow-up. *J Arthroplasty* 2016; 31:S110-4.
6. Crowe JF, Mani VJ, Ranawat CS. Total hip replacement in congenital dislocation and dysplasia of the hip. *J Bone Joint Surg Am* 1979; 61:15-23.
7. Harris WH. Traumatic arthritis of the hip after dislocation and acetabular fractures: treatment by mold arthroplasty. An end-result study using a new method of result evaluation. *J Bone Joint Surg Am* 1969; 51:737-55.
8. Apolone G, Mosconi P. The Italian SF-36 Health Survey: translation, validation and norming. *J Clin Epidemiol* 1998; 51:1025-36.
9. Linn BS, Linn MW, Gurel L. Cumulative illness rating scale. *J Am Geriatr Soc* 1968; 16:622-6.
10. Lewinnek GE, Lewis JL, Tarr R, Compere CL,

- Zimmerman JR. Dislocations after total hip-replacement arthroplasties. *J Bone Joint Surg Am* 1978; 60:217-20.
11. Malizos KN, Bargiotas K, Papatheodorou L, Hantes M, Karachalios T. Survivorship of monoblock trabecular metal cups in primary THA: midterm results. *Clin Orthop Relat Res* 2008; 466:159-66.
 12. DeLee JG, Charnley J. Radiological demarcation of cemented sockets in total hip replacement. *Clin Orthop Relat Res* 1976; 121:20-32.
 13. Brooker AF, Bowerman JW, Robinson RA, Riley LH Jr. Ectopic ossification following total hip replacement. Incidence and a method of classification. *J Bone Joint Surg Am* 1973; 55:1629-32.
 14. Massin P, Schmidt L, Engh CA. Evaluation of cementless acetabular component migration. An experimental study. *J Arthroplasty* 1989; 4:245-51.
 15. Clohisy JC, Calvert G, Tull F, McDonald D, Maloney WJ. Reasons for revision hip surgery: a retrospective review. *Clin Orthop Relat Res* 2004; 429:188-92.
 16. Xenakis TA, Macheras GA, Stafilas KS, Kostakos AT, Bargiotas K, Malizos KN. Multicentre use of a porous tantalum monoblock acetabular component. *Int Orthop* 2009; 33:911-6.
 17. Gasparini G, De Gori M, Calonego G, Della Bora T, Caroleo B, Galasso O. Drug elution from high-dose antibiotic-loaded acrylic cement: a comparative, in vitro study. *Orthopedics* 2014; 37:e999-1005.
 18. Söderman P, Malchau H. Is the Harris hip score system useful to study the outcome of total hip replacement? *Clin Orthop Relat Res* 2001; 384:189-97.
 19. Singh JA, Schleck C, Harmsen S, Lewallen D. Clinically important improvement thresholds for Harris Hip Score and its ability to predict revision risk after primary total hip arthroplasty. *BMC Musculoskelet Disord* 2016; 17:256.
 20. Galasso O, Mariconda M, Donato G, et al. Histopathological, clinical, and electrophysiological features influencing postoperative outcomes in carpal tunnel syndrome. *J Orthop Res* 2011; 29:1298-304.
 21. Mariconda M, Galasso O, Secondulfo V, Cozzolino A, Milano C. The functional relevance of neurological recovery after lumbar discectomy: a follow-up of more than 20 years. *J Bone Joint Surg Br* 2008; 90:622-8.
 22. Ayers DC, Greene M, Snyder B, Aubin M, Drew J, Bragdon C. Radiostereometric analysis study of tantalum compared with titanium acetabular cups and highly cross-linked compared with conventional liners in young patients undergoing total hip replacement. *J Bone Joint Surg Am* 2015; 97:627-34.
 23. Wegrzyn J, Kaufman KR, Hanssen AD, Lewallen DG. Performance of porous tantalum vs. titanium cup in total hip arthroplasty: randomized trial with minimum 10-year follow-up. *J Arthroplasty* 2015; 30:1008-13.
 24. Gruen TA, Poggie RA, Lewallen DG, et al. Radiographic evaluation of a monoblock acetabular component: a multicenter study with 2- to 5-year results. *J Arthroplasty* 2005; 20:369-78.
 25. Mariconda M, Galasso O, Costa GG, Recano P, Cerbasi S. Quality of life and functionality after total hip arthroplasty: a long-term follow-up study. *BMC Musculoskelet Disord* 2011; 12:222.

HOW CARTILAGE STATUS CAN BE RELATED TO JOINT LOADS IN ANTERIOR CRUCIATE LIGAMENT RECONSTRUCTION: A PRELIMINARY ANALYSIS INCLUDING MRI T2 MAPPING AND JOINT BIOMECHANICS

G. CASSIOLAS¹, M. BERNI¹, G. MARCHIORI¹, M. BONTEMPI¹,
L. BRAGONZONI², G. VALENTE¹, A. GRASSI¹, S. ZAFFAGNINI¹,
A. VISANI¹ and N. F. LOPOMO³

¹IRCCS Istituto Ortopedico Rizzoli, Bologna, Italy; ²Alma mater studiorum, Università di Bologna, Bologna; ³Università degli Studi di Brescia, Brescia, Italy

The knee is the largest and most complex joint in the human body. Traumatic events, such as anterior cruciate ligament (ACL) tear, can lead to an alteration of joint tissues homeostasis. Literature reports an evident correlation between abnormal joint biomechanics and the status of articular tissues. These alterations, due to a sub-optimal ACL reconstruction, may result in an increasing risk of developing degenerative pathologies, such as osteoarthritis. Thus, the identification of the optimal surgical technique is a highly demanding issue in ACL reconstruction. The aim of this study was to analyze the correlation between joint cartilage conditions and knee biomechanics in ACL reconstructions, by integrating MRI T2 mapping investigations, radiostereophotogrammetry-based gait analysis and subject-specific musculoskeletal modelling.

Due to its prominent loading-supporting role, human knee joint is very often subjected to traumatic injuries. Among them, anterior cruciate ligament (ACL) lesion presents the highest rate of incidence (1). Surgical ACL reconstruction is indeed one of the most widespread approach, used to restore joint biomechanics and reduce pain. Nevertheless, the choice of the optimal surgical technique and graft represents a critical issue. In fact, there is evidence that the knee often incurs in altered conditions affecting the homeostatic state of the articular tissues and definitely increasing the risk of developing degenerative pathologies, such as osteoarthritis (OA); this alteration of the joint biomechanical condition can occur due to both ACL injury and, above all, a sub-optimal reconstruction (2). In particular, articular cartilage can demonstrate the signs of alteration, with OA that is radiologically reported to occur in 10% to 90% of the cases, after few years from the

surgery (3). However, early stages of OA appear as physiological modifications within the collagen fiber network, which can be hardly detectable by standard radiographic control, thus precluding the possibility of any conservative treatment. The progression of these altered conditions has been attributed to a variation of joint biomechanics after ACL reconstruction, which may therefore contribute to the onset of the disease itself (4).

In this perspective, the integration of novel medical imaging technologies and protocols such as those provided by magnetic resonance imaging (MRI) and innovative approaches for estimating knee biomechanics, such as subject-specific multi-body models and radiostereophotogrammetry-based gait analysis system, may prove to be effective in detecting critical conditions leading to early OA development. Actually, MRI can be exploited for an early detection of cartilage changes (5). Several

Key words: cartilage status, joint biomechanics, T2 mapping, ACL reconstruction.

Corresponding author:

Ing. Giorgio Cassiolas
Laboratorio di Biomeccanica e Innovazione Tecnologica,
IRCCS Istituto Ortopedico Rizzoli,
Via di Barbiano 10/2, 40136 Bologna, Italy
Tel.: +39 0516366042
e-mail: g.cassiolas@unibs.it

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MR sequences have been proposed as “biomarkers” specific for early OA detection; particularly, transverse relaxation time T2 revealed to be able to evaluate the collagen content-fiber orientation and the loss of type II collagen, which are the first signs of cartilage degeneration (6). On the other side, multi-body modelling has been extensively used to simulate musculoskeletal system during different tasks (7), specifically concerning also knee joint and ACL issues (8).

The main goal of this preliminary study was to investigate – by integrating MRI T2 mapping, gait analysis via radiostereophotogrammetry (RSA) and subject-specific multi-body lower limb modelling, whether the changes in joint biomechanics, necessarily occurring after ACL reconstruction, can be significantly correlated to the knee cartilage physiological condition. This approach specifically aims to provide a reliable tool able to support the optimization of the reconstructive procedure, leading to an effective surgical prevention of OA development.

MATERIALS AND METHODS

This study was approved by local IRB and written informed consent was obtained from all participants. Overall, clinical trial included 42 patients undergoing ACL reconstruction. Out of these subjects, in this preliminary analysis, we considered 9 patients (3 females and 6 males, age = 22.2±6.1 years), who underwent 3 different surgical techniques: single-bundle (SB) (9), non-anatomic double-bundle (naDB) (9) and single-bundle with lateral plasty (SBLP) (10); each technique included 3 subjects. For each subject, MRI T2 mapping sequences were performed via 1.5 T scanner (GE Healthcare) both before (baseline) and 18 months after the surgery (FU18). At the same time, stance phase was monitored during standard level gait by means of a radiostereophotogrammetric system. Knee biomechanics was then estimated by multi-body musculoskeletal modeling (7). Graphical details on the workflow are reported in Fig. 1.

Cartilage assessment

Imaging parameters for T2 mapping were: repetition time (RT) = 1000 ÷ 1100 ms, echo time (TE) = 8 ÷ 72

ms, field of view (FOV) = 60; slice thickness = 3 mm, slice spacing = 3.5 mm, matrix = 512 x 512, Band Width (BW) = 122 Hz/pixel. Sagittal T2 sequences, resulting from both baseline and follow-up acquisitions, were imported into an open-source DICOM viewer (OsiriX, Pixmeo Sarl), firstly to obtain the specific range of RT TE and secondly to define the cartilage regions of interest (ROIs). Each sequence was divided on a slice-by-slice basis and by precise anatomical markers, i.e. menisci (11). The mid-sagittal slice through the centre of each condyle, composed by eight different echo images, was selected for the T2 analysis (12). MR images were then processed using a dedicated software (MRI Processor Toolbox, ImageJ). Posterior lateral tibial plateau (pLTP), central medial tibial plateau (cMTP) and central medial femoral condyle (cMFC) were selected as ROIs; the contours of each ROI were manually outlined on the mask obtained by overlaying the starting slice to the corresponding region in each T2 mapping image (12). T2 values of more than 200 ms were considered outliers and automatically excluded from the analysis (6, 13).

Kinematics Acquisition

The stance phase was acquired for each patient at baseline and at follow-up by means of a custom-made radiostereophotogrammetry system. The radiographic equipment was constituted by two synchronized 100 kHz radiographic tubes and two 430x430 mm flat panels. Acquisition frame rate was optimized to 8 Hz, to limit patient radiological exposition. The CCD sensors system had a resolution of 2280x2280 pixels, with pixel dimension equal to 148 µm. A force platform (P6000, BTS Bioengineering) synchronized with the radiological system was also used to acquire ground reaction forces (GRF). Force platform data-rate was set to 400 Hz.

Musculoskeletal Modelling

A complete subject-specific multi-body model of the lower limb (from thigh to foot) was created for each patient, keeping into account inertial and anatomical inter-subject differences. MRI sagittal proton density (PD) sequences were imported in 3DSlicer (14) and virtual models of tibia, fibula and femur were generated by segmentation. To complete the limb, a general foot model (7) was also imported and scaled according to each patient anthropometry. Furthermore, the inertial properties

and joint relationships were defined via nmsBuilder (15), including:

i): inertial properties of body segments (comprehensive of soft tissue contribution) were adjusted according to patients' anthropometry starting from a general 10-segments, 23-degrees-of-freedom (DOF) model (16);

ii): knee joint was modelled as a free joint (6 DOF) to highlight all the translational and rotational capabilities;

iii): ankle joint was modelled as a 1 DOF hinge joint, thereby permitting only the flexion-extension movement.

Since the radiostereophotogrammetric field-of-view was focused at the knee joint, ankle flexion-extension movement was modelled according to standard references (17). The complete lower limb models were then imported in an open source biomechanics simulation software (OpenSim, SimTK), where kinematic data and GRF information were exploited to perform inverse dynamic simulations, thus to obtain reliable information about the loads acting at joint.

Table I legend:

*pLTP: posterior lateral tibial plateau; *cMTP: central medial tibial plateau; *cMFC: central medial femoral condyle.

			AGE	Δ T2			
				*pLTP	*cMTP	*cMFC	average
				<i>p</i> =0.0311 <i>r</i> =0.733	<i>p</i> =0.0138 <i>r</i> =0.8	<i>p</i> =0.0369 <i>r</i> =0.717	<i>p</i> =0.0172 <i>r</i> =0.783
Δ DYNAMICS	peak	Medio-lateral force	<i>p</i> =-0.8432 <i>r</i> =0.08	<i>p</i> =0.7756 <i>r</i> =-0.12	<i>p</i> =0.7756 <i>r</i> =-0.12	<i>p</i> =0.4366 <i>r</i> =-0.3	<i>p</i> =0.81 <i>r</i> =-0.1
		Anterior-posterior force	<i>p</i> =0.2298 <i>r</i> =0.45	<i>p</i> =0.2298 <i>r</i> =0.45	<i>p</i> =0.1618 <i>r</i> =0.52	<i>p</i> =0.5206 <i>r</i> =0.25	<i>p</i> =0.2125 <i>r</i> =0.47
		Vertical force	<i>p</i> =0.3586 <i>r</i> =-0.35	<i>p</i> =0.7081 <i>r</i> =-0.15	<i>p</i> =0.7756 <i>r</i> =-0.12	<i>p</i> =0.3853 <i>r</i> =-0.33	<i>p</i> =0.6777 <i>r</i> =-0.17
		Flexion moment	<i>p</i> =0.9116 <i>r</i> =0.05	<i>p</i> =1 <i>r</i> =0	<i>p</i> =0.8432 <i>r</i> =-0.08	<i>p</i> =0.81 <i>r</i> =-0.1	<i>p</i> =0.9484 <i>r</i> =-0.03
		Abduction moment	<i>p</i> =0.6436 <i>r</i> =0.18	<i>p</i> =0.4101 <i>r</i> =0.32	<i>p</i> =0.6436 <i>r</i> =0.18	<i>p</i> =0.5809 <i>r</i> =0.22	<i>p</i> =0.463 <i>r</i> =0.28
		Axial moment	<i>p</i> =0.3586 <i>r</i> =-0.25	<i>p</i> =0.3586 <i>r</i> =0.35	<i>p</i> =0.3363 <i>r</i> =0.37	<i>p</i> =0.2696 <i>r</i> =0.42	<i>p</i> =0.2912 <i>r</i> =0.4
	impulse	Medio-lateral force	<i>p</i> =0.6134 <i>r</i> =0.2	<i>p</i> =0.2912 <i>r</i> =0.4	<i>p</i> =0.3853 <i>r</i> =0.33	<i>p</i> =0.3586 <i>r</i> =0.35	<i>p</i> =0.3125 <i>r</i> =0.38
		Anterior-posterior force	<i>p</i> =0.7756 <i>r</i> =-0.12	<i>p</i> =0.9816 <i>r</i> =0.02	<i>p</i> =0.9484 <i>r</i> =0.03	<i>p</i> =0.9116 <i>r</i> =-0.05	<i>p</i> =0.9116 <i>r</i> =0.05
		Vertical force	<i>p</i> =0.3853 <i>r</i> =0.33	<i>p</i> =0.2912 <i>r</i> =0.4	<i>p</i> =0.2125 <i>r</i> =0.47	<i>p</i> =0.8801 <i>r</i> =0.07	<i>p</i> =0.2696 <i>r</i> =0.42
		Flexion moment	<i>p</i> =0.81 <i>r</i> =0.1	<i>p</i> =0.7756 <i>r</i> =0.12	<i>p</i> =0.8801 <i>r</i> =0.07	<i>p</i> =0.8801 <i>r</i> =0.07	<i>p</i> =0.7081 <i>r</i> =0.15
		Abduction moment	<i>p</i> =0.3363 <i>r</i> =-0.37	<i>p</i> =0.2125 <i>r</i> =-0.47	<i>p</i> =0.2912 <i>r</i> =-0.4	<i>p</i> =0.0857 <i>r</i> =-0.617	<i>p</i> =0.1777 <i>r</i> =-0.5
		Axial moment	<i>p</i> =0.8432 <i>r</i> =-0.08	<i>p</i> =0.2125 <i>r</i> =0.47	<i>p</i> =0.2912 <i>r</i> =0.4	<i>p</i> =0.7081 <i>r</i> =0.15	<i>p</i> =0.3125 <i>r</i> =0.38

Kinetics Assessment

Inverse dynamic simulation provided full information of forces and moments acting at the joint during the stance phase of the performed level gait. This preliminary analysis was focused on peak and impulse of forces and moments acting on the principal directions (antero-posterior, medio-lateral and vertical forces, and flexion, abduction and axial moments). Force values were normalized in percentage by subject weight, whereas moments were normalized in percentage by weight and height. Impulses were calculated as the area under the time curve of the absolute values of the corresponding kinetic quantity.

Statistical Analysis

Differences between FU18 and baseline values of T2 signal for each specific ROI, as well as of kinetic quantities, were evaluated. Keeping into account the sample size, Spearman correlation analysis between T2 values for each ROI and dynamic parameters was carried out. Correlations were considered significant when p -value < 0.1. Furthermore, the relationships between patients' anthropometry (height, mass and age), and T2 values for each ROI and dynamics variations were investigated.

RESULTS

No correlation between baseline or FU18 relaxation time and patients' anthropometric data was found. As highlighted in figure 2, a significant correlation (p -value=0.0857) between the normalized abduction moment impulse and the T2 signal in the medial femoral condyle region was evidenced, with a negative trend (correlation coefficient $r=-0.6167$). Age correlated with the longitudinal evolution of T2 signal, but not with the variation of impulse. Specific p and r values for all the analysed quantities and ROIs are reported in Table I.

DISCUSSION

Usually, after a traumatic injury such as an ACL tear, cartilage presents higher T2 values compared to healthy conditions (18). Therefore, one of the first aims that ACL reconstruction should pursue, in addition to effectively restore knee stability, would be to recover pre-traumatic cartilage homeostasis,

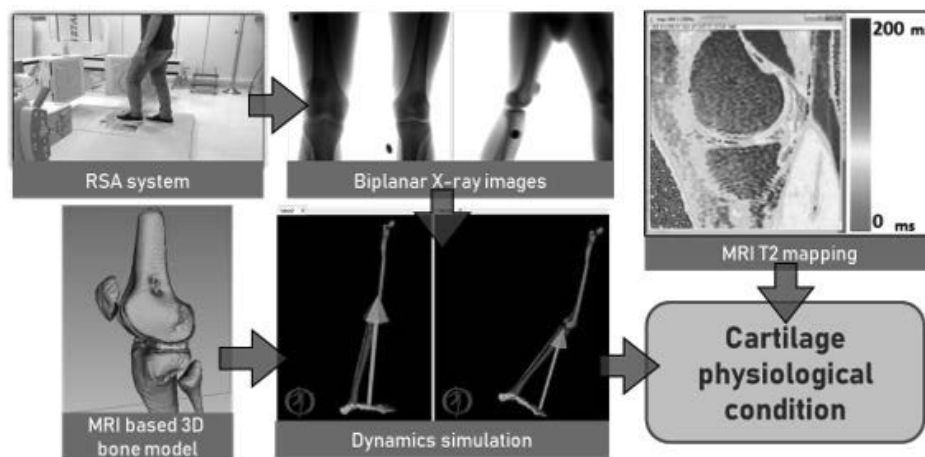


Fig. 1. Workflow of the study. The integration of MRI T2 mapping, RSA and subject-specific modeling allowed to evaluate the physiological status of cartilage after ACL reconstruction.

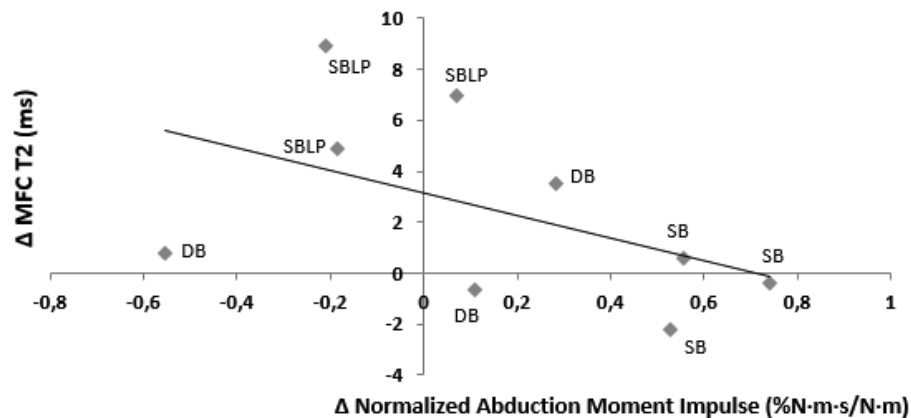


Fig. 2. Distribution of variation for T2 in medial femoral condyle and normalized abduction moment impulse for the subjects of the study. SB, single-bundle; naDB, non-anatomic double-bundle; SBLP, single-bundle lateral plasty.

thus reducing the risk of early tissue degeneration. In this sense, a reduction of T2 values after 18 months from the intervention would be advisable. Despite the small sample size, a statistically significant correlation was found between the variation of the normalized abduction moment and the variation of the T2 signal in the medial femoral condyle region, one of the riskiest region for early OA (19). The negative correlation coefficient highlighted how an increment of the impulse was related to a decrement of T2 values; this specific finding could underline that an increase of the loading in the knee coronal

plane may provide a precious impact in aiding the cartilaginous tissue to restore its biochemical equilibrium on the long time after the surgery (20). Furthermore, age seemed to contribute to the tissue's capacity to restore itself, but the lack of a statistically significant correlation with the moment parameter indicated that age cannot explain the variation by itself; probably, this restoring capacity is multifactor-dependent where age, gender, bone shape and surgical technique represent only some of variables which could influence the final outcome (21). Particularly, follow-up time appears

to be determinant for homogenous analyses and comparisons between studies: the study by Kumar et al (22) reported a significant correlation between T2 increase and medial knee loading increase over the first 6 months after the surgery, but an opposite trend from 6 to 12 months.

More patients will be included in the analysis to validate these preliminary results. Despite this limitation, the presented procedure represents a valuable tool to highlight the influence of ACL reconstruction on both biomechanics and tissue conditions by integrating MRI evaluation, assessing the evolution of knee cartilage status, a reliable motion analysis system, accurately recording limb movements soft-tissue artifacts and subject-specific biomechanical modelling.

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REFERENCES

1. Bollen S. Epidemiology of knee injuries: diagnosis and triage. *Br J Sports Med* 2000; 34:227-8.
2. Van Meer BL, Oei EH, Meuffels DE, van Arkel ER, Verhaar JA, Bierma-Zeinstra SM, Reijnen M. Degenerative Changes in the Knee 2 Years after Anterior Cruciate Ligament Rupture and Related Risk Factors: A Prospective Observational Follow-up Study. *Am J Sports Med* 2016; 44(6):1524-33.
3. Barrenius B, Ponzer S, Shalabi A, Bujak R, Norlén L, Eriksson K. Increased Risk of Osteoarthritis after Anterior Cruciate Ligament reconstruction. *Am J Sports Med* 2014; 42(5):1049-57.
4. Amano K, Pedoia V, Su F, Souza RB, Li X, Ma CB. Persistent Biomechanical Alterations After ACL Reconstruction Are Associated with Early Cartilage Matrix Changes Detected by Quantitative MR. *Orthop J Sports Med* 2016; 4(4):2325967116644421.
5. Gold GE, McCauley TR, Gray ML, Disler DG. What's New in Cartilage? *RadioGraphics* 2003; 23(5):1227-42.
6. Koff MF, Amrami MD, Kaufman KR. Clinical evaluation of T2 values of patellar cartilage in patients with osteoarthritis. *Osteoarthritis Cartilage* 2007; 15:198-204.
7. Delp SL, Anderson FC, Arnold AS, Loan Habib PA, John CT, Guendelman E, Thelen DG. OpenSim: Open-source Software to Create and Analyze Dynamic Simulations of Movement. *IEEE Transactions on Biomedical Engineering* 2007.
8. Bersini S, Sansone V, Frigo CA. A dynamic multibody model of the physiological knee to predict internal loads during movement in gravitational field. *Comput Methods Biomech Biomed Engin* 2016; 19(5):571-9.
9. Zaffagnini S, Bruni D, Marcheggiani Muccioli GM, Bonanzinga T, Lopomo N, Bignozzi S, Marcacci M. Single-bundle patellar tendon versus non-anatomical double-bundle hamstrings ACL reconstruction: a prospective randomized study at 8-year minimum follow-up. *Knee Surg Sports Traumatol Arthrosc* 2011; 19:390-7.
10. Marcacci M, Zaffagnini S, Iacono F, Neri MP, Loreti I, Petitto A. Arthroscopic intra- and extra-articular anterior cruciate ligament reconstruction with gracilis and semitendinosus tendons. *Knee Surgery* 1998; 6:68-75.
11. Gupta R, Virayavanich W, Kuo D, Su F, Link T, Ma B, Li X. MR T1 ρ quantification of cartilage focal lesions in acutely injured knees: correlation with arthroscopic evaluation. *Magn Reson Imaging* 2014; 32:1290-6.
12. Nishioka H, Hirose J, Nakamura E, et al. Detecting ICRS grade 1 cartilage lesions in anterior cruciate ligament injury using T1 ρ and T2 mapping. *Eur J Radiol* 2013; 82:1499-505.
13. Dunn TC, Lu Y, Jin H, Ries MD, Majumdar S. T2 relaxation time of cartilage at MR imaging: comparison with severity of knee osteoarthritis. *Radiology* 2004; 232(2):592-8.
14. Fedorov A, Beichel R, Kalpathy-Cramer J, et al. 3D Slicer as an Image Computing Platform for the Quantitative Imaging Network. *Magnetic Resonance Imaging* 2012; 30:1323-41.
15. Valente G, Pitto L, Testi D, Seth A, Delp SL, Stagni R, Viceconti M, Taddei F. Are Subject-Specific Musculoskeletal Models Robust to the Uncertainties

- in Parameter Identification? PLoS ONE 2014; 9(11):e112625.
16. Anderson FC, Pandy MG. A Dynamic Optimization Solution for Vertical Jumping in Three Dimensions. *Comput Methods Biomech Biomed Engin* 1999; 2(3):201-31.
 17. Bovi G, Rabuffetti M, Mazzoleni P, Ferrarin M. A multiple-task analysis approach: kinematic, kinetic and healthy young and adult subjects. *Gait & Posture* 2011; 33:6-13.
 18. Xu J, Xie G, Di Y, Bai M, Zhao X. Value of T2-mapping and DWI in the diagnosis of early knee cartilage injury. *Musculoskeletal Radiology* 2011; 5(2):13-8.
 19. Bae JH, Hosseini A, Wang Y, Torriani M, Gill TJ, Grodzinsky AJ, Li G. Articular cartilage of the knee 3 years after ACL reconstruction. A quantitative T2 relaxometry analysis of 10 knees. *Acta Orthop*. 2015; 86(5):605-10.
 20. Wellsandt E, Gardinier ES, Manal K, Axe MJ, Buchanan TS, Snyder-Mackler L. Decreased knee joint loading associated with early knee osteoarthritis after anterior cruciate ligament injury. *Am J Sports Med* 2016; 44(1):143-51.
 21. Webster KE, McClelland JA, Palazzolo SE, Santamaria LJ, Feller JA. Gender differences in the knee adduction moment after anterior cruciate ligament reconstruction surgery. *Br J Sports Med* 2012; 46:355-9.
 22. Kumar D, Su F, Wu D, Pedroia V, Heitkamp L, Ma B, Souza RB, Li X. Frontal Plane Knee Mechanics and Early Cartilage Degeneration in People with Anterior Cruciate Ligament Reconstruction. *Am J Sports Med* 2018; 46(2):378-87.

BIOMATERIALS AND BIOPHYSICAL STIMULI FOR BONE REGENERATION

L. FASSINA¹, N. BLOISE², G. MONTAGNA^{1,2}, L. VISAI², M.E. MOGNASCHI¹,
F. BENAZZO³ and G. MAGENES¹

¹Dipartimento di Ingegneria Industriale e dell'Informazione, Università di Pavia, Pavia, Italy; ²Dipartimento di Medicina Molecolare, Università di Pavia, Pavia, Italy; ³Dipartimento di Scienze Clinico-Chirurgiche, Diagnostiche e Pediatriche, Università di Pavia, Pavia, Italy

First, we review basic concepts of Tissue Engineering, that is, how the tensegrity is able to modulate the cell behavior. Then, we review our experimental results regarding the bone tissue engineering via biomaterials and bioreactors.

Basic concepts of tissue engineering

Tissue Engineering has been defined as follows: “Tissue engineering is the application of principles and methods of engineering and life sciences toward fundamental understanding of structure-function relationships in normal and pathological mammalian tissues and development of biological substitutes to restore, maintain, or improve tissue functions”.

The fundamental concept that emerges from the previous definition is the so-called relationship between structure and function. In other words, cells are characterized, both inside and outside, by a structural context (called ‘tensegrity’) able to modulate proliferation and differentiation. In particular:

- 1) There is structural connection between extracellular matrix proteins, integrins of the plasma membrane, cytoskeleton, nuclear membrane and DNA macromolecules (heterochromatin, euchromatin during the interphase of the cell cycle).
- 2) In addition, transcription factors, mRNA and miRNAs pass through the pores of the nucleus.
- 3) Forces and deformations (concentrated, distributed, perpendicular to the plasma

membrane, and of shear, i.e. parallel to the plasma membrane) are transmitted along the cell tensegrity structure directly to the interphase DNA, influencing the transcription.

- 4) These forces and deformations, thus transmitted, are also able to modulate the permeability of the pores of the nucleus, thus influencing the trafficking of transcription factors, mRNA and miRNAs.
- 5) The main ‘biochemical actors’ are almost all connected to the tensegrity structure (e.g., G protein, PIP₂, DAG, enzymes localized on membranes). It has been shown that these molecules drive biochemical reactions not only from the chemical viewpoint, but also from the spatial one; in other words, biochemical reactions are possible because they occur in a very precise spatial organization.
- 6) A functioning cytoskeleton (i.e. not destroyed or fixed) is a necessary condition for various biochemical reactions (e.g., G-PIP₂-DAG-IP₃ pathway).

As a consequence, it is clear how Tissue Engineering is interdisciplinary: Tissue Engineering uses concepts and methods of both biology and

Key words: bone regeneration, bone tissue engineering, biomaterials, bioreactors

Corresponding Author:

Lorenzo Fassina, Ph.D.,
Dipartimento di Ingegneria Industriale e dell'Informazione,
Università di Pavia, Via Ferrata 1, 27100 Pavia, Italy
Tel.: +390382985352
E-mail: lorenzo.fassina@unipv.it

engineering allowing to study how the variations of the structural context (tensegrity) in cells, tissues, organs induce variations in biological functions (e.g., cell growth and differentiation, tissue formation and remodeling, morphogenesis, organ working).

An example of the tensegrity effect was studied by Ingber (1). As the adhesion of the cell culture surface increases, the adhesiveness caused by the deposition of fibronectin increases the area of cell spreading; the consequence is a greater growth [measured, in proliferated cells, in terms of the incorporation of radioactive (^3H)-Thymidine nucleoside].

Another case is represented by the seeding of

distinct hepatocytes on distinct areas obtained with photochemical techniques (2). It is experimentally shown that small-area-adherent hepatocytes produce albumin and have a low proliferation (i.e. they maintain their phenotype), whereas, if the same cells are adhered to larger areas, they proliferate more and produce less albumin (i.e. they do not maintain the characteristic phenotype).

Another example by Ingber et al. is the building, via endothelial cells, of *in vitro* linear and capillary-like structures (3). In this experiment, the authors tried to understand in which particular tensegrity the formation of capillaries is fostered; the purpose was to find how to disadvantage the neo-angiogenesis in tumor tissues. Only particular concentrations of fibronectin and collagen IV (basal lamina collagen) can foster the formation of capillary-like structures (3).

Bone tissue engineering

Regarding our experience, we have designed and used biomaterials and bioreactors suitable to implement specific tensegrity conditions (4-25). For example, a biocompatible and porous polyurethane has geometrical, mechanical, surface and biocompatibility features promoting the attachment, the proliferation and the differentiation of osteoblasts (4).

Nevertheless, in Tissue Engineering, the design of a biocompatible material is only the first step to create a tissue substitute *in vitro*. In particular, an electromagnetic bioreactor (Igea, Carpi, Italy) was designed for the biophysical stimulation of cells *in vitro* (4). This device consists of two electrical windings (called solenoids) at the center of which the cell culture is positioned. The bioreactor is able to stimulate the cells with a time-varying magnetic induction field (maximum intensity = 3.3 mT, frequency = 75 Hz) and with an induced time-varying electric field.

As seen in the scanning electron microscope images (4), the electromagnetic stimulation is able, with respect to the untreated control, to promote the cell proliferation with the formation of extensive 3D clusters formed by cells and by extracellular bone matrix. Furthermore, the electromagnetic stimulation increases the degree of calcification of the extracellular bone matrix.

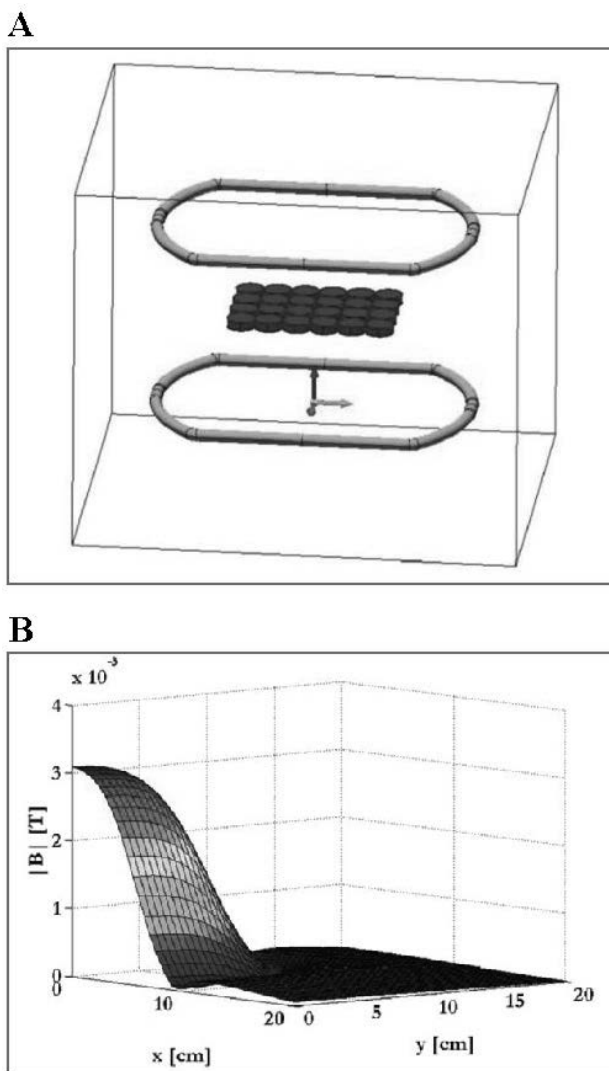


Fig. 1. *In silico* model of the electromagnetic bioreactor (A) and 3D distribution of the magnetic induction field when it is maximum (B).

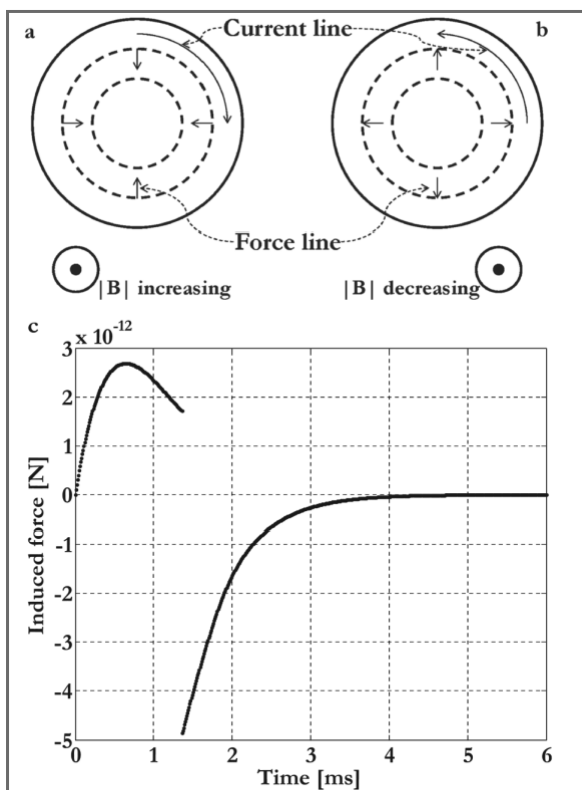


Fig. 2. Induced electric currents (*top*) and local compression and traction vs time (*bottom*).

To explain the previous biological effects, an *in silico* model of the electromagnetic bioreactor has been created (5). In Fig. 1, we see the model of the solenoids with the geometry of a multiwell plate in the center; we also observe the 3D distribution of the magnetic induction field when it is maximum.

The biological effects can be explained by calculating the electric currents induced in the culture medium (Fig. 2). The time-varying magnetic induction field induces time-varying electrical currents, which, in turn, by the law of Faraday-Neumann-Lenz, generate local mechanical forces of compression and traction (5). In other words, the cells inside the electromagnetic bioreactor undergo mechanical stimulation, which is nothing more than a mechanical vibration at the frequency of 75 Hz and with the force intensity of the pN order.

Similar results (in terms of proliferation,

differentiation and production of bone extracellular matrix) have been obtained using different types of biophysical stimulus such as: i) the perfusion of the culture medium (25) emulation of the movement of the interstitial fluid in the compact bone: ii); the ultrasound stimulus (24), which is known to heal bone fractures (Igea, Carpi, Italy).

REFERENCES

1. Ingber DE. Fibronectin controls capillary endothelial cell growth by modulating cell shape. *Proc Natl Acad Sci USA* 1990; 87:3579-83.
2. Singhvi R, Kumar A, Lopez GP, Stephanopoulos GN, Wang DI, Whitesides GM, Ingber DE. Engineering cell shape and function. *Science* 1994; 264:696-8.
3. Ingber DE, Folkman J. Mechanochemical switching between growth and differentiation during fibroblast growth factor-stimulated angiogenesis *in vitro*: role of extracellular matrix. *J Cell Biol* 1989; 109:17-330.
4. Fassina L, Visai L, Benazzo F, et al. Effects of electromagnetic stimulation on calcified matrix production by SAOS-2 cells over a polyurethane porous scaffold. *Tissue Eng* 2006; 12:1985-99.
5. Mognaschi ME, Di Barba P, Magenes G, Lenzi A, Naro F, Fassina L. Field models and numerical dosimetry inside an extremely-low-frequency electromagnetic bioreactor: the theoretical link between the electromagnetically induced mechanical forces and the biological mechanisms of the cell tensegrity. *Springerplus* 2014; 3:473.
6. Di Barba P, Fassina L, Magenes G, Mognaschi ME. Shape synthesis of a well-plate for electromagnetic stimulation of cells. *International Journal of Numerical Modelling: Electronic Networks, Devices and Fields* 2017; <https://doi.org/10.1002/jnm.2259>.
7. Bloise N, Petecchia L, Ceccarelli G, et al. The effect of pulsed electromagnetic field exposure on osteoinduction of human mesenchymal stem cells cultured on nano-TiO₂ surfaces. *PLoS One* 2018; 13:e0199046.
8. Fassina L, Cornacchione M, Pellegrini M, et al. Model of Murine Ventricular Cardiac Tissue for *In Vitro* Kinematic-Dynamic Studies of Electromagnetic and β -Adrenergic Stimulation. *J Healthc Eng* 2017; 2017:4204085.

9. Capelli E, Torrisi F, Venturini L, Granato M, Fassina L, Lupo GFD, Ricevuti G. Low-Frequency Pulsed Electromagnetic Field Is Able to Modulate miRNAs in an Experimental Cell Model of Alzheimer's Disease. *J Healthc Eng* 2017; 2017:2530270.
10. Pasi F, Fassina L, Mognaschi ME, Lupo GFD, Corbella F, Nano R, Capelli E. Pulsed Electromagnetic Field with Temozolomide Can Elicit an Epigenetic Pro-apoptotic Effect on Glioblastoma T98G Cells. *Anticancer Res* 2016; 36:5821-6.
11. Cornacchione M, Pellegrini M, Fassina L, et al. β -Adrenergic response is counteracted by extremely-low-frequency pulsed electromagnetic fields in beating cardiomyocytes. *J Mol Cell Cardiol* 2016; 98:146-58.
12. Falone S, Marchesi N, Osera C, Fassina L, Comincini S, Amadio M, Pascale A. Pulsed electromagnetic field (PEMF) prevents pro-oxidant effects of H_2O_2 in SK-N-BE(2) human neuroblastoma cells. *Int J Radiat Biol* 2016; 92:281-6.
13. Patrucco A, Cristofaro F, Simionati M, et al. Wool fibril sponges with perspective biomedical applications. *Mater Sci Eng C Mater Biol Appl* 2016; 61:42-50.
14. Osera C, Amadio M, Falone S, et al. Pre-exposure of neuroblastoma cell line to pulsed electromagnetic field prevents H_2O_2 -induced ROS production by increasing MnSOD activity. *Bioelectromagnetics* 2015; 36:219-32.
15. Fassina L, Magenes G, Gimmelli R, Naro F. Modulation of the cardiomyocyte contraction inside a hydrostatic pressure bioreactor: *in vitro* verification of the Frank-Starling law. *Biomed Res Int* 2015; 2015:542105.
16. Marchesi N, Osera C, Fassina L, et al. Autophagy is modulated in human neuroblastoma cells through direct exposition to low frequency electromagnetic fields. *J Cell Physiol* 2014; 229:1776-86.
17. Ceccarelli G, Bloise N, Mantelli M, et al. A comparative analysis of the *in vitro* effects of pulsed electromagnetic field treatment on osteogenic differentiation of two different mesenchymal cell lineages. *Biores Open Access* 2013; 2:283-94.
18. Fassina L, Saino E, Visai L, et al. Electromagnetic stimulation to optimize the bone regeneration capacity of gelatin-based cryogels. *Int J Immunopathol Pharmacol* 2012; 25:165-74.
19. Saino E, Fassina L, Van Vlierberghe S, Avanzini MA, Dubruel P, Magenes G, Visai L, Benazzo F. Effects of electromagnetic stimulation on osteogenic differentiation of human mesenchymal stromal cells seeded onto gelatin cryogel. *Int J Immunopathol Pharmacol* 2011; 24(1Suppl2):1-6.
20. Saino E, Grandi S, Quartarone E, et al. *In vitro* calcified matrix deposition by human osteoblasts onto a zinc-containing bioactive glass. *Eur Cell Mater* 2011; 21:59-72.
21. Fassina L, Saino E, Cusella De Angelis MG, Magenes G, Benazzo F, Visai L. Low-power ultrasounds as a tool to culture human osteoblasts inside cancellous hydroxyapatite. *Bioinorg Chem Appl* 2010; 2010:456240.
22. Saino E, Maliardi V, Quartarone E, et al. *In vitro* enhancement of SAOS-2 cell calcified matrix deposition onto radio frequency magnetron sputtered bioglass-coated titanium scaffolds. *Tissue Eng Part A* 2010; 16:995-1008.
23. Fassina L, Saino E, Visai L, Magenes G. Electromagnetically enhanced coating of a sintered titanium grid with human SAOS-2 osteoblasts and extracellular matrix. *Conf Proc IEEE Eng Med Biol Soc* 2008; 2008:3582-5.
24. Fassina L, Saino E, Sbarra MS, et al. Ultrasonic and electromagnetic enhancement of a culture of human SAOS-2 osteoblasts seeded onto a titanium plasma-spray surface. *Tissue Eng Part C Methods* 2009; 15:233-42.
25. Fassina L, Visai L, Asti L, Benazzo F, Speziale P, Tanzi MC, Magenes G. Calcified matrix production by SAOS-2 cells inside a polyurethane porous scaffold, using a perfusion bioreactor. *Tissue Eng* 2005; 11:685-700.

SINGLE DOSE CEFAZOLIN IS SAFE AND EFFECTIVE FOR PRE-OPERATIVE PROPHYLAXIS IN ORTHOPAEDIC ONCOLOGY

A. ZIRANU¹, M. LILLO¹, M. FANTONI¹, N. MAFFULLI^{2,3} and G. MACCAURO¹

¹*Orthopedic and Traumatology Institute, Fondazione Policlinico Universitario A. Gemelli IRCCS - Università Cattolica del Sacro Cuore, Roma;* ²*Department of Trauma and Orthopaedics, University of Salerno School of Medicine, Surgery and Dentistry, Salerno, Italy;* ³*Centre for Sport and Exercise Medicine, Queen Mary University of London, Barts and The London School of Medicine, London, England*

Surgical site infections (SSI) are a common potentially preventable complication after surgical procedures. A standardized antibiotic prophylaxis in elective orthopaedic surgery plays a major role in lowering SSI. At present, there is little published evidence regarding standardized antibiotic prophylaxis in orthopaedic oncological surgery. We introduced a prophylactic antibiotic protocol for orthopaedic oncological surgery in our hospital. The proposed protocol consists in “one-shot” intravenous administration of Cefazolin 2g, 30 min before surgery. In our setting, this preoperative antibiotic prophylaxis regimen was associated with a markedly lower rate of SSI’s. There is no current evidence in favour of greater effectiveness of prophylaxis beyond 24/48 h after surgery compared to our pre-surgical “one-shot” administration; by contrast, prolonged post-surgical prophylaxis is likely to undermine the patient’s bacterial flora and select resistant pathogens. These results are preliminary and should be used to start planning a standardised prophylactic protocol to prevent SSI’s after orthopaedic oncological surgery.

Surgical site infections (SSI) are a common potentially preventable complication after surgical procedures. SSI occur within 30 days after the surgery, in the part of the body where the surgery took place. If the surgical operation involves *in situ* implantation of a foreign body of non-human origin, the length of time to define an SSI is extended to one year after the index surgery (1, 2).

Among hospital-acquired infections (HAI), SSI’s rank 3rd for incidence, representing 14.6% of all infections observed in hospital patients and 38% of infections in surgical patients (3, 4). Their impact on health resource consumption is remarkable: patients with SSI spend an average 11 days in hospital, i.e. almost twice the average 6-day hospital stay of non-infected patients (5). In addition, the probability of

re-admission and overall post-surgical mortality are higher in patients with SSI. Given the increase in costs and the mortality from SSI, their reduction is a major goal of modern surgical practice. Together with non-pharmacological prevention, antibiotic prophylaxis effectively reduces the incidence of SSI.

Inappropriate or excessive antibiotic prophylaxis favour the development of resistant pathogens (6, 7). For example, an increase in colitis or diarrhea caused by *Clostridium difficile* in hospital patients has been linked to the inconsiderate use of inappropriately long antibiotics administration (8-11).

A standardized antibiotic prophylaxis in elective orthopaedic surgery plays a major role in lowering SSI (12). At present, there is little published evidence regarding standardized antibiotic

Key words: orthopedic oncological surgery; surgical site infection; antibiotic prophylaxis; cefazolin

Corresponding author:

Dr Marco Lillo,
Orthopedic and Traumatology Institute,
Fondazione Policlinico Universitario A. Gemelli IRCCS,
Università Cattolica del Sacro Cuore,
00188 Rome, Italy
e-mail: marcolillo.01@gmail.com

prophylaxis in orthopaedic oncological surgery. SSI are serious complications in orthopaedic oncological surgery, with potentially devastating and life-threatening consequences, especially taking into account the impaired immunological status and the general conditions of these patients (13, 14). The immunosuppression induced by anti-neoplastic chemotherapy is a major factor of higher rate of SSI compared to general orthopaedic surgery patients (15-20).

It is presently unknown whether in this field a “single-shot” prophylactic approach is preferable to long-term post-surgical antibiotic therapy.

We introduced a prophylactic antibiotic protocol for orthopaedic oncological surgery in our hospital. Adherence to such protocol was not compulsory, and we evaluated the effects of the application of the protocol in our patients.

MATERIAL AND METHODS

The proposed protocol consists in “one-shot” intravenous administration of Cefazolin 2g, 30 min before surgery. We identified two different groups of patients according to the risk related to surgical procedure (Table I).

In high risk patients (Group 2) in whom a prosthesis is implanted, and may require a drain at surgical site, we continued to administer intravenous Cefazolin 1g every 12 h until drain removal, occurring within 24 h from surgery.

From January 2015 to January 2016, 93 patients with a primary musculoskeletal tumor underwent surgery in our setting. The administration of all drugs, including antibiotics, is recorded prospectively in the relevant section of the hospital records, and the occurrence of SSI was recorded in a routine fashion in the medical records by the attending surgeons. In all the 93 patients, the antibiotic administered, the time of administration, the relevant dosage, possible additional administration of antibiotic during long-surgeries, and use of antibiotic beyond 24 h after the index operation was recorded (Table II).

RESULTS

We divided the patients into two groups: in 36 patients the antibiotic prophylaxis regimen was in strict accordance with the described protocol; the remaining 57 patients received a different prophylaxis regimen with regard to timing, doses and employed antibiotics.

Table I. Two different groups of patients according to the risk related to surgical procedure.

Group A: <u>Low risk</u>	Bone/soft tissues biopsy (35 patients)	Cefazolin i.v. 2 gr. “one shot” 30 min before surgery
	Bone resections and curettage with or without autologous bone grafts and/or utilization of local adjuvants (liquid nitrogen, cement, hydrogen peroxide, ect) (24 patients)	Cefazolin i.v. 2 gr. “one shot” 30 min before surgery
Group B: <u>High risk</u>	Osteosynthesis with intramedullary nails and/or plates (15 patients)	Cefazolin i.v. 2 gr. “one shot” 30 min before surgery
	Prosthesis (19 patients)	Cefazolin i.v. 2 gr. “one shot” 30 min before surgery. Then Cefazolin i.v. 1 gr. every 12 hours until the removal of drainages

Table II. *Features of the 93-patient sample of the study.*

VARIABLES
-Weight (BMI): 30,25-34,23
-Age: 3-82 yrs
-Comorbidities: diabetes, RA, cardiovascular diseases, allergies, pulmonary diseases, ASA score
-Oncological diagnosis
-Type and duration of surgical procedure
-Radio/chemotherapy
-Type and duration of antibiotic prophylaxis
-Smoke

Table IIIa. *Numbers of infected patients in two groups.*

93	Group 1	Group 2
Infected	3	8
Not infected	33	49

Table IIIb. *Common variables in infected patients of group 2.*

Diabetes (2, 1 from group A and 1 from group B)
Radio/chemotherapy (2, both from group B)
ASA score >2 (5, 2 from group A and 3 from group B)
Rheumatoid arthritis (1, from group B)
Obesity (2, 1 from group A and 1 from group B)
Surgical duration >90 min (6, all from group B)
“One shot” prophylactic antibiotic administration < 30 min before surgery (6, 2 from group A and 4 from group B)

In the patients in whom the protocol had been followed, the incidence of SSI was 8.3% (3 of 36, with an absolute risk of 0.083), while the patients treated with a different prophylaxis developed an SSI in 14.1% of cases (8 of 57, with an absolute risk of 0.141) (Tables III a, b).

DISCUSSION

Antibiotic prophylaxis in orthopaedic surgery is hotly debated, and there is no high level of evidence proving greater effectiveness of prophylaxis exceeding 24/48 h after surgery compared to a single pre-surgical “one-shot” administration. By contrast, prolonged post-surgical administration of antibiotics likely undermines the patient’s bacterial flora, exposing them to the risk of *Clostridium difficile*, colitis or resistant pathogens, with consequent rise in costs and mortality.

Many risk factors are involved in the occurrence of SSI in orthopaedic oncology, including the presence of comorbidities, smoking, ASA score >2, not to mention the quality of sterilization, the amount of foot traffic in the operating room or the presence of laminar air flow. Antibiotic prophylaxis should supplement the general principles of SSI prevention, not replace appropriate practice.

In our national setting, there is wide variability in peri-surgical antibiotic treatments in the main orthopaedic oncological centres and discrepancies emerged when trying to precisely distinguish between its prophylactic (to prevent SSI’s) use and therapeutic use (to cure an already developed SSI). Many orthopaedic oncological centres use prophylactic schemes that overlap with schemes designed for tackling frank infection: this approach is economically unsustainable, and it contributes to the emergence of multi-resistant microbial strains. The simple antibiotic prophylaxis protocol proposed in this investigation is a concrete improvement in the prevention of SSI in these difficult patients.

In patients with allergy, intolerance or hypersensitivity to beta-lactam antibiotics, intravenous Vancomycin (15mg/kg and up to 1 g) or intravenous Clindamycin (600 mg) are valid alternatives. It should be kept in mind that many

patients present with MRSA colonization (21): in these patients, Cefazolin may be ineffective. Administration of intravenous Vancomycin (15mg/kg and up to 1 g) combined with nasal decolonization based on Mupirocin (3 times/day for 5 days) and dermal washing with Chlorhexidine soap should be considered.

These data are only preliminary and are reassuring about the validity of our prophylactic protocol. The study showed a clear decrease in incidence of SSI associated to the adherence to the proposed scheme. No doubt large studies should be conducted, though, given the present results, it could be easily argued that it would be unethical to conduct randomised controlled trials in orthopaedic oncological patients.

There is no doubt that the present investigation is preliminary, given the relatively small size of our sample, yet it gives evidence of the benefits of a standardised prophylactic protocol in terms of prevention of SSIs after orthopaedic oncological surgery.

Given the population incidence of primary bone cancer, it is difficult to enroll large patient cohorts from a single centre. Therefore, other orthopaedic centres are being enrolled to further standardise the implementation of the protocol presented in this investigation. Enrolling more patients would allow to study the clinical relevance and the impact of each variable at play, allowing to develop and refine a reliable protocol for safe effective prophylaxis of infection in musculoskeletal oncological surgery.

REFERENCES

1. Sands K, Vineyard G, Platt R. Surgical site infections occurring after hospital discharge. *J Infect Dis* 1996; 173(4):963-70.
2. Horan TC, Gaynes RP, Martone WJ, Jarvis WR, Emori TG. CDC definitions of nosocomial surgical site infections, 1992: a modification of CDC definitions of surgical wound infections. *Infect Control Hosp Epidemiol* 1992; 13(10):606-8.
3. Nichols RL. Preventing surgical site infections: a surgeon’s perspective. *Emerg Infect Dis* 2001; 7:220-4.
4. Empori T. G., Gaynes R.P. An overview of

- nosocomial infections, including the role of the microbiology laboratory. *Clin Microbiol Rev* 1993; 6(4):428-42.
5. Kirkland KB, Briggs JP, Trivette SL, Wilkinson WE, Sexton DJ. The impact of surgical-site infections in the 1990s: attributable mortality, excess length of hospitalization, and extra cost. *Infect Control Hosp Epidemiol* 1999; 20(11):725-30.
 6. Gold HS, Moellering RC. Antimicrobial drug resistance. *N Engl J Med* 1996; 335:1445-53.
 7. American Society for Microbiology. Report of the ASM Task Force on antimicrobial resistance. Washington: The Society, 1994.
 8. Austin DJ, Kakehashi M, Anderson RM. The transmission dynamics of antibiotic-resistant bacteria: the relationship between resistance in commensal organisms and antibiotic consumption. *Proc R Soc Lond Biol Sci* 1997; 264:1629-38.
 9. Schwartz B, Bell DM, Hughes JM. Preventing the emergence of antimicrobial resistance. A call for action by clinicians, public health officials, and patients. *JAMA* 1997; 278: 944-45.
 10. McCain LF, Hughes JM. Trends in antimicrobial drug prescribing among office-based physicians in the United States. *JAMA* 1995; 273: 214-19.
 11. Starks I, Ayub G, Walley G, Orendi J, Roberts P, Maffulli N. Single-dose cefuroxime with gentamicin reduces *Clostridium difficile*-associated disease in hip-fracture patients. *J Hosp Infect* 2008; 70(1):21-6.
 12. Dougall TW, Duthie R, Maffulli N, Hutchison JD. Antibiotic prophylaxis: theory and reality in orthopaedics. *J R Coll Surg Edinb* 1996; 41(5):321-2.
 13. Umer HM, Umer M, Qadir I, Abbasi N, Masood N. Impact of unplanned excision on prognosis of patients with extremity soft tissue sarcoma. *Sarcoma* 2013; 2013:498604.
 14. Nakamura T, Matsumine A, Matsubara, Asanuma K, Uchida A, Sudo A. The symptom-to-diagnosis delay in soft tissue sarcoma influence the overall survival and the development of distant metastasis. *J Surg Oncol* 2011; 104(7):771-5.
 15. Jeys L, Grimer R. The long-term risks of infection and amputation with limb salvage surgery using endoprostheses. *Recent Results Cancer Res* 2009; 179:75-84.
 16. Morii T, Yabe H, Morioka H, et al. Postoperative deep infection in tumor endoprosthesis reconstruction around the knee. *J Orthop Sci* 2010; 15(3):331-9.
 17. Morii T, Mochizuki K, Tajima T, Ichimura S, Satomi K. Surgical site infection in malignant soft tissue tumors. *J Orthop Sci* 2012; 17(1):51-7.
 18. Donati F, Di Giacomo G, D'Adamio S, Ziranu A, Careri S, Rosa M, Maccauro G. Silver-Coated Hip Megaprosthesis in Oncological Limb Salvage Surgery. *Biomed Res Int* 2016; 2016:9079041.
 19. Piccioli A, Donati F, Di Giacomo G, et al. Infective complications in tumour endoprostheses implanted after pathological fracture of the limbs. *Injury* 2016; 47(Suppl4):S22-8.
 20. Donati F, Di Giacomo G, Ziranu A, Spinelli S, Perisano C, Rosa MA, Maccauro G. Silver coated prosthesis in oncological limb salvage surgery reduce the infection rate. *J Biol Regul Homeost Agent*. 2015; 29(4Suppl):149-55.
 21. Walley G, Orendi J, Bridgman S, Davis B, Ahmed el-N, Maffulli N. Methicillin resistant *Staphylococcus aureus* (MRSA) is not always caught on the orthopaedic ward. *Acta Orthop Belg* 2009; 75(2):245-51.

BIOMARKERS AND INFECTIONS IN ORTHOPEDICS: OUR EXPERIENCE AND LITERATURE REVIEW

P. DI BENEDETTO, M. M. BUTTIRONI and A. CAUSERO

Clinic of Orthopedics, Academic Hospital of Udine, Udine, Italy

The issue of infections in Orthopedics is rising in importance day by day. In management of Periprosthetic Joint Infection (PJI), classical biomarkers involved in diagnosis process are C-reactive protein (CRP) and Erythrocyte Serum Rate (ESR). Although wide use in PJI diagnosis process, CRP and ESR are not sensitive and specific enough for a correct diagnosis. Orthopedic surgeons, Microbiologists and Infectivologists are very interested in research about new (or rediscovered) biomarkers that can help in the diagnosis of orthopedics infections. In our Institution, a rigid protocol is applied to face suspicious PJI, implemented with LE testing routinely. In our retrospective observational study, we have compared reliability of LE test in relation ICM criteria and the comparison with other diagnostic tests or exams. Our results show that LE is a reliable method especially for intra-operatively PJI diagnosis.

Infection in Orthopedics is a problem and is increasing in importance. Osteomyelitis and septic arthritis are present in every orthopedic ward, but to date the most important issue is probably Periprosthetic Joint Infection (PJI) (1, 2). PJI diagnosis remains a challenge for orthopedic surgeons; there is much debate in literature about this topic. The criteria of diagnosis, defined and revised in the last International Consensus Meetings (ICM) about PJI, are divided in Major and Minor criteria (3); it is important to highlight that some biomarkers are present as Minor criteria.

A biomarker is defined as “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention” by Biomarkers Definition Working Group in 2001 (4). In orthopedics, we can categorize biomarkers in serum and synovial kinds. A serum biomarker is less invasive to obtain, and less hazardous for iatrogenic articular infections. Moreover, serum

biomarkers are useful for monitoring trend of the pathology and the therapy response. Advantages of a synovial biomarker is the ability to reflect the local response of the organism, although is very sensitive to empiric antibiotic therapy.

In management of PJI, classical biomarkers involved in diagnosis process are C-reactive protein (CRP) and Erythrocyte Serum Rate (ESR). The first is a non-specific acute phase protein that normalizes in two or three weeks post-operatively, while the second is a non-specific inflammation measure that normalizes between three and twelve months post-operatively (5). Although wide used in PJI diagnosis process, CRP and ESR are not enough: indeed the sensitivity is estimated relatively at 88% and 77% while specificity relatively of 74% and 70% (6). Some studies indicate that CRP and ESR cannot be used for monitoring persistence of infection after revision surgery (7).

According to the results, orthopedic surgeons, microbiologists and infectivologists are very

Key words: infection, periprosthetic, joint, biomarkers

Corresponding author:

Paolo Di Benedetto, MD, PhD,
Clinica Ortopedica,
Azienda Sanitaria Universitaria Integrata di Udine,
P.le S.Maria della Misericordia, 15, 33100 Udine, Italy
Tel.: +39 0432 559464 - Fax: +39 0432 559298
e-mail: paolo.dibenedetto@asuud.sanita.fvg.it

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interested in research about new (or rediscovered) biomarkers that can help diagnose and monitor infections in Orthopedics.

In our department, revision surgery for PJI is not a rare procedure. We apply a rigid protocol to approach PJI, known as “Udine Strategy” (8). From a few years, it has been implemented routinely with the use of the Leucocyte Esterase (LE) test. The aim of our retrospective observational study is to demonstrate reliability of LE in relation to ICM criteria and the comparison with other diagnostic tests or exams.

MATERIALS AND METHODS

Between December 2016 and September 2017, we enrolled 38 patients with suspicion of PJI; 22 females and 16 males, mean age at the time of surgery 71.1 years.

These patients underwent revision surgery, respectively, 26 for revision of total knee arthroplasty (TKA) and 12 for revision of total hip arthroplasty (THA). All patients underwent routine preoperative blood tests, LE test, histological and cultural synovial exam, frozen section (FS) exam on peri-prosthetic tissue samples (9), histological and cultural test on peri-prosthetic tissue samples and cultural exam on sonicated fluid from prosthetic components. For the LE test, “Chemistrip 7 Urine Test Strips” (Roche Diagnostics, Indianapolis, Indiana) was utilized. According to literature, tests that had results of 2+ and 3+ were positive, while 1+ was to be considered negative. All strips were read at 1 and 2 min as recommended by manufacturer.

All cultural exams on synovial fluid, peri-prosthetic tissue samples and sonicated fluid had been conducted for aerobic, anaerobic and fungal organisms.

RESULTS

In 9 of the 38 patients, micro-organisms were isolated from cultural exams on synovial fluid or peri-prosthetic tissue samples, 5 cases were *Staficococcus aureus*, 3 cases *Stafilococcus epidermidis* and 1 case *Stafilococcus gallolyticus* according to local epidemiology (Table I).

LE test was considered negative in 20 patients (52.6%), 1+ in 4 patients (10.5%), 2+ in 2 patient (5.2%), 3+ in 12 patients (31.6%). None of the LE tests were excluded for blood contamination.

Frozen section examination resulted negative in 28 patients (73.6%) and positive in 10 patients (26.3%). In 1 of 10 positive frozen section exams, at the histological examination, 25 PMN/HRF were found, highlighting a false negative case.

Sonicated fluid culture resulted positive in 10 cases, according to the cultural exam on synovial fluid or tissue sample findings. In 1 patient, the culture resulted positive only at sonicated fluid culture exam. In 27 cases, it resulted negative.

The comparison between LE test and the other diagnostic methods, considered individually, was carried out both descriptively, evaluating the proportion of cases in which the tests agreed positively or negatively (positive culture-positive test or negative culture-negative test) on the total, using Cohen’s Kappa index.

The concordance between LE test and peri-prosthetic or synovial fluid culture resulted of 88.24% with $\kappa=0,721$. Performing the same operation between LE test and frozen section exam, the concordance was of 87.5% with $\kappa=0,714$.

Tab I. Results of cultural exams.

Oragnism	Tissue simple	Sonicated fluid
Staphylococcus aureus	5	5
Staphylococcus epidermidis	3	4
Streptococcus gallolyticus	1	1

Finally, the concordance between LE test and culture of the sonicated fluid exam resulted of 86.67% with $\kappa=0,700$.

Applying ICM criteria (3) on our patients, 10 of them were considered affected by PJI. Eight of these met the major criteria, presenting two or more positive cultures for the same organism; one of these also presented a sinus tract. Two patients met 3 minor criteria: a positive LE test (3+) and synovial leukocyte count (3300/ μ L) (considered as a single criterion), elevated synovial PMN (82%) and elevated synovial RCP (6.6 mg/L).

Comparing these results, accuracy of LE test was assessed, demonstrating a sensitivity of 100%, a specificity of 85.71%, a positive predictive value (PPV) of 71.43% and a negative predictive value (NPV) of 100%. The same was carried out for the other tests examined. According to our data, the culture exams on peri-prothetic tissue samples reported sensitivity, specificity, PPV and NPV respectively of 90%, 100%, 100% and 96.55%, frozen section examination of 90%, 100%, 100%, 96.55% and the culture on sonication fluid of 100%, 96.43%, 90.91% and 100% (Table II).

In our Institution, a small analysis on the α -defensin test was conducted which has reported sensitivity, specificity, PPV and NPV values respectively of 50%, 100%, 100% and 87.5%.

DISCUSSION

Our growth in knowledge regarding specific pathways of articular infections has created new potential biomarkers. The majority of these are pro-

inflammatory cytokines found both in serum and in synovial fluid. The hope is that the novel biomarkers should improve the diagnostic process in rapidity and accuracy, overcoming the more traditional tests (10). In literature there are concerns regarding this, indeed we can find many papers about serum and synovial biomarker.

Serum biomarkers

IL-6 is secreted by macrophages and T-cell and it is a regulator of acute phase response that stimulate hepatocytes to produce other markers including CRP. Therefore IL-6 increase before CRP, with the advantage to have the possibility to detect PJI early. In addition to this IL-6 rise down quicker than other classical biomarkers after surgery (11). IL-4 is a biomarker found higher in knee and hip septic revision compared to aseptic revision (12). Both IL-6 and IL-4 have high specificity, respectively of 95% and 90%, but a lower sensitivity, respectively of 46.7% and 60%. Some authors suggest combining these biomarkers to improve their diagnostic accuracy (6).

From the first years of the new millennium, there is concern about procalcitonin (PCT) as a reliable marker for sepsis. PCT rise in importance because it seems able to discriminate between a septic or inflammatory disease (13). The issue about real sensitivity and specificity of PCT and about the cost-effectiveness of these tests does not support its role in PJI diagnosis (14).

Recently, D-dimer take place in a cohort of serum biomarkers. It is a product of fibrin degradation with a short half-life. During early post-operative period,

Tab II. Exam accuracy.

	Sensitivity	Specificity	PPV	PNV
Leucocyte Esterase test	100%	85.71%	71.43%	100%
Cultural exam	90%	100%	100%	96.56%
Frozen section exam	90%	100%	100%	96.56%
Sonicated fluid cultural exam	100%	96.43%	90.91%	100%

D-dimer rise rapidly in day 1 and decrease just as quickly at base level in day 2; a second peak is present at 2 weeks post-operatively. For this reason, D-dimer value, combined with level of CRP and ESR, might be effective in early detection of PJI (15).

Synovial biomarkers

A recent meta-analysis has shown the increasing interest of synovial biomarkers in scientific literature: the number of potential biomarkers analyzed is vast, but the number of publications is poor (16). The majority of these studies do not have a real role in clinical practice. Some institutions do not have facilities that can run specific tests and the early PJI diagnosis is mandatory.

Synovial biomarkers can also be detected through a point-of-care test for accelerate diagnosis: nowadays only LE and α -defensin has a point-of-care test validate and tested on the market. Among many biomarkers analyzed, CRP, LE, α -defensin, IL-6, IL-1b, and IL-17 are the most important.

LE has been largely studied in literature: it is an enzyme produced by neutrophils in synovial fluid during an infection (17). The LE test is simple and with low costs (0.17\$ per test). Sensitivity and Specificity of LE for diagnosing PJI has 81% and 97% respectively but this test has also the disadvantage to be unreadable if blood is contaminated (18).

The α -defensin has demonstrated to have greatest diagnostic odd ratio (16). This biomarker is released by activated neutrophils (19); its sensitivity was 100% and specificity was 96% in a recent meta-analysis (18). An important drawback is the cost of this test, much higher than LE test.

As previously mentioned, in our Institution a rigid protocol is applied to face suspicious PJI (8). The “Udine Strategy” has been implemented with LE testing routinely and in our experience during the last years, seems to give good results. Also for this reason, this retrospective observational study has made.

Comparing concordance between LE test and others is significant. Probably, the most important comparison has been done with frozen section examination, because these tests are executed, in our protocol, at the same time during surgery and often

FS guides subsequent clinical and surgical decisions. In our investigation, as previously mentioned, a sample analyzed by FS examination resulted a false negative, indeed >25 PMN/HRF were found at subsequent histological exam. The corresponding intra-operatively LE test resulted 3+. Hence, we deduce the importance to have more than one test available, able to be considered a reliable guidance during our activity. Indeed this topic is widely debated in the literature (12, 20).

A major limitation of our study is certainly the restricted number of patients examined, which could reflect a selected subpopulation of patients and could therefore invalidate the investigation. In this regard, it would be necessary to increase the observation time and a greater number of patients should be enrolled.

A further limitation regards the characteristic of the LE test to obtain indirect results, compared to the leukocyte-count examination, which, according to these assumptions, should have the same accuracy. Specifically, it has been demonstrated that the LE test may be excessively sensitive in the first postoperative period, when compared with the leukocyte count (21).

The potential benefits of this diagnostic method include large availability, low costs, and the ability to provide reliable diagnosis within 60-120 sec (22). As already mentioned, the LE test is part of a battery of tests present in a colorimetric strip, used in the diagnosis of pathologies of the urinary tract. Considering that this test is easily available and economic, it can be used almost anywhere, even in structures where budget is limited.

The manageability of the test also allows its use both in the surgery room and in the ward and allows the reading by para-medicals. However, this last point could turn into a disadvantage, since untrained staff could introduce a bias in the result.

Another important advantage is rapidity of results; most of the other validated tests for the diagnosis of PJI require a greater turnaround time. The simple leukocyte count in the synovial fluid takes 30 min in the best laboratories and the culture of the microorganism, the diagnostic gold standard, can take days.

Analysis of literature and our study indicate that LE is a reliable test for PJI diagnosis, especially intra-operatively. Further studies are necessary to clarify the role of the large number of potential biomarkers identified and what their benefits are, used both individually and in association.

REFERENCES

1. Kurtz SM, Lau E, Watson H, Schmier JK, Parvizi J. Economic burden of periprosthetic joint infection in the United States. *J Arthroplasty* 2012; 27(8Suppl):61-5.
2. Di Benedetto P, Di Benedetto ED, Buttironi MM, et al. Two-stage revision after total knee arthroplasty. *Acta Biomed* 2017; 88(2S):92-7.
3. Parvizi J, Tan TL, Goswami K, Higuera C, Della Valle C, Chen AF, et al. The 2018 definition of periprosthetic hip and knee infection: an evidence-based and validated criteria. *J Arthroplasty* 2018; 33(5):1309-14.
4. Biomarkers Definitions Working G. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. *Clin Pharmacol Ther* 2001; 69(3):89-95.
5. Aalto K, Osterman K, Peltola H, Rasanen J. Changes in erythrocyte sedimentation rate and C-reactive protein after total hip arthroplasty. *Clin Orthop Relat Res* 1984; (184):118-20.
6. Berbari E, Mabry T, Tsaras G, et al. Inflammatory blood laboratory levels as markers of prosthetic joint infection: a systematic review and meta-analysis. *J Bone Joint Surg Am* 2010; 92(11):2102-9.
7. Bejon P, Byren I, Atkins BL. Serial measurement of the C-reactive protein is a poor predictor of treatment outcome in prosthetic joint infection. *J Antimicrob Chemother* 2011; 66(7):1590-3.
8. Bassetti M, Cadeo B, Villa G, Sartor A, Cainero V, Causero A. Current antibiotic management of prosthetic joint infections in Italy: the 'Udine strategy'. *J Antimicrob Chemother* 2014; 69(Suppl1):i41-5.
9. Di Benedetto P, Povegliano L, Cainero V, Gisonni R, Beltrame A, Causero A. The role of intraoperative frozen section in arthroplasty revision surgery: our experience. *Acta Biomed* 2016; 87(Suppl1):34-40.
10. Deirmengian C, Hallab N, Tarabishy A, Della Valle C, Jacobs JJ, Lonner J, et al. Synovial fluid biomarkers for periprosthetic infection. *Clin Orthop Relat Res* 2010; 468(8):2017-23.
11. Heinrich PC, Castell JV, Andus T. Interleukin-6 and the acute phase response. *Biochem J* 1990; 265(3):621-36.
12. Gollwitzer H, Dombrowski Y, Prodinger PM. Antimicrobial peptides and proinflammatory cytokines in periprosthetic joint infection. *J Bone Joint Surg Am* 2013; 95(7):644-51.
13. Talebi-Taher M, Shirani F, Nikanjam N, Shekarabi M. Septic versus inflammatory arthritis: discriminating the ability of serum inflammatory markers. *Rheumatol Int* 2013; 33(2):319-24.
14. Bottner F, Wegner A, Winkelmann W, Becker K, Erren M, Gotze C. Interleukin-6, procalcitonin and TNF-alpha: markers of peri-prosthetic infection following total joint replacement. *J Bone Joint Surg Br* 2007; 89(1):94-9.
15. Lee YS, Lee YK, Han SB, Nam CH, Parvizi J, Koo KH. Natural progress of D-dimer following total joint arthroplasty: a baseline for the diagnosis of the early postoperative infection. *J Orthop Surg Res* 2018; 13(1):36.
16. Saleh A, Ramanathan D, Siqueira MBP, Klika AK, Barsoum WK, Rueda CAH. The diagnostic utility of synovial fluid markers in periprosthetic joint infection: a systematic review and meta-analysis. *J Am Acad Orthop Surg* 2017; 25(11):763-72.
17. Parvizi J, Jacovides C, Antoci V, Ghanem E. Diagnosis of periprosthetic joint infection: the utility of a simple yet unappreciated enzyme. *J Bone Joint Surg Am* 2011; 93(24):2242-8.
18. Wyatt MC, Beswick AD, Kunutsor SK, Wilson MJ, Whitehouse MR, Blom AW. The alpha-Defensin immunoassay and leukocyte esterase colorimetric strip test for the diagnosis of periprosthetic infection: a systematic review and meta-analysis. *J Bone Joint Surg Am* 2016; 98(12):992-1000.
19. Lehrer RI, Ganz T. Defensins: endogenous antibiotic peptides from human leukocytes. *Ciba Found Symp* 1992; 171:276-90.
20. Deirmengian C, Kardos K, Kilmartin P, Cameron A, Schiller K, Parvizi J. Combined measurement of synovial fluid alpha-Defensin and C-reactive protein levels: highly accurate for diagnosing periprosthetic joint infection. *J Bone Joint Surg Am* 2014; 96(17):1439-45.

21. Bedair H, Ting N, Jacovides C, Saxena A, Moric M, Parvizi J, Della Valle CJ. The Mark Coventry Award: diagnosis of early postoperative TKA infection using synovial fluid analysis. *Clin Orthop Relat Res* 2011; 469(1):34-40.
22. Wetters NG, Berend KR, Lombardi AV, Morris MJ, Tucker TL, Della Valle CJ. Leukocyte esterase reagent strips for the rapid diagnosis of periprosthetic joint infection. *J Arthroplasty* 2012; 27(8Suppl):8-11.

A LOCAL ANESTHESIA WITHOUT TOURNIQUET FOR DISTAL FIBULA HARDWARE REMOVAL AFTER OPEN REDUCTION AND INTERNAL FIXATION: THE SAFE USE OF EPINEPHRINE IN THE FOOT. A RANDOMIZED CLINICAL STUDY

A. POGGETTI¹, A. DEL CHIARO², M. NICASTRO³, P. PARCHI²,
N. PIOLANTI² and M. SCAGLIONE²

¹Hand and Reconstructive Microsurgery Unit, AOU Careggi, Italy; ²Intensive Unit Care, University of Pisa, Italy; ³Orthopaedic and Trauma Division, University of Pisa, Italy

Hardware removal after surgical treatment fracture is one of the most common procedures in orthopaedic daily activity. A percentage from 14.5 to 21 of total removal involves the ankle joint. Trying to reduce the important socio-economic impact of this surgical procedure, we thought to perform it using the Wide Awake Local Anaesthesia Without Tourniquet (WALANT), a particular technique presented by D. Lalonde that associated a local anaesthetic drug with epinephrine in order to obtain an effective haemostatic effect despite the lack of a tourniquet. Nowadays, the WALANT efficiency and safety in hand surgery is widely demonstrated in literature but there are no data about its use in lower limb extremity surgeries. Authors performed a randomized study with 60 patients whom underwent distal fibula hardware removal between 2014 and 2016; they were divided into two groups: Group A under loco-regional anaesthesia with tourniquet and Group B under WALANT. We did not find significant differences in term of maximum pain level felt during the anaesthesiologic and surgical procedure. However, the use of WALANT significantly reduced post-operative pain levels. The WALANT procedures also reduced the number of hospitalization days. No differences in term of post-operative complication rates were found. In conclusion, the WALANT can be considered as a suitable option for distal fibula hardware removal in selected patients; it shows important clinical and economic advantages compared to the traditional loco-regional anaesthesia with tourniquet. This study also lays the foundation for the use of the WALANT beyond the field of hand surgery only.

Level of clinical evidence: II

Hardware surgical removal after Open Reduction and Internal Fixation (ORIF) of bone fracture is one of the most common procedures in orthopaedic daily practice (1, 2), although the indications for this operation are not yet defined in literature (3-5). Most of these (from 15 to 21%) are performed after ORIF for distal tibia or distal fibula fractures (6, 7). Indications for hardware removal in order of frequency are: hardware related pain or ankle

stiffness, hardware infections, patient's insistence even if asymptomatic or implant failure (like screw breakages or peri-device fractures) (6-9). In an era of increasing interest in economic impact of medical procedures, the important socio-economic effect of this frequent surgical procedure must be taken into consideration (1). We thought that the introduction of the Wide Awake Local Anaesthesia without Tourniquet (WALANT) - that is already largely employed in hand surgery (10-12) could reduce its

Key words: Local anesthesia without tourniquet, WALANT, foot and ankle hardware removal, epinephrine

Corresponding author:

Andrea Poggetti MD,
Hand and Reconstructive Microsurgery Unit,
AOU Careggi, Italy, Largo Palagi 2,
50036, Florence, Italy
Tel.: +39 3272031697
E-mail: poggetti.andrea@gmail.com

significant economic impact even for this kind of surgical procedure. To our knowledge, literature presents only one article suggesting the use of local anaesthesia for ankle trauma surgery (13). In order to evaluate clinical results of the use of WALANT in hardware removal, Authors created a monocentric randomized study dividing a total of 60 patients whom underwent distal fibula plate removal between January 2014 and December 2016 into two groups: the former using a traditional loco-regional anaesthesia (group A) and the latter using the WALANT (group B). The primary outcome of this study is to highlight differences in pain scores between the two groups in pain scores measured before and during the surgical procedures throughout the five days after surgery.

MATERIALS AND METHODS

From January 2014 and December 2016 Authors evaluated 60 patients whom underwent lateral malleolus hardware removal.

Inclusion criteria were: isolated distal fibula fracture (no other associated fractures or intra-operative demonstrated tibio-fibular syndesmosis imbalance); ORIF (Open Reduction and Internal Fixation) surgical procedure for the original distal fibula fracture performed under loco-regional anaesthesia (in particular, a “bi-block” anaesthesia); X-Ray confirmed fracture healing; hardware removal for local pain (also mild pain levels) or ankle stiffness; more than 12 months between the ORIF and the hardware removal surgical procedure.

Exclusion criteria were: preference for procedure under intravenous sedation anaesthesia (IVSA) or general endotracheal anaesthesia (GETA); medical contraindication for the WALANT use (substantially history of peripheral vascular disorder due to occlusive peripheral arteries disease diabetes, etc. and history of allergic reaction to local anaesthetic drugs); clinical and X-Ray signs of hardware infection; X-Ray documented hardware failure; delayed healing of the surgical scar or tardive distal fibula skin coverage suffering (clinically documented by an ulcer development) after ORIF procedure; inability to sign the informed consent. Our local Ethics Committee approved this study.

Eligible patients were informed in detail about the differences between the general, loco-regional and the

WALANT anaesthesia and all of them signed a written informed consent to be admitted into our study. They were then randomly divided into two groups of 30 patients: group A performed the hardware removal under loco-regional anaesthesia (in particular, a “bi-block” anaesthesia) and group B performed the same surgical procedure under WALANT. Both groups underwent the surgical procedures into the main operative room.

For each patient, we collected demographic data such as age, gender, distal fibula fracture AO/OTA classification and type of hardware for ORIF, hardware removal indication and time between ORIF and hardware removal procedure. We also registered the number of hospitalization days; we considered 2 days of hospitalization if patient had to stay in the hospital for the night after surgery. We then collected data about maximum perceived pain using the Numeric Pain Rating Scale (NRS). The bi-block anaesthesia was performed by an anaesthesiologist using 1% mepivacaine. A tourniquet was placed in the middle third of the thigh (14). Using an electric bipolar signal in order to locate the femoral and the sciatic nerve local anaesthetic was injected around these two nerves to reach a complete lower limb sensitivity and motor block (14). The WALANT was instead performed by the same surgeon using a mixture of 0.5% ropivacaine (40 ml) and 1:1000 epinephrine (0.2 ml). The first 30 ml was injected into subcutaneous tissues of the middle point of the before-created surgical scar and the last 10 ml was injected with the contact of the needle with the distal fibula bone (Fig. 1 and Fig. 2). We injected the mixture not only near the lateral fibula surface but also

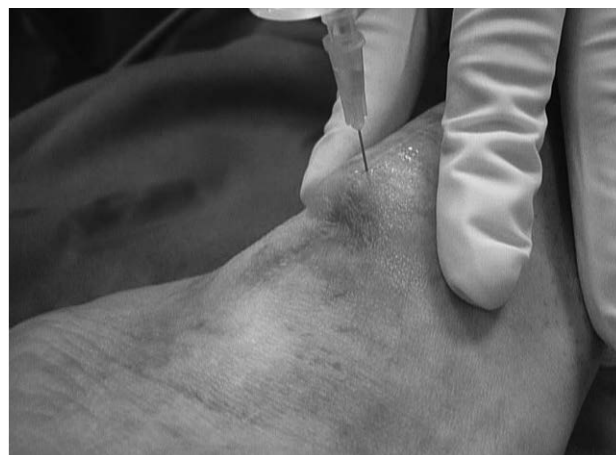


Fig. 1. Details of the technique we used for the injection of the mixture of ropivacaine and epinephrine.

near its anterior and posterior surface in order to reach the medial surface by periosteal membrane diffusion phenomena as described by D. Lalonde (11). In order to reduce the pain related to the local anaesthetic injection, we warmed the mixture (15) and we added 1 ml of 8.4% bicarbonate (16). We waited 20-30 min from the injection before starting surgical procedure (11, 17).

All patients received antibiotic prophylaxis with 2 g of sodic cefazoline administered 30 min before the surgical incision. A team of three surgeons performed all the hardware removal procedures.

For each patient we fixed a medical examination five days after the surgery in order to control the wound conditions and the post-operative maximum pain score. We also planned another examination at 15 days after surgery to remove stitches and point out possible cases of delayed wound healing.

Statistical analysis was performed using the Statistical Package for Social Sciences, Version 13 (SPSS Inc., Chicago, Illinois). Continuous variables were showed as mean $\pm$ standard deviation and discrete variables were expressed as frequency percentages. The non-parametric Mann-Whitney test was used to analyse variable differences between the two groups of patients. We used a 5% level of confidence for the test.

RESULTS

Patients' mean age at the time of hardware removal was 32 years (from 21- to 59-years-old), consisting of 49 males (81.6%) and 11 female (18.4%). The mean



Fig. 2. The surgical site aspect after the injection of the mixture of ropivacaine and epinephrine.

time between the ORIF and the hardware removal procedure was 15 months (with a range between 12 and 21 month). Using the AO/OTA classification, 51 fractures (85%) were 44-B1.1/2 and 9 (5%) fractures were 44-B1.3. ORIF procedure was performed using Syntes LCP Lateral Distal Fibula Plates in 43 (71.6%) cases and Intrauma O'Nil Kite Plates in 17 (28.4%) cases. Data about type of hardware removal indications showed that 43 (71.6%) patients reported distal fibula local pain (with a mean NRS of 2.7 and a range from 1 to 7) while 17 patients (28.4%) suffered both for pain and ankle stiffness related to the hardware presence; differences in demographic and clinical data between two groups are shown in Table I. No differences in term of mean age, gender ratio and time between ORIF and hardware removal procedures were found.

Statistically significant differences were instead found in term of days of hospitalization. After the surgical operation under WALANT, no patient had to remain in hospital for the night against the 10 patients after the bi-block anaesthesia. Patients remained into the hospital after the bi-block anaesthesia fundamentally because they had a persistence of the lower limb motor nerve block (and inability in free deambulation), beyond the term of the surgical block closure (8 pm); this was the reason why the surgeon preferred not to discharge them before the following day after surgical activity (8 am). No difference in mean maximum NRS evaluated during the anaesthetic procedure was found (Table I). Patients from Group A referred they felt the worst pain during the anaesthetic infiltration around the sciatic nerve; less pain was instead reported during the infiltration around the femoral nerve. Patients from Group B however referred the worst pain during the infiltration of the first 10-20 ml of local anaesthetic and epinephrine mixture; local pain decreased after almost 2 min so the surgeon could deeply infiltrate the mixture near the periosteal membrane also, without further patient suffering. No differences were found in terms of local pain felt during the surgical procedure of hardware removal. Some patients from Group B (26%) referred the persistence of a tactile perception during the surgery that they described as "strange" but not painful. Patients from Group A did

not refer any particular perception during the surgical operation. We highlighted a significant difference in terms of maximum NRS score during the 5 days after surgery. Patients from Group B reported a lower NRS than patients from group A during our first clinical examination. We did not find any cases of peripheral ischemic infarction. Moreover, we did not find any cases of delayed wound healing at our second clinical examination (fixed 15 days after surgery) in either group of patients.

DISCUSSION

Hardware surgical removal after open reduction and internal fixation (ORIF) of bone fractures represents a significant part of elective orthopaedic surgery. Different authors reported variable percentage from 15 to 30% of total planned orthopaedic surgeries (1, 18). Three different reviews show that the ankle joint is the most frequently involved anatomic district, with percentages from 14.5 to 21 of total hardware removals (6-8). Although, there is little information regarding the incidence and indications for implant removal after ORIF for ankle fractures. Previous reports indicate that hardware-related complaints represent the most common indication (19, 20). Removal due to infection varies from 1.3 to 4.8% and hardware failure from 0.8 to 1.2% (21, 22). In addition, the patient's personal preference is a recurring indication but it is frequently hidden under a mild hardware-related pain or ankle stiffness. However, the indications and the clinical need for routine hardware removal after ORIF are disputed (23); surgeons express different viewpoints on this argument with some advocating the routine removal and many opposing the routine removal (24). This frequent surgical procedure obviously represents an important item of expenditure for the national health system. In an era of increasing attention for socio-economic impact of medical procedure, we thought to perform distal fibula hardware surgical removal under the Wide Awake Local Anaesthesia without Tourniquet (WALANT), a particular anaesthesiologic technique presented by D. Lalonde that is actually largely employed in hand surgery (10, 11, 25). Nowadays, there are many articles in literature demonstrating that WALANT offers a wide range of clinical and

economic advantages in hand surgery, compared to traditional loco-regional anaesthesia with tourniquet. In particular, the main advantages are: no need for tourniquet (because of the haemostatic effect of the epinephrine); the anaesthesiologist presence is not essential for its use (because "only" local anaesthesia is applied); the possibility for an intraoperative motoric check (because it blocks only the sensitive nervous fibres); a more rapid patient discharge after surgery (10, 11, 25). Many articles also highlighted the substantial safety of WALANT, also the widely mentioned risk of peripheral ischemic infarction due to the epinephrine injection into upper limb extremities has been disproven (11, 26). Moreover, an antidote (phentolamine) is largely available if this rare side effect appears after the injection. Obviously, literature reports that the use of this particular kind of local anaesthesia also has some contraindications like the history of peripheral vascular disorder (due to occlusive peripheral arteries disease, diabetes etc.) or the history of allergic reaction to local anaesthetic drugs. Despite the large number of articles about the use of the WALANT in hand surgery, literature presents no data about its application in lower limb extremity surgeries such as ankle hardware removal.

Authors therefore planned a single centre, randomized clinical study including 60 patients that underwent distal fibula hardware removal from January 2014 to December 2016, divided into two groups: Group A performed the surgery under a traditional loco-regional anaesthesia with tourniquet and Group B performed the surgery under the WALANT technique. The two groups showed no significant differences in term of demographic and trauma-features data. We excluded patients that preferred another kind of anaesthesia (like IVSA or GETA) from our study along with patients that were not able to sign the informed consent. We did not include patients with a history of allergic reactions to local anaesthetic drugs, history of peripheral vascular disorder due to occlusive peripheral arteries disease, diabetes, vasculitis etc. and patients that, after the ORIF procedure, developed a delayed wound healing or tardive distal fibula skin coverage suffering. Indeed, this fact clearly reveals a local microcirculation impairment that could worsen with

the use of epinephrine (11, 25). Finally, we also excluded patients with clinical and radiographic signs of hardware infection and/or failure because the removal procedure could be more invasive requiring a more extended anaesthetized area.

We did not find differences in terms of perceived mean maximum NRS during the anaesthesiology procedures. Indeed, the pain that patients from group A referred to during the peri-sciatic nerve injection was statistically comparable with the one that patients from group B experienced during the infiltration of the first 10-20 ml of local anaesthetic and epinephrine mixture. No differences were highlighted in terms of intraoperative maximum pain. Despite the use of local

anaesthesia, patients from group B did not denounce particular pain during the initial unscrewing from the medial fibula cortical bone. This fact is probably due to the periosteal diffusion of the local anaesthetic drugs that were injected at least 30 min before starting surgical procedures. For this reason, in our experience, it is important to insert the needle very close to the periosteal membrane in order to permit its medial diffusion. Mean maximum post-operative NRS referred at the 5-day follow-up showed a statistically significant difference instead. This fact is probably linked to the difference in local anaesthetic drugs pharmacokinetics. Indeed, the mepivacaine used for loco-regional anaesthesia generally allows an almost

Table I. Differences in demographic and clinical data between the two groups of patients.

	GROUP A	GROUP B	p value
Number of patients	30	30	
Mean age (years)	31 $\pm$ 0.8	32 $\pm$ 1.3	>0.05
Gender Ratio (M/F)	25:5	24:6	>0.05
Time between ORIF and hardware removal	15 $\pm$ 0.9	13 $\pm$ 1.4	>0.05
1 day hospitalization	20 (66%)	30 (100%)	<0.05
2 days hospitalization	10 (33%)	0 (0%)	<0.05
Mean maximum NRS during anaesthesitic procedure	6.23 $\pm$ 1.36 (range 4-9)	6.07 $\pm$ 1.34 (range 3-9)	>0.05
Mean maximum NRS during surgical procedure	0.63 $\pm$ 0.67 (range 0-2)	0.67 $\pm$ 0.72 (range 0-2)	>0.05
Mean maximum NRS after surgery	4.51 $\pm$ 1.04 (range 2-6)	3.30 $\pm$ 1.09 (range 1-6)	<0.05
Delayed wound healing	0	0	>0.05

10 h sensitive block while the ropivacaine used for WALANT, an almost 24 h block with a valid post-operative analgesic effect (27).

It is important to underline that, despite its limits in sensitive nerve block duration, we chose mepivacaine for the loco-regional anaesthesia because it is one of the local anaesthetic drugs with the shorter and the more stable period of motor nerve block (that is almost 3-4 h) (27). Nevertheless, the use of mepivacaine did not prevent the presence of a significant difference in number of hospitalization days between the two groups. This difference represents an important economic advantage in the use of WALANT that permits a more rapid patient discharge, given the lack of peripheral motor nerve block.

Finally, authors did not found significant difference even in terms of wound healing delay. This is an important evidence against WALANT's detractors that says the local swelling due to the injection of epinephrine and local anaesthetic could cause difficulties in surgical suture and then increase the risk of wound healing complications. We also highlighted no cases of peripheral ischemic infarction confirming the WALANT substantial safety in upper and lower limb extremity surgical procedures despite the use of epinephrine.

In conclusion, the WALANT can be considered as a viable solution for distal fibula hardware removal procedures in carefully selected patients. This particular anaesthesiologic setting can offer important clinical and economic advantages compared to the traditional loco-regional anaesthesia with a significant reduction in term of post-operative pain and recovery duration, without increase in pre- and intra-operative pain levels and in post-surgical complications rate. Moreover, this study lays the foundations for the use of WALANT, not only for hands but also for other types of extremities in surgical procedures. Furthermore, we propose to increase the clinical relevance of this study enlarging the number of enrolled patients and improve the efficacy in post-operative pain control using the new bupivacaine liposomal formulation (13).

REFERENCES

1. Bostman O, Pihlajamäki H. Routine implant removal after fracture surgery: a potentially reducible consumer of hospital resources in trauma units. *The Journal of trauma* 1996; 41(5):846-9.
2. Ochs BG, Gonser CE, Baron HC, Stockle U, Badke A, Stuby FM. Refracture of long bones after implant removal. An avoidable complication? *Der Unfallchirurg* 2012; 115(4):323-9.
3. Unno Veith F, Ladermann A, Hoffmeyer P. Is hardware removal a necessity? *Revue medicale suisse* 2009; 5(201):977-80.
4. Busam ML, Esther RJ, Obrebsky WT. Hardware removal: indications and expectations. *J Am Acad Orthop Surg* 2006; 14(2):113-20.
5. Muller-Farber J. Metal removal after osteosyntheses. Indications and risks. *Der Orthopäde* 2003; 32(11):1039-57.
6. Reith G, Schmitz-Greven V, Hensel KO, Schneider MM, Tinschmann T, Bouillon B, et al. Metal implant removal: benefits and drawbacks--a patient survey. *BMC surgery* 2015; 15:96.
7. Haseeb M, Butt MF, Altaf T, Muzaffar K, Gupta A, Jallu A. Indications of implant removal: A study of 83 cases. *Int J Health Sci (Qassim)* 2017; 11(1):1-7.
8. Naumann MG, Sigurdson U, Utvag SE, Stavem K. Incidence and risk factors for removal of an internal fixation following surgery for ankle fracture: A retrospective cohort study of 997 patients. *Injury* 2016; 47(8):1783-8.
9. Jung HG, Kim JI, Park JY, Park JT, Eom JS, Lee DO. Is Hardware Removal Recommended after Ankle Fracture Repair? *BioMed research international* 2016; 2016:5250672.
10. Leblanc MR, Lalonde J, Lalonde DH. A detailed cost and efficiency analysis of performing carpal tunnel surgery in the main operating room versus the ambulatory setting in Canada. *Hand* 2007; 2(4):173-8.
11. Lalonde D, Bell M, Benoit P, Sparkes G, Denkler K, Chang P. A multicenter prospective study of 3,110 consecutive cases of elective epinephrine use in the fingers and hand: the Dalhousie Project clinical phase. *J Hand Surg Am* 2005; 30(5):1061-7.
12. Bismil M, Bismil Q, Harding D, Harris P, Lamyman E, Sansby L. Transition to total one-stop wide-awake hand surgery service-audit: a retrospective review. *JRSM short reports* 2012; 3(4):23.
13. Herbst SA. Local infiltration of liposome bupivacaine

- in foot and ankle surgery: case-based reviews. *Am J Orthop* (Belle Mead NJ) 2014; 43(10 Suppl):S10-2.
14. Di Massa A, Orlandini N, Di Don Francesco P, Simone ME. Electric nerve stimulation (ENS): 70 clinical cases of bi-block aided by an electric bipolar signal. *Minerva Anestesiol* 1997; 63(10):315-9.
 15. Hogan ME, vanderVaart S, Perampaladas K, Machado M, Einarson TR, Taddio A. Systematic review and meta-analysis of the effect of warming local anesthetics on injection pain. *Ann Emerg Med*. 2011; 58(1):86-98.e1.
 16. Vossinakis IC, Stavroulaki P, Paleochorlidis I, Badras LS. Reducing the pain associated with local anaesthetic infiltration for open carpal tunnel decompression. *Journal of hand surgery* 2004; 29(4):399-401.
 17. Davison PG, Cobb T, Lalonde DH. The patient's perspective on carpal tunnel surgery related to the type of anesthesia: a prospective cohort study. *Hand* 2013; 8(1):47-53.
 18. Rutkow IM. Surgical operations in the United States. Then (1983) and now (1994). *Archives of surgery* 1997; 132(9):983-90.
 19. Brown OL, Dirschl DR, Obrebsky WT. Incidence of hardware-related pain and its effect on functional outcomes after open reduction and internal fixation of ankle fractures. *Journal of orthopaedic trauma* 2001; 15(4):271-4.
 20. Jacobsen S, Honnens de Lichtenberg M, Jensen CM, Torholm C. Removal of internal fixation--the effect on patients' complaints: a study of 66 cases of removal of internal fixation after malleolar fractures. *Foot & ankle international* 1994; 15(4):170-1.
 21. Miller AG, Margules A, Raikin SM. Risk factors for wound complications after ankle fracture surgery. *The Journal of bone and joint surgery American volume* 2012; 94(22):2047-52.
 22. SooHoo NF, Krenke L, Eagan MJ, Gurbani B, Ko CY, Zingmond DS. Complication rates following open reduction and internal fixation of ankle fractures. *The Journal of bone and joint surgery American volume* 2009; 91(5):1042-9.
 23. Williams AA, Witten DM, Duester R, Chou LB. The benefits of implant removal from the foot and ankle. *The Journal of bone and joint surgery American volume* 2012; 94(14):1316-20.
 24. Hanson B, van der Werken C, Stengel D. Surgeons' beliefs and perceptions about removal of orthopaedic implants. *BMC musculoskeletal disorders* 2008; 9:73.
 25. Lalonde D, Martin A. Epinephrine in local anesthesia in finger and hand surgery: the case for wide-awake anesthesia. *J Am Acad Orthop Surg* 2013; 21(8):443-7.
 26. Thomson CJ, Lalonde DH. Randomized double-blind comparison of duration of anesthesia among three commonly used agents in digital nerve block. *Plastic and reconstructive surgery* 2006; 118(2):429-32.
 27. Becker DE, Reed KL. Essentials of local anesthetic pharmacology. *Anesthesia progress*. 2006; 53(3):98-108.

CRYOTHERAPY EFFICACY AND SAFETY AS LOCAL THERAPY IN SURGICAL TREATMENT OF MUSCULOSKELETAL TUMOURS. A RETROSPECTIVE CASE SERIES OF 143 PATIENTS

S. COLANGELI¹, P. PARCHI¹, L. ANDREANI¹, G. BELTRAMI², G. SCOCCIANI², F. SACCHETTI¹, M. CECCOLI¹, F. TOTI², D.A. CAMPANACCI² and R. CAPANNA¹

¹Second Orthopedic Division, Department of Translational Research and New Technology in Medicine and Surgery, University of Pisa, Italy; ²Department of Orthopedic Oncology, Azienda Ospedaliero-Universitaria Careggi, Firenze, Italy

Cryotherapy, also called Cryoablation (CA), is a technique that provides a local treatment to various pathological conditions. In Musculoskeletal tumours management, Cryoablation is well accepted and validated as a treatment in palliative cures for metastatic patients. Recently, CA has been proposed also as an alternative to radiofrequency ablation in osteoid osteoma and other benign tumour treatment with promising results. Cryotherapy with argon ice-balls as local adjuvant in open surgery is a tool that can provide enlargement of surgical margins if used properly. There is still not enough evidence supporting use of cryotherapy as local adjuvant in Musculoskeletal open surgery as the series cited above are very small and there is no comparative RCT between local adjuvant therapies including CA. One-hundred-and-eighty-three patients were treated with Cryoablation from 2000 and 2018 in the Musculoskeletal Tumours Surgery Unit of Careggi (Florence) and the University 2nd Clinic of Pisa. In our study group, 38 patients (26.6%) were affected by bone metastasis, 16 patients (11.1%) by aneurismal bone cysts or angiomas, 22 patients (15.4%) by low-grade malignant musculoskeletal tumours, 2 patients (1.4%) by fibromatosis, 63 patients (44.1%) by benign musculoskeletal tumours (principally Giant Cell Tumours-GCT) and 2 patients (1.4%) by Osteosarcomas. In 125 cases (87.4%), CA has been used as an adjuvant therapy, in 12 cases (8.4%) as a percutaneous ablation therapy and in 6 cases (4.2%) as adjuvant to remove tumoral lesions ‘en bloc’ or as a ‘poor technique’ for its sterilizing effect on previously resected bones. Mean follow-up was 10 years. Twenty-three patients (16%) were classified as Alive with Disease (AWD) due to local recurrence or tumour progression (14 metastases, 5 low-grade malignant bone tumours, 4 Giant Cell Tumours). Eight patients died due to the disease (6 metastases, 2 osteosarcomas), while 1 died from leukaemia. One-hundred-and-eleven patients (78%) were classified as Continues Disease Free (CDF). All patients reported decrease in pain-related symptoms after surgery and all surgeons reported better control of blood loss. Three cases (2%) of local skin necrosis or wound dehiscence were reported. No local recurrences were reported after fibromatosis ablation. Our results confirm that CA could be considered as a safe and effective technique to treat various conditions as adjuvant and palliative therapy. In particular, in open surgery, cryotherapy as an adjuvant treatment could lead to very low rates of recurrence in locally aggressive tumours like Giant Cell Tumours. These results could be generalized but a better understanding about indications and outcomes can be reached studying CA in specific populations with comparison to other adjuvant techniques.

Key words: cryotherapy, adjuvant therapies, musculoskeletal tumours, local therapies, case series

Corresponding author:

Federico Sacchetti, MD,
Department of Orthopaedic and Trauma Surgery,
University of Pisa,
Via Paradisa 2,
56124, Pisa, Italy
e-mail: federico.sacchetti1989@gmail.com

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Cryotherapy, also called Cryoablation (CA), is a relatively novel technique that provides a local treatment to various pathological conditions. CA induces coagulating necrosis by using rapid freezing, slow thawing, and repetition of the freeze-thaw cycle (1). It works by rapidly decompressing a gas (usually argon) through a needle-like probe. As the argon flows through the needle, a ball of ice crystals forms around the tip of the probe, thus immediately leading to the cellular death of the surrounding tissues. In Musculoskeletal tumours management, Cryoablation is well accepted and validated as a treatment in palliative cures for metastatic patients (2,3). Painful bone metastasis in patients that can be treated only with “low-risk” approaches are addressed to percutaneous Scan images guided cryoablations.

In recent years, CA has been proposed also as an alternative to radiofrequency ablation in osteoid osteoma and other benign tumour treatments with promising results (4-8). Cryotherapy with argon ice-balls as local adjuvant in open surgery is a tool that can provide enlargement of surgical margins if used properly. In locally aggressive tumours such as Giant Cell Tumours, aneurismal bone cyst and Chondroblastoma, local recurrence still represents a

major challenge (9,10). Cryotherapy has been tested in some case series showing good results in terms of local recurrences rates (11-14). There is still not enough evidence supporting use of cryotherapy as local adjuvant in Musculoskeletal open surgery since the series cited above are very small and there is no comparative RCT among local adjuvant therapies including CA. Our study includes 143 cases of local aggressive tumours, metastasis and other conditions managed with the aid of CA with a median follow-up of 10 years.

MATERIALS AND METHODS

One-hundred-and-forty-three patients were treated with Cryoablation between May 2000 and June 2018 in the Musculoskeletal Tumours Surgery Unit of Careggi (Florence) and the 2nd University Clinic of Pisa. Each patient underwent Computed Tomography Scan and Magnetic Resonance Images to plan surgical intervention and positioning of the needles before surgery. Each patient underwent intra-operative fluoroscopy and Post-operative Ct-Scan controls. Regular clinical and radiological follow-up was planned at 2, 6 and 12 months and then each 6 months after surgery to detect any recurrence,

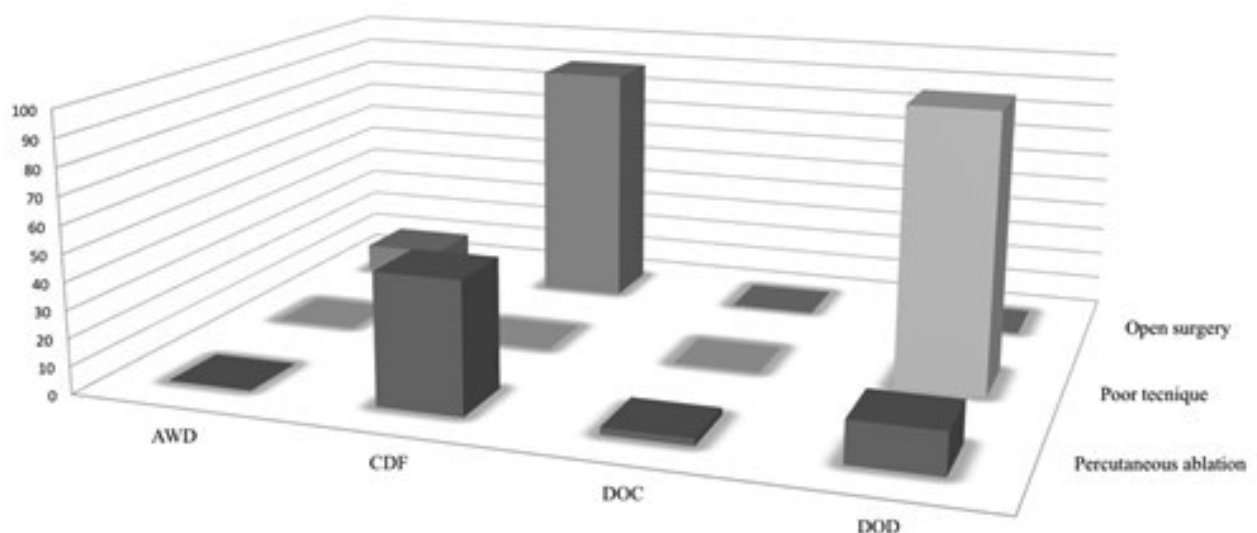


Fig. 1. Graphical interpretation of clinical follow-up results by surgery type.

complication or disease progression. Each patient was classified as:

- alive with disease (AWD) if there was a local recurrence or tumour progression after surgery;
- died of disease (DOD) if the patient died due to neoplasm progression or neoplasm-related complications;
- died for other causes (DOC);
- continues disease free (CDF) if there was no progression or recurrence of the tumour since the last clinical and radiological follow-up.

All results were expressed as percentages (rates). A questionnaire with self-reporting of residual pain (NRS scale) was delivered to each patient, and each surgeon was asked to report difficulties in blood loss checking during surgical intervention. Cryotherapy was used as an adjuvant in open surgery to enlarge surgical margins, as a “poor technique” to sterilize bone after tumour resections and as percutaneous Ct-Scan guided therapy for small lesions.

As adjuvant in open surgery, CA was performed using usually two or more probes positioned in a previously planned spatial configuration after lesion curettage to enlarge surgical margins without compromising bone structural resistance. After having done bone curettage,

a gel-like solution was generally used to completely fill the cavity as a thermal conductor. The probes were then immersed in the gel-like solution with the studied configuration in order to freeze all the cavity thanks to the argon flow (Fig. 1, 2). After first freezing cycle, the resulting solid gel solution was then melted with hot water. These cycles were repeated three times for ten minutes each or 8 minutes in some cases when there were risks of skin freezing or of damaging nerves and vessels. In this manner, thermal shock of the freeze-thaw cycles should be well conducted and regularly distributed around the cavity walls enlarging surgical margins.

During the procedure, constant skin irrigation and protection with sterile glove filled with warm water was performed in order to avoid skin necrosis. As an ablation therapy, the needle-like probe was positioned with a Ct-Scan in radiology department or with an amplifier in operative room guidance to reach the lesion and to perform circumferential coagulation necrosis. Ablation of the mass and the surrounding tissues necrosis was verified during the procedure. As a ‘poor technique’, used to sterilize bones after bone resections, Cryotherapy was applied to the surgical specimen after macroscopic removal of all tumour tissue. A dedicated team performed 3 cycles of freezing and thawing 10 minutes each putting



Fig. 2. Probes disposition after aneurismal bone cyst curettage.

the specimen in liquid nitrogen in order to sterilize it from all tumoral cells. After this procedure, the resected and sterilized bone could be positioned in its anatomical position to perform tissues reconstruction. When the 'poor technique' were applied to long segments of weight bearing bone like femur, surgeons used to fill the sterilized bone with cement to improve mechanical resistance of the construct. In our study group, 38 patients (26.6%) were affected by bone metastasis (usually oligometastatic patients), 16 patients (11.1%) by aneurismal bone cysts or angiomas, 22 patients (15.4%) by low-grade malignant musculoskeletal tumours, 2 patients (1.4%) by fibromatosis, 63 patients (44.1%) by benign musculoskeletal tumours (principally Giant Cell Tumours-GCT) and 2 patients (1.4%) by Osteosarcomas. In 125 cases (87.4%), CA has been used as an adjuvant therapy, in 12 cases (8.4%) as a percutaneous ablation therapy and in 6 cases (4.2%) as adjuvant to remove tumoral lesions 'en bloc' or as a 'poor technique' for its sterilizing effect on previously resected bones. Mean follow-up was 10 years.

RESULTS

Only 23 patients (16%) were classified as alive with disease (AWD) due to local recurrence or tumour progression (14 metastases, 5 low-grade malignant bone tumours, 4 Giant Cell Tumours). 8 patients died due to the disease (6 metastases, 2 osteosarcomas), 1 died of a different disease (leukemia). 111 patients

(78%) were classified as continuous disease free (CDF). In the open surgery group our patients were classified as AWD: 10.5%, DOD: 0%, DOC: 0% and CDF: 89.4%; in the 'poor technique' group they were classified as AWD: 0%, DOD: 100%, DOC: 0%, CDF: 0%; in the percutaneous ablation group patients of our study group resulted as AWD: 35%, DOD: 15%, DOC: 2.5%, CDF: 47.5%. All patients reported diminution of pain related symptoms after surgery and all surgeons reported better control of blood loss. Complications rates were very low, only 3 cases (2%) of local skin necrosis or wound dehiscence were reported, all these 3 patients healed with periodical medications. No local recurrences were reported after fibromatosis ablation (Table II, Fig. 3).

DISCUSSION

Cryotherapy has a broad range of applications in medical field. Palliative therapies using CA are well accepted and proven to be safe and effective. In particular, image guided percutaneous ablations of metastases is very useful to treat lesions otherwise managed only with follow-up and pain related drugs (2). In patients who are not suitable for surgical excision of metastasis due to bad general conditions or to particular locations of the lesion, percutaneous CA could be the only available option.

Table I. *Clinical follow-up results by tumor type.*

Tumor's Type	Total Patients	AWD	DOD	DOC	CDF
Benign Musculoskeletal tumors	63	4	0	0	59
Bone Metastasis	38	14	6	0	18
Low-grade malignant musculoskeletal tumors	22	5	0	1	16
Aneurismal bone cysts or angiomas	16	0	0	0	16
Fibromatosis	2	0	0	0	2
Osteosarcomas	2	0	2	0	0

Table II. *Clinical follow-up results by surgery type.*

Surgery Type	AWD (%)	DOD (%)	DOC (%)	CDF (%)
Open Surgery	10,5	0	0	89,5
Poor technique	0	100	0	0
Percutaneous ablation	35	15	2,5	47,5

As radiofrequency, cryotherapy also could be used in small lesion ablations, in particular, osteoid osteomas and fibromatosis loci could be treated with the same techniques of image guided percutaneous ablation with radiofrequencies. Our results confirm optimal outcomes for fibromatosis loci ablations being 0% our rate of local recurrence with a long-term follow-up.

Given the very small subgroup of patients treated with CA as a 'poor technique', we could not generalize our results in this particular field of application. However, in our series, sterilizing bone resulted very useful in surgical interventions to improve reconstruction time. Using the same resected bone allowed us to perfectly recreate the anatomical

site eliminating problems of different sizes or shapes that are usually linked to allotransplants. The use of custom made titanium prostheses could be a better alternative in some cases but the costs are greater and their production process requires long time that could be significantly shortened by the use of bone sterilized with "poor technique. Thus improving patients' post-operative recovery and their quality of life. Complications rate resulted very low.

The most promising therapeutic area of CA is the field relative to CA as adjuvant after open surgery to enlarge surgical margins. Our recurrence rate after low-extremities GCT curettages is very low being around 6% and being the recurrence rates reported in literature after curettage of low-extremities CGTs (15) around 15-20% using different adjuvant therapies. Our results confirms that CA used as an adjuvant therapy after CGT open surgery is a very useful technique. Blood loss seems to be more controllable as subjective reports of surgeons suggest. Further research, with prospective comparative study designs is needed to evaluate superiority of CA adjuvant therapies in regards to the other currently used techniques. Complication rates are also very low. If the surgeon applies the appropriate procedures being very careful about distances between the probe and the skin, no complications should be expected.

In our series, only 3 skin necrosis were reported (2% of the patients), with complete resolution after periodical medications. No bone fractures were reported in our study group. This one was one of the most feared complications when CA was introduced due to the weakening of the surrounding bone after curettages. Our results demonstrate that CA is effective but also safe. The very key point is the needle positioning. With a correct positioning, surgeons can enlarge surgical margins without



causing excessive weakening of the surrounding bone or skin necrosis. A careful preoperative planning is essential to reach these goals of efficacy and safety. In the future, the introduction of surgical navigation tools, in particular with the aid of Augmented Reality technology, should allow us to improve precision of the needle positioning.

Our results confirm that CA could be considered as a safe and effective technique to treat various conditions as an adjuvant or palliative therapy. In particular, in open surgery, cryotherapy as an adjuvant treatment could lead to very low rates of recurrence in locally aggressive tumours like Giant Cell Tumours. These results could be generalized but a better understanding about indications and outcomes can be reached studying CA in specific populations with comparison to other adjuvant techniques.

REFERENCES

1. Gage AA, Baust J. Mechanisms of tissue injury in cryosurgery. *Cryobiology* 1998; 37(3):171-86.
2. McMenomy BP, Kurup AN, Johnson GB, et al. Percutaneous cryoablation of musculoskeletal oligometastatic disease for complete remission. *J Vasc Interv Radiol* 2013; 24(2):207-13.
3. Cappuccio M, Bandiera S, Babbi L, et al. Management of bone metastases. *Eur Rev Med Pharmacol Sci* 2010; 14(4):407-14.
4. Tsoumakidou G, Too CW, Garnon J, Steib JP, Gangi A. Treatment of a spinal aneurysmal bone cyst using combined image-guided cryoablation and cementoplasty. *Skel radiol* 2015; 44(2):285-9.
5. Griauzde J, Gemmete JJ, Farley F. Successful treatment of a Musculoskeletal Tumor Society grade 3 aneurysmal bone cyst with N-butyl cyanoacrylate embolization and percutaneous cryoablation. *JVIR* 2015; 26(6):905-9.
6. Havez M, Lippa N, Al-Ammari S, et al. Percutaneous image-guided cryoablation in inoperable extra-abdominal desmoid tumors: a study of tolerability and efficacy. *Cardiovasc Intervent Radiol* 2014; 37(6):1500-6.
7. Schmitz JJ, Schmit GD, Atwell TD, Callstrom MR, Kurup AN, Weisbrod AJ, Morris JM. Percutaneous Cryoablation of Extraabdominal Desmoid Tumors: A 10-Year Experience *AJR Am J Roentgenol* 2016; 207(1):190-5.
8. Mahnken AH, Konig AM, Figiel JH. Current Technique and Application of Percutaneous Cryotherapy. *RoFo* 2018; 190(9):836-46.
9. Wang EH, Marfori ML, Serrano MV, Rubio DA. Is curettage and high-speed burring sufficient treatment for aneurysmal bone cysts? *Clin Orthop Relat Res* 2014; 472(11):3483-8.
10. Errani C, Ruggieri P, Asenzio MA, et al. Giant cell tumor of the extremity: A review of 349 cases from a single institution. *Cancer Treat Rev* 2010; 36(1):1-7.
11. Souna BS, Belot N, Duval H, Langlais F, Thomazeau H. No recurrences in selected patients after curettage with cryotherapy for grade I chondrosarcomas. *Clin Orthop Relat Res* 2010; 468(7):1956-62.
12. Donati D, Wafa H, Di Bella C, Colangeli M, Colangeli S, Bertoni F. Management of pelvic giant cell tumours involving the acetabular bone. *Acta orthop B* 2008; 74(6):773-8.
13. Robinson D, Yassin M, Nevo Z. Cryotherapy of musculoskeletal tumors--from basic science to clinical results. *Tech canc res treat* 2004; 3(4):371-5.
14. Mashhour MA, Abdel Rahman M. Lower recurrence rate in chondroblastoma using extended curettage and cryosurgery. *Int ortho* 2014; 38(5):1019-24.
15. Raskin KA, Schwab JH, Mankin HJ, Springfield DS, Hornicek FJ. Giant cell tumor of bone. *J Am Acad Orthop Surg* 2013; 21(2):118-26.

POLYMETHYLMETHACRYLATE-AUGMENTED FENESTRATED PEDICLE-SCREW FIXATION IN LOW BONE QUALITY PATIENTS: A CASE SERIES AND LITERATURE REVIEW

R. GHERMANDI¹, V. PIPOLA¹, S. COLANGELI², P. PARCHI², L. ANDREANI², R. CAPANNA², and A. GASBARRINI¹

¹*Department of Oncologic and Degenerative Spine Surgery, IRCCS Istituto Ortopedico Rizzoli – Bologna, Italy;* ²*Second Orthopedic Division, Department of Translational Research and New Technology in Medicine and Surgery, University of Pisa, Pisa, Italy*

The use of pedicle screws in low bone quality patients implicates risks of secondary implant loosening for grip lack. In fact, the result is a reduced mechanical stability at bone-screw interface and consequently an increased chance of pullout and hardware failure. Augmentation techniques have been described for many years and fenestrated screws that allow cement injection is one of them. This is a retrospective observational study of patients treated at our department with polymethylmethacrylate- (PMMA) augmented fenestrated screws. Indications for posterior instrumentation were traumatic fracture in osteoporotic spine, oncological disease, post-traumatic deformity, degenerative disease, revision surgery and sickle cell disease fractures. Implant stability was evaluated with X-Rays and CT scan performed 3 days after surgery and every 3 months during the follow-up. Accuracy of screw placement was evaluated with Heary classification. Fifty-three surgical treatments in 52 patients were performed and 247 PMMA augmented fenestrated screws were placed. According to the Heary classification, 96.21% resulted Grade I, 1.8% Grade II, 2% Grade IV. A total of 17 complications occurred. Fenestrated screw augmentation should be performed in selected patients in whom the bone quality is insufficient to guarantee implant stability. These screws may result useful in complex cases as revision surgeries, osteoporosis and tumour affections where bone quality is highly compromised.

While indications for spine instrumentation are increasing, the population is aging and finding patients with poor bone quality or elderly patients with implant failure is becoming a common problem. Alternatives to traditional pedicle screws have spread in the last decades not only in revision surgery but also in primary surgery. Many solutions have been proposed in order to reduce the risk of screw loosening: expandable screws, hydroxyapatite-coated screws, bicortical screws, larger diameter screws and PMMA augmentation. PMMA augmentation can be achieved by direct technique through cement injection before screw

inserting or through fenestrated pedicle screws with holes designed for cement injection.

In low bone quality patients, the bone-screw interface is greatly affected reducing the mechanical grip of the screws and their integration at the interface between bone and metal. Polymerization of PMMA, once extruded through the screw holes, creates a continuous mass between the core of the screw and the cancellous bone in the vertebral body (1).

MATERIALS AND METHODS

This is a retrospective observational study of patients

Key words: fenestrated pedicle screws, osteoporosis, low bone quality, hardware failure, polymethylmethacrylate, PMMA

Corresponding Author:

Riccardo Ghermandi, MD,
Department of Oncologic and Degenerative Spine Surgery,
IRCCS Istituto Ortopedico Rizzoli,
Via Giulio Cesare Pupilli, 1, 40136 Bologna, Italy
Tel.: +39 051 6366493 - Fax: +39 051 6366337
e-mail: riccardoghermandi@gmail.com

with low bone quality treated at our department from September 2006 to September 2012, who received a posterior fixation with PMMA augmented fenestrated screws. Indications for posterior instrumentation were traumatic fracture in osteoporotic spine, oncological disease, post-traumatic deformity, degenerative disease, revision surgery and sickle cell disease fractures. Implant stability was evaluated with X-Rays and CT scan performed 3 days after surgery and every 3 months during the follow-up. Accuracy of screw placement was evaluated with Heary classification (2). After the first 12 months, radiologic controls were taken once a year in all surviving patients. Clinical evaluation was performed with the VAS score. Complications were recorded and were classified according to the temporal distribution in intraoperative, early post-operative and late post-operative complications (3).

RESULTS

From September 2006 to September 2012, 53 surgical treatments in 52 patients with low bone density were recorded. Mean age was 64.53 years old (range

22-85 years old). Indications for posterior fixation were traumatic fracture in osteoporotic spine in 5 cases; oncological disease in 28 cases; post-traumatic deformity in 3 cases; degenerative disease in 3 cases; revision surgery in 12 cases; sickle cell disease fractures in 1 case (2 interventions). Mean Follow-up period was 23.9 months. A total of 247 fenestrated screws were implanted and cement augmentation was performed with no more than 2 cc of PMMA per screw. According to the Heary classification (2) 96.21% of the screws were Grade I, 1.8% Grade II, 2% Grade IV. VAS score decreased significantly after the procedure, the pain was relieved and quality life greatly improved, mainly in walking ability.

We recorded 6 intraoperative complications (35.3%); 1 early post-operative complication (5.9%) and 10 late post-operative complications (58.8%). Six patients had an intracanal cement leakage. In 5 cases cement leakage was detected by post-operative CT scan; it did not require any treatments because patients had no symptoms in 4 cases and 1 case radicular pain referred the remission of symptoms after 14 months of physiotherapeutic treatment. In 1 case



Fig.1. A, b): Forty-nine-year-old female rheumatoid arthritis and L1 burst fracture; rapid worsening of neurological function, ASIA C. She was submitted to a decompression and T11-L3 fixation with PMMA augmented fenestrated screws and vertebroplasty of level above and below. c): CT scan shows the circumferential distribution of cement around the screw; d, e): post-operative LL and AP X-Ray; ASIA: American Spinal Injury Association score.

Table I. *Features of population enrolled.*

Nº	Age	Date	Fenestreted Screws	Complications
1	66	09/2006	1	
2	76	12/2006	4	Cauda equina
3	68	01/2007	2	
4	73	02/2007	4	
5	85	05/2007	4	Infection
6	57	05/2007	4	
7	75	06/2007	1	
8	56	06/2007	7	
9	57	06/2007	10	Infection
10	62	06/2007	4	
11	70	08/2007	2	Leakage
12	76	08/2007	4	Leakage
13	77	09/2007	4	
14	58	11/2007	3	
15	79	02/2008	4	
16	70	02/2008	4	
17	72	02/2008	4	
18	55	03/2008	4	
19	55	03/2008	4	Rod Breakage
20	58	04/2008	4	
21	66	04/2008	5	
22	60	09/2009	2	
23	63	10/2009	5	
24	58	11/2009	4	
25	64	11/2009	3	Rod Breakage
26	67	12/2009	2	
27	73	01/2010	4	
28	56	03/2010	4	
29	52	05/2010	2	
30	63	06/2010	12	Leakage/Dehiscence
31	65	06/2010	4	

32	71	07/2010	4	Leakage
33	45	08/2010	2	
34	70	12/2010	1	
35	51	01/2011	8	Rod breakage
36	57	02/2011	4	
37	80	02/2011	4	Traumatic Fracture
38	51	02/2011	4	Leakage
39	59	02/2011	4	
40	71	03/2011	12	Leakage
41	63	07/2011	6	
42	68	07/2011	10	Traumatic Fracture
43	79	08/2011	4	
44	80	08/2011	4	
45	72	08/2011	4	
46	63	09/2011	6	
47	54	09/2011	4	Infection
48	68	11/2011	4	
49	22	11/2011	11	
50	55	11/2011	4	Screw Pull-Out
51	46	12/2011	8	
52	59	02/2012	14	

cement leakage was detected intraoperatively with subsequent laminectomy and cement removal without any sequelae after surgery. No cases of pulmonary embolism were detected. There was one cauda equina syndrome due to a postsurgical hematoma that appeared three days after surgery and required a re-intervention. Two patients had accidents (car crash and ladder fall down) with adjacent levels fractures that required a revision surgery with extension of fixation to the compromised levels. Four surgical site infections were recorded that were successfully

treated by antibiotic therapy; three-rod breakages and 1 case of screw pull out (0.4 % of all screws). Three patients with metastases died of the disease 13, 21 and 24 months after treatment (Table I).

DISCUSSION

It has been widely demonstrated that demineralized bone has direct relationship with pedicle screw loosening. Increased spaces in osteoporotic cancellous bone framework limits

the immediate mechanical grip of the screws and compromises integration at the interface between bone and metal, favouring the weakness of the implant. The increasing age of the population and the spreading of spine surgical indications are the reasons that encourage many kinds of research in this field. Ozturk et al (4) presented a series of 1454 screws (not fenestrated) with 48 months follow up in cement augmentation technique, concluding that this method provides rigid fixation and prevents implant failure, however cement leakage and pulmonary embolism can be a problem and should be carefully monitored. With respect to the cement leakage, Frankel et al (5) detected extravasations in 39% of their series with 1 pulmonary embolus and no relationship with the amount of cement injected. Sawakami et al (6) instrumented 38 patients with pseudoarthrosis from osteoporotic fractures, deciding that reinforcement of pedicle screws using PMMA augmentation may be a feasible surgical technique in poor bone quality spine.

In a study conducted on bone specimens obtained from 5 adult humane spines, Leichtle et al (7) have shown no difference in main failure load between solid cemented screw and fenestrated screws and similar failure pattern between the two groups. The main advantage of fenestrated screws is that the cement can be injected directly through the screw avoiding the removal of the screw required, instead, for the solid screws. Furthermore, the use of fenestrated screws minimize the operative time, above all during multilevel instrumentations, and it is associated with a circumferential distribution of cement around the screw and not predominantly at the screw tip in the anterior part of the vertebral body.

The optimal cement volume for a proper screw fixation is controversial. A cement volume of 1 ml to 3 mL was suggested as optimal; instead volume >3 ml are not associated to an improvement of fixation strength but a greater risk of cement leakage (8, 9). Alternatives to PMMA have also been assayed: Derincek et al (10) revised 17 cadaveric vertebrae with calcium sulphate augmentation and with straightforward technique, finding that mean pull-out strength ratio of revised screws to original was 0.71 for anatomic trajectory screws and 1.8 for

graft-augmented screws, a statistically significant difference ($P=0.002$) (6). In 6 cadaveric sacrum the PMMA augmentation provided increased resistance to cyclic loading compared with the CTBC (calcium triglyceride bone cement) for pedicle screw fixation, but both augmentations well exceeded previously published findings for non-augmented screws (11).

Bioactive glass can significantly improve bone microstructure of the interface in osteoporosis condition and increase the hold-strength of the pedicle screw, concluded an experimental study in ovariectomized sheeps (12). Even if non-cemented, pedicle screws augmentation techniques with polyethylene (Nesplon) tape applied sublaminar or subpars are described to improve fixation stiffness (13). Double-threaded pedicle screws (14) and expansive pedicle screws showed to significantly improve biomechanical fixation strength in bone of compromised quality (15) along with longer screws that reach the anterior cortex (16).

Parè et al (17) observed that a significant increase in pullout strength for both thoracic and lumbar vertebrae could be achieved by injecting a limited quantity of cement through a fenestrated pedicle screw when the bone mineral density is severely compromised. The median removal torque was 0.34 Nm for the standard and 1.83 Nm for the augmented screws (18). Fenestrated screws have the capacity to confine cement placement in the vertebral body and may provide enhanced safety from cement extrusion into the spinal canal. It is feasible to inject high-viscosity PMMA through this fenestration geometry, and higher-viscosity cement may improve the fixation effect (18).

CONCLUSION

Fenestrated screw augmentation should be performed in selected patients in whom bone quality is insufficient to guarantee implant stability. These screws may result useful in complex cases such as osteoporotic and tumour patients and revision surgery. Cement leakage and its related complications as neural injuries and pulmonary embolism represent the main limitation of their use even if they can be controlled by cement volume between 1 and 3 mL per screw.

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REFERENCES

1. Amendola L, Gasbarrini A, Fosco M, Simoes C.E, Terzi S, De Iure F, Boriani S. Fenestrated pedicle screws for cement-augmented purchase in patients with bone softening: a review of 21 cases. *J Orthop Traumatol* 2011; 12:193-9.
2. Heary RF, Bono CM, Black M. Thoracic pedicle screws: postoperative computerized tomography scanning assessment. *J Neurosurg* 2004; 100:(4SupplSpine)325-31.
3. Bandiera S, Boriani S, Donthineni R, Amendola L, Cappuccio M, Gasbarrini A. Complications of en bloc resections in the spine. *Orthop Clin N Am* 2009; 40:125-31.
4. Ozturk C, Alanay A, Enercan M, Karadereler S, Tezer M, Hamzooglu A. Cement Augmented Pedicle Screw Fixation In Osteopenic Patients: Minimum Two-Year Follow-Up Of 1,454 Pedicle Screws. *Spine* 2011; 2011(Supplement 2011):114.
5. Frankel BM, Jones T, Wang C. Segmental polymethylmethacrylate augmented pedicle screw fixation in patients with bone softening caused by osteoporosis and metastatic tumor involvement: a clinical evaluation. *Neurosurgery* 2007; 61(3):531-7.
6. Sawakami K, Yamazaki A, Ishikawa S, Ito T, Watanabe K, Endo N. Polymethylmethacrylate augmentation of pedicle screws increases the initial fixation in osteoporotic spine patients. *J Spinal Disord Tech* 2012; 25(2):E28-35.
7. Leichtle CI, Lorenz A, Rothstock S, Happel J, Walter F, Shiozawa T, Leichtle UG. Pull-out strength of cemented solid versus fenestrated pedicle screws in osteoporotic vertebrae. *Bone Joint Res* 2016; 5(9):419-26.
8. Bullmann V, Liljenqvist UR, Rodl et al). Pedicle screw augmentation from a biomechanical perspective. *Othopade* 2010; 39:673-8.
9. Frankel BM, D'Agostino S, Wang C. A biomechanical cadaveric analysis of polymethylmethacrylate-augmented pedicle screw fixation. *J Neurosurg Spine* 2007; 7:47:53.
10. Derincek A., Wu C., Mehbod A., Transfeldt E. Biomechanical comparison of anatomic trajectory pedicle screw versus injectable calcium sulfate graft-augmented pedicle screw for salvage in cadaveric thoracic bone. *J Spinal Disord Tech* 2006; 19(4):286-91.
11. McLachlin SD, Al Saleh K, Gurr KR, Bailey SI, Bailey CS, Dunning CE. (2011) Comparative assessment of sacral screw loosening augmented with pmma versus a calcium triglyceride bone cement. *Spine* 36: E699–E704.
12. Li Y, Cheng H, Liu ZC, Wu JW, Yu L, Zang Y, He Q, Lei W, Wu ZX. (2013) In vivo study of pedicle screw augmentation using bioactive glass in osteoporosis sheep. *J Spinal Disord Tech*. 26: E118-123.
13. Hamasaki T, Tanaka N, Kim J, Okada M, Ochi M, Hutton WC. (2010) Pedicle Screw Augmentation With Polyethylene Tape A Biomechanical Study in the Osteoporotic Thoracolumbar Spine. *J Spinal Disord Tech*. 23:127–132.
14. Mummaneni PV, Haddock SM, Liebschner MA, Keaveny TM, Rosenberg WS. (2002) Biomechanical Evaluation of a Double-Threaded Pedicle Screw in Elderly Vertebrae. *J Spinal Disord Tech*. 15: 64-68.
15. Cook SD, Salkeld SL, Whitecloud TS 3rd, Barbera J. (2000) Biomechanical Evaluation and Preliminary Clinical Experience with an Expansive Pedicle Screw Design. *J Spinal Disord*. 13: 230-236.
16. Zindrick MR, Wiltse LL, Widel EH et al. (1986) A biomechanical study of intrapeduncular screw fixation in the lumbosacral spine. *Clin Orthop* 203:99-112.
17. Paré PE, Chappuis JL, Rampersaud R. (2011) Biomechanical Evaluation of a Novel Fenestrated Pedicle Screw Augmented With Bone Cement in Osteoporotic Spines. *SPINE* 36: E1210–E1214.
18. Choma TJ, Pfeiffer FM, Swope RW, Hirner JP. (2012) Pedicle Screw Design and Cement Augmentation in Osteoporotic Vertebra. Effects of Fenestrations and Cement Viscosity on Fixation and Extraction. *Spine* 37: E1628–E1632.

A RARE CASE OF HOFFA'S FAT PAD HERNIATION IN A YOUNG PATIENT: DYNAMIC ULTRASOUND AND MRI DIAGNOSIS

I. SIMONETTI¹, V. CHIANCA², M. ZAPPÀ³, M. CARFORA⁴,
E. JANNELLI⁵ and F. DI PIETTO⁶

¹Department of Advanced Biomedical Sciences, Università degli Studi Federico II, Naples, Italy; ²Unit of Diagnostic and Interventional Radiology, I.R.C.C.S. Istituto Ortopedico Galeazzi, Milano, Italy; ³Department of Medicine and Health Sciences, Università del Molise, Campobasso, Campobasso, Italy; ⁴Department of Diagnostic Imaging, "P.O. Maddaloni", Maddaloni (CE), Italy; ⁵Clinica Ortopedica e Traumatologica, IRCCS Policlinico San Matteo, Università degli Studi di Pavia, Pavia, Italy; ⁶Department of Diagnostic Imaging, "A. Cardarelli" Hospital, Naples, Italy

Abstract Hoffa's fat pad (HFP) is part of the knee structure interposed between the joint capsule and the synovium. Recent studies have classified infrapatellar fat pad (IFP) disorders on a pathogenesis-based classification: traumatic disorders, post-traumatic disorders, lesions secondary to adjacent disorders. Masses or pseudo-masses may also be found within the IFP, however these alterations are much less frequent. Diagnostic imaging plays an important role in the diagnosis of masses and pseudo-masses of IFP and the first choice exam is a dynamic ultrasound study. We presented a rare case of Hoffa's fat pad herniation through the joint capsule, in a young child without history of knee injuries that has never been described before in the literature.

The infrapatellar fat pad (IFP), also known as Hoffa's fat pad (HFP), is an intracapsular and extrasynovial structure located in the infrapatellar region, posterior to patellar tendon and anterior to the capsule (1). IFP constitutes a true crossroad between patella, femur and tibia (2).

Infrapatellar Fat Pad injuries are divided into intrinsic and extrinsic. Intrinsic lesions such as hoffitis, anterolateral impingement, plica syndrome, post-arthroscopic changes, trauma, patellar dislocation and extrasynovial tumors are less frequent. Differently, extrinsic lesions are more frequent and may affect the synovium, patellar ligament, vascular structures, and bursae. Mucoid and parameniscal cysts may also develop in the infrapatellar space (1).

In this article, we present a rare case of Hoffa's fat pad herniation in a child that shows a palpable mass in the anterolateral aspect of knee exclusively during maximal flexion of the knee.

Case report

An 8-year-old male performed a magnetic resonance (MR) exam at our radiological department for a palpable mass at the anterolateral aspect of knee, without history of knee injuries. The patient did not present any symptoms or restriction of range of movement (ROM). Informed consent was obtained from all individual participants included in the study.

The clinical examination showed a mass, soft, mobile and not painful, visible and palpable exclusively during maximal flexion of the knee in

Key words: Hoffa's fat pad, infrapatellar fat pad disorders, infrapatellar fat pad, ultrasound, dynamic sonography, magnetic resonance imaging

Corresponding author:

Igino Simonetti MD,
Department of Advanced Biomedical Sciences,
University of Naples Federico II.
Via Sergio Pansini, 5
80131 Napoli, Italy
e-mail: igino.simonetti@gmail.com

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the anterolateral aspect of the infrapatellar region. The mass was not visible or palpable during full knee extension. The skin over the suspected mass did not present any changes.

MRI protocol was performed with a 1.5 T (Toshiba Vantage Titan System), with sagittal Turbo Spin Echo (TSE) T1-weighted in sagittal plane (TR/TE 420/10mS, 17x17cm FOV, 4mm thickness), axial and coronal TSE proton density-weighted with fat saturation (TR/TE 2688/18mS, 17x17cm FOV, 4mm slice thickness), and sagittal and axial TSE T2-weighted (TR/TE 6000/120mS, 17x17cm FOV, 4mm slice thickness).

The MRI images showed a little area of low signal on T1-weighted sagittal images (Fig. 1) and focal hyperintensity on axial T2-weighted sequences at the anterolateral margin of the joint capsule. These aspecific findings did not explain the clinical mass and we decided to performed dynamic ultrasound (US) evaluation.

The US examination performed with linear

probe (15-18 Mhz) showed partial herniation of Hoffa's fat pad through the capsular defect during flexion movement of the knee (Fig. 2). US Color-Doppler examination showed no signs of hypervascularization of the herniated tissue or signs of fat necrosis. Diagnosis of hernia of Hoffa's fat pad was made and no treatment was necessary. The patient returned for observation one year later and the mass was clinically and sonographically stable and unchanged.

DISCUSSION

The infrapatellar fat pad (IFP), also known as Hoffa's fat pad (HFP), is an intracapsular and extrasynovial structure located in the infrapatellar region and is made of fat lobules separated by thin fibrous cords (1). It is located in the anterior compartment of the knee and is limited anteriorly by the patellar tendon, the patellar retinacula and the joint capsule, superiorly by the inferior pole of the

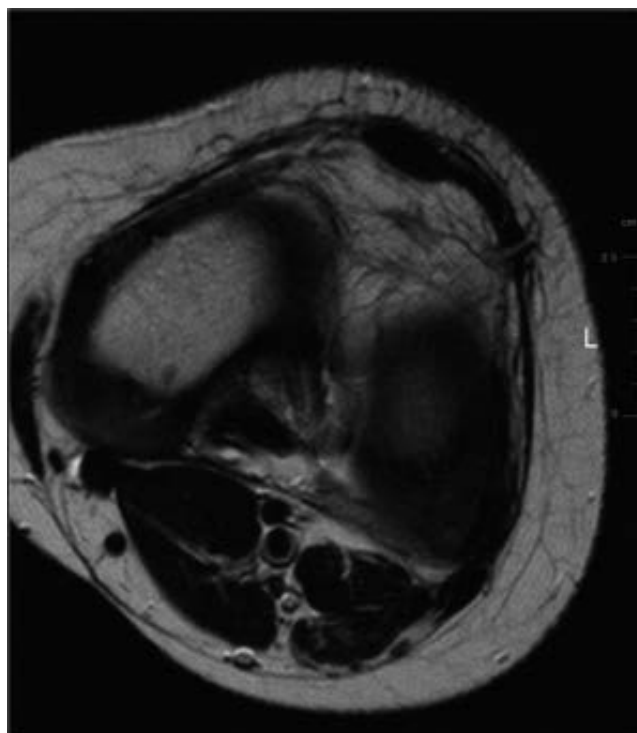


Fig.1. MRI - Axial T2w show the presence of a focal hyperintensity on axial T2w sequences at the anterolateral margin of the joint capsule.

patella, inferiorly by the proximal tibia and the deep infrapatellar bursa, posteriorly by the horns of the menisci, femoral condyles and joint synovium (3).

IFP is attached to the intercondylar notch of the femur by the infrapatellar synovial plica also known as the ligamentum mucosum that is the remnants of embryologic synovial membranes. It is the most common plica of the knee and sometimes it can become symptomatic. A further ligament that courses within the posterior aspect of the fat pad between the anterior horns of both menisci is the transverse (geniculate) ligament (4).

The IFP is a flexible and displaced structure; the main functions are: to increase the synovial area; to facilitate the distribution of synovial fluid; reduce friction between the patella, patellar tendon and deep skeletal (5). Moreover, it prevents pinching of the synovial membrane and it facilitates vascularization of adjacent structures (5).

Recent studies have shown that IFP is a metabolically active producer of cytokines, cellular growth factors and adipokines; it is also a stem cell reservoir and an ideal source of mesenchyme-lineage stem cells for tissue-engineering strategies (6).

Vascularity of IFP is supplied by the superior and inferior genicular arteries from which two vertical vascular branches located at the posterolateral margins

of the patellar tendon branch out. These vertical arteries are interconnected by two horizontal arteries that are positioned superior and inferior to the IFP (7). Additional anastomotic arteries may be present at the level of the tibial plateau and within infrapatellar synovial fold, connecting the medial genicular artery to the middle or superior horizontal artery (7). While the peripheral area of IFP is well supplied, there is a paucity of vascularity in the central one (7). The IFP is richly innervated and is therefore a source of anterior knee pain; it receives branches of the femoral, common peroneal and saphenous nerves (1).

The IFP injuries are divided into intrinsic and extrinsic. Intrinsic lesions such as Hoffa's disease, anterolateral impingement, plica syndrome, post-arthroscopic changes, trauma, patellar dislocation and extrasynovial tumors are less frequent. Differently, extrinsic lesions are more frequent and may affect the synovium, patellar ligament, vascular structures, and bursae. Muroid and parameniscal cysts may also develop in the infrapatellar space (2). Recent studies have classified IFP disorders on a pathogenesis-based classification:

- Traumatic disorders: (traumatic lesions involving the anterior knee, impingement syndrome, patellar dislocations);
- Post-traumatic disorders: (post-traumatic or post-

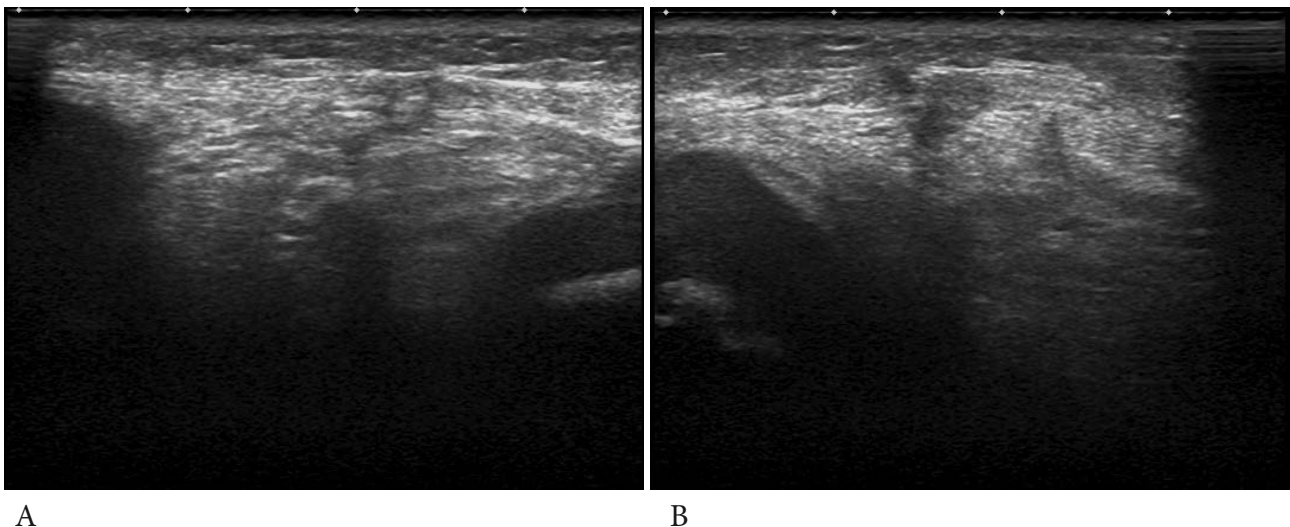


Fig. 2. US long-axis view. Extension (a) and flexion (b) show partial herniation of Hoffa's fat pad through the capsular defect during flexion movement of the knee.

arthroscopic fibrosis, Cyclops lesion and post-surgical fibrosis);

- Lesions secondary to adjacent disorders: (meniscal and ligamentous injuries, trauma of the patellar tendon, articular disorders, meniscal cysts and synovial abnormalities), masses and pseudo-masses (ganglion cysts, chondroma, synovial hemangioma).

Masses or pseudo-masses abnormalities rarely occur within the IFP. Patients with oedema and abnormalities of the IFP are often symptomatic, even if sometimes asymptomatic (1). The reason why some subjects do not feel pain is still unknown, even though they present MRI findings that are similar to those in patients with local pain (6).

Imaging

US imaging is a safe and effective evaluation of superficial soft-tissue abnormalities and is typically the initial imaging modality requested (8). Moreover, US compared with MRI enables dynamic and comparative assessment of the clinical structures (8). The optimal acoustic window of the IFP is with the knee joint fully extended or at 30° flexion (8). It is possible to identify two discernable well-defined tissue layers within the IFP: the superficial layer, which generally resembles the adjacent extra-articular subcutaneous fat, and the homogeneously hypoechoic deeper layer, distinguishable by the absence of adipose tissue septae (6).

Additionally, compression sonoelastography showed greater elasticity in the superficial HFP layer than in the deeper zone (6). It can reliably distinguish between cystic and solid masses and with the use of color or Power Doppler, determine the amount of vascularity within the mass. Dynamic US imaging can be performed to assess abnormalities that are present only during provocative maneuvers (9). Contralateral imaging can also be performed to evaluate symmetry. Information obtained by US can be used to dictate further imaging or can be used to guide biopsies or surgical excisions (9).

MRI

MRI is the diagnostic modality of choice for lesions when US has not been diagnostic (10). MRI protocol generally includes sagittal proton density

(PD)-weighted, three-orthogonal-plane sequences proton density (PD)-weighted with fat saturation, axial T2w.

At MRI, the IFP appears predominantly hyperintense on T1w and T2w sequences and structurally similar to subcutaneous fat. The internal fibrous septa are hypointense on T1w sequence and hypointense on fat saturation sequences (10).

The infrapatellar plica is well imaged at MRI as a low-signal-intensity structure of variable size and thickness on T1w images. It can be followed from its femoral origin (in the anterior part of the intercondylar notch) since its distal attachment into the inferior pole of the patella (11).

Hoffa's fat pad hernia is a new entity, described in literature only previously from Chauvin et al. Our case describes a Hoffa's fat pad herniation through the joint capsule of a young child without history of knee injuries that has never been described before in the literature. In our case, the painless herniation, caused by a defect of the joint capsule, was detected exclusively with dynamic ultrasound examination. MRI did not show the hernial mass but only the defect of the joint capsule on the lateral margin. Although the etiology remains still understood, it seems to be related to a capsular defect.

It is possible that IFP herniation may be linked to the presence of a locus minoris resistentiae in the anterolateral margin of the joint capsule at the point of passage of vessels from intra to extra-articular, easily detectable at MRI.

CONCLUSION

We described an interesting case of IFP herniation through a capsular defect without any traumatic event.

Radiologist and orthopedic surgeons must remember that the IFP should be always investigated for pathologic changes not only with an MR exams but specially with a US evaluation. US allows the detection of several pathology, which remains underdiagnosed without a dynamic evaluation.

REFERENCES

1. Draghi F, Ferrozzi G, Urciuoli L, Bortolotto C,

- Bianchi S. Hoffa's fat pad abnormalities, knee pain and magnetic resonance imaging in daily practice. *Insights Imaging Internet* 2016; 7(3):373-83.
2. Maurel B, Le Corroller T, Cohen M, et al. [Infrapatellar fat pad: anterior crossroads of the knee]. *J Radiol* 2010; 91(9 Pt 1):841-55.
 3. Larbi A, Cyteval C, Hamoui M, Dallaudiere B, Zarqane H, Viala P, et al. Hoffa's disease: A report on 5 cases. *Diagn Interv Imaging Internet* 2014; 95(11):1079-84.
 4. LaPrade RF. The anatomy of the deep infrapatellar bursa of the knee. *Am J Sports Med* 1998; 26(1):129-32.
 5. Park JH, Park JH, Lee AH, Lee DH. An unusual presentation of Hoffa's disease in an elderly patient with no trauma history: A case report. *Acta Orthop Traumatol Turc.* 2011; 45(3):195-9.
 6. Vera-Pérez E, Sánchez-Bringas G, Ventura-Ríos L, Hernández-Díaz C, Cortés S, Gutiérrez M, et al. Sonographic characterization of Hoffa's fat pad. A pilot study. *Rheumatol Int* 2017; 37(5):757-64.
 7. Kohn D, Deiler S, Rudert M. Arterial blood supply of the infrapatellar fat pad - Anatomy and clinical consequences. *Arch Orthop Trauma Surg.* 1995; 114(2):72-5.
 8. Zappia M, Oliva F, Chianca V, Di Pietto F, Maffulli N. Sonographic Evaluation of the Anterolateral Ligament of the Knee: A Cadaveric Study. *J Knee Surg* 2018; 7-10.
 9. Chauvin NA, Khwaja A, Epelman M, Callahan MJ. Imaging findings of Hoffa's fat pad herniation. *Pediatr Radiol* 2016; 46(4):508-12.
 10. Saddik D, McNally EG, Richardson M. MRI of Hoffa's fat pad. *Skeletal Radiol* 2004; 33(8):433-44.
 11. García-Valtuille R, Abascal F, Cerezal L, García-Valtuille A, Pereda T, Canga A, et al. Anatomy and MR Imaging Appearances of Synovial Plicae of the Knee. *RadioGraphics* 2002; 22(4):775-84.

MUSCULOSKELETAL HYDATID DISEASE: REPORT OF A RARE CASE WITH PRIMARY AND EXCLUSIVE LOCALIZATION IN RIGHT HEMI-PELVIS AND FEMUR

P. LOMORO¹, I. SIMONETTI², A. NANNI³, R. CASSONE⁴, G. VINCI⁵, V. FICHERA⁶, E. JANNELLI⁷ and L. SAMMARCHI⁸

¹Department of Diagnostic Medicine Institute of Radiology, IRCCS San Matteo University Hospital Foundation, Pavia, Italy; ²Department of Advanced Biomedical Sciences, University of Naples Federico II, Naples, Italy; ³Department of Diagnostic Medicine Institute of Radiology, IRCCS San Matteo University Hospital Foundation, Pavia, Italy; ⁴Section of Radiological Sciences, Department of Biomedical and Dental Sciences and of Morphofunctional Imaging, University of Messina, Messina, Italy; Nuclear Medicine Unit, University Hospital "G. Martino", Messina, Italy. ⁵Department of Nephrology, University of Campania "Luigi Vanvitelli", Naples, Italy; ⁶Department of Pediatrics, Anna Meyer Children's University Hospital, Florence, Italy; ⁷Clinica Ortopedica e Traumatologica - IRCCS Policlinico San Matteo, Università degli Studi di Pavia, Pavia, Italy; ⁸Department of Diagnostic and Interventional Radiology and Neuroradiology of IRCCS San Matteo University Hospital Foundation, Pavia, Italy

Hydatid Disease (HD), also called *Echinococcosis* or *Hydatidosis*, is a parasitic infection caused by the larval stage of the tapeworm *Echinococcus*: *E. granulosus* or *E. multilocularis*. HD occur most frequently in liver or lungs, rarely in brain, skeletal muscles, bones, kidneys, spleen. Bone infestation of *Echinococcosis hydatid* cysts occurs respectively by haematogenous seeding and progressive invasion into bone by lesions in the adjacent soft tissues. Patients with musculoskeletal HD clinically show the disease in adulthood because the lesions develop very slowly. In some cases, HD is an uncommon cause of soft tissue mass, pain and neurovascular symptoms due to compression or to secondary infection. Diagnostic imaging plays an important role in the diagnosis of HD and in the differential diagnosis with soft tissue tumors. We present a rare case of male patient of 42 year-old with diagnosis of HD with primary and exclusive localization in right hemi-pelvis and femur.

Hydatid Disease (HD), also called *Echinococcosis* or *Hydatidosis*, is a parasitic infection caused by the larval stage of the tapeworm *Echinococcus*: *E. granulosus* (cystic hydatid disease) or *E. multilocularis* (alveolar hydatid disease) (1). High prevalence of *E. granulosus* is reported in South America, Mediterranean countries, Russian Federation, Middle East, Australia, Central Asia, Northern and Eastern regions of Africa, while *E. multilocularis* is limited to some areas of Central

Europe, Canada and Alaska (2). A few cases of infections due to *E. oligarthrus* or *E. vogeli* (polycystic HD) have also been described in South America (3). However, in non-endemic areas, some cases of infections can occur as a result of the emigration of infected persons and the diagnosis is often delayed because of a low prevalence and consequently a low clinical suspicion for the disease (3).

HD is a zoonosis. The adult tapeworm lives in

Key words: Echinococcus granulosus, Echinococcus multilocularis, Echinococcosis, Hydatid disease, bone cysts, computer tomography, magnetic resonance imaging

Corresponding author:

Igino Simonetti MD,
Department of Advanced Biomedical Sciences,
University of Naples Federico II,
Via Sergio Pansini 5, 80131 Napoli, Italy
e-mail: igino.simonetti@gmail.com

the small bowel of the definitive hosts (dogs, foxes and other carnivores) and delivers the eggs that are excreted in the environment with the stool, where they are ingested by an intermediate hosts (sheep, cattle, pigs, rodents and humans) (2).

The embryos that are liberated from the eggs in the bowel of the intermediate hosts penetrate the intestinal wall entering the portal blood stream and are carried to the liver (68-75%) or to the lungs (15-25%) (4); within these organs they develop into cysts, which can be single or, in some cases, multiples (5). Infrequently, HD occurs in the brain, the skeletal muscles, the bones, the kidneys, and the spleen or in other tissues (5).

Case report

In 2003, in another hospital, a 42-year-old male patient, from Morocco was diagnosed with an osteo-muscular *Echinococcosis* in his right-thigh, presented as a spontaneous femoral fracture. He had been treated with surgical correction and antibiotic

therapy giving him a temporary benefit until 2007, when he had a worsening of the HD, which, among other symptoms, included the development of cutaneous fistulas sovrainfected by *S. aureus* and *S. epidermidis*, causing two episodes of osteomyelitis. He then underwent multiple percutaneous drainage and antiparasitic therapies. In February 2016, a curettage of the anterior and posterior compartment of the thigh was performed. Others locations of the disease were excluded with imaging techniques during the follow-up.

In April 2018, he arrived at our hospital complaining of onset of fever, important worsening of edema and functional impotence in his right thigh. After the admission, laboratory and imaging examination were performed. Laboratory tests were normal except for microcytic anemia and for the presence of antibodies against *E. granulosus*. A fistula swab culture was performed and resulted positive for Methicillin-Sensitive *S. aureus*, consequently an intravenous antibiotic therapy with

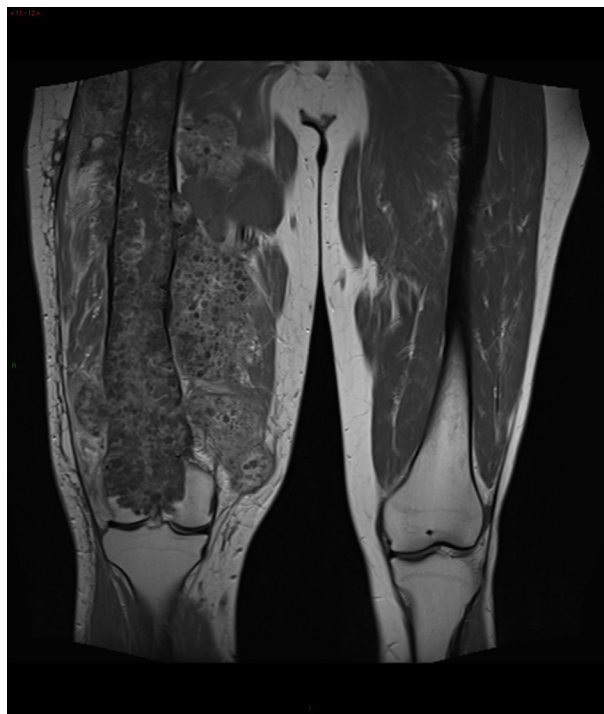


Fig. 1. Coronal TSE T1W images show the presence of multiple cystic formations involving the right femur and the muscles of the anterior and posterior compartment of the thigh. The right femur is deformed, swollen with a cortical thinning. The right thigh shows an increased size compared to the contralateral with signs of fat infiltration of the muscles. The knee and the hip are spared from lesions.

cefazolin and rifampicin was started.

Chest X-Ray and abdominal ultrasonography (US) showed no abnormalities. Magnetic resonance imaging (MRI) of the hip, right thigh and knee with a contrast agent revealed a pathological tissue consisting of multiple polylobulated formations of variable sizes; mother cysts that started from a few millimeters to some centimeters; daughter cysts, with a multi-micro cystic internal structure at the level of the femur, from the trochanteric region to the distal epiphyseal and in the soft tissue surrounding the femur. The pathological tissue replaced most of the extensor and adductor compartment of the musculature, except a part of the flexor compartment; the femoral bone component showed diffused thinning of the cortical bone with some cortical interruptions and segmental swelling (Fig.1, 2). The diagnosis of recurrence of the HD was made based on the clinical manifestations, laboratory findings and radiological imaging. The orthopedist decided for a conservative management with medical (pharmacological o Antibiotic therapy) treatment and a strict follow-up, the only curative option was the amputation of the right thigh with reconstruction of a part of the hip.

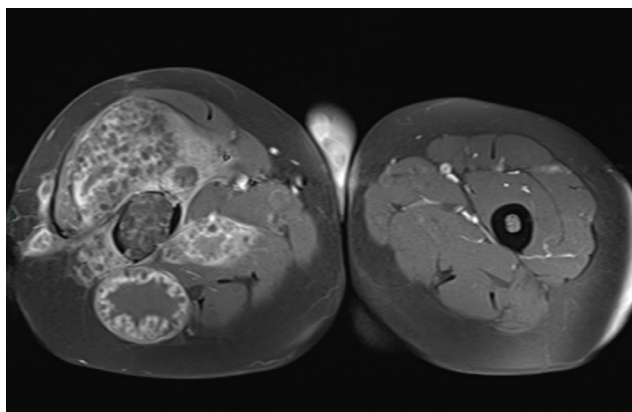
Many patients with musculoskeletal hydatid

disease clinically show the disease in adulthood because the lesions develop very slowly. Males and females are equally affected. Bone infestation of *E. granulosus* and *E. multilocularis* cysts occurs respectively by haematogenous seeding and progressive invasion into bone by lesions in the adjacent soft tissues (6).

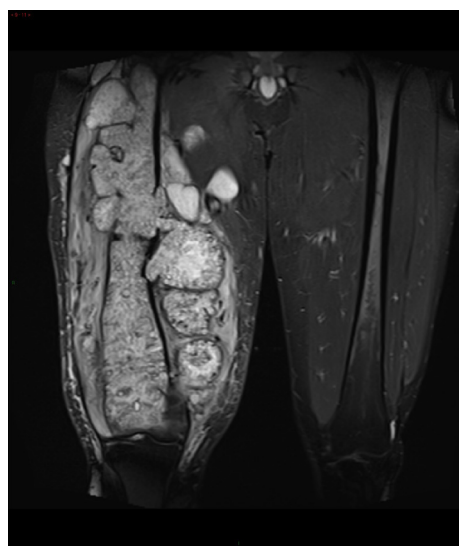
Clinically, musculoskeletal HD is asymptomatic; in some cases, it is an uncommon cause of soft tissue mass, pain and neurovascular symptoms due to compression or to secondary infection (7).

Immunodiagnosis, through the detection of circulating *E. granulosus* antibodies, is useful in primary diagnosis and patient's follow-up after the treatment (8). Imaging appearance of hydatid cysts (HCs) depends on the stage of the cyst growth (5).

On US, the presence of multiple vesicles (daughter cysts) in the mother cyst (cysts inside a cyst) is characteristic of the HD; unilocular or complex cyst formation or solid lesion mimicking a tumor may also be found. The rim surrounding the cyst is a characteristic sign of HD of the liver and the lungs. It is composed of parasitic membranes (endocyst and ectocyst) that manifests as hypoechoic layer, and of a vascularized membrane (pericyst), that is formed as a response to the host and it manifests



A



B

Fig. 2. Axial (a) and Coronal (b) STIR images show “cyst within a cyst sign”, i.e. multiple multilocular cystic formations characterized by daughter cysts (hypointense) within the mother cysts (hyperintense). There is no evidence of simple cysts.

as echoic line (9). In the complex cysts, multiple echogenic foci due to hydatid sand may be seen (hydatid sand sign). Wall calcification, which is a sign of the parasite's inactivity, usually manifests as a hyperechoic contour with a cone-shaped acoustic shadow (10). Calcifications may be encountered at radiography, both as an incidental finding or on scans performed to evaluate musculoskeletal pain (11).

Computer Tomography (CT) may show a closed sac containing fluid (3-30 HU) that can have some rim calcifications. In complex cysts, generally the mother cyst's fluid has a high attenuation compared to the daughter vesicles (11).

Initial findings on US can be confirmed with MRI that shows unilocular hydatid cyst as a heterogeneous low-to-intermediate-signal-intensity on T1- and high-signal-intensity on T2-weighted images. In multilocular cysts, the daughter vesicles are hypointense on T1-w and hypo- or hyperintense on T2-w images when compared to the mother cyst (12). Endocysts and ectocysts membranes are hypointense on T2-w images, while pericysts membranes is hyperintense on T2-w images. After intravenous administration of gadolinium, contrast enhancement is seen at the vascularized pericyst (12). Two characteristic signs of cyst complications are the "water-lily sign" and "fluid-fluid level sign" (13). The first may be observed when there is a separation of the endocyst from the pericyst, which produces a floating membrane appearance (12).

Imaging of soft tissue and bone HD

High levels of lactate make the muscle an unfavorable site for hydatid cyst infestation (prevalence of primary presentation 0.5-4%) (14). Paravertebral, pelvic, gluteus and the lower extremity muscles are the most frequent location of muscular involvement. The definitive diagnosis is generally confirmed by histopathological examination after surgery (13).

Bone involvement is present in approximately the 0.8-4% of patients affected by H, and the more frequently involved bone sites are the spine (35-50%) and the hip (21%), followed by the femur (16%) and the tibia (10%). In the spine, the thoracic vertebrae are most often affected (50%), followed, with decreasing frequency, by the lumbar (20%),

sacral (20%) and cervical (10%) vertebrae (5).

The diagnosis of bone HD is made by appearance of radiological characteristics. The radiographic changes show erosion, destruction and deformity of the bone; periosteal reaction, sclerosis and intracystic calcification are uncommon. The differential diagnosis includes nonspecific or specific osteomyelitis (tuberculosis or actinomycosis), aneurysmal bone cyst, giant cell tumor, myeloma, atypical osteomyelitis, cystic metastases, and fibrous dysplasia (14).

In the presence of vertebral involvement, typical elements that can help to differentiate HD from tuberculous spondylitis are the lack of bone sclerosis and disc damage, the involvement of the ribs, and the extension into spinal canal and spinal cord (5).

CT is the best-suited modality for a clear demarcation of the lesion and for evaluating the osseous involvement. CT appearance of bone lesions is similar to those demonstrated on radiography (5).

MRI is the preferred imaging modality for the evaluation and characterization of the extension of the extraosseous diffusion and within the soft tissues of HD (14).

Treatment

Bone HD is commonly managed with surgical treatment. For the large and extensive lesions, curettage and/or aspiration of cysts are performed and chemotherapy (albendazole) is administered to prevent recurrences (15). Complete excision, or even amputation, is needed in some cases. The fluid of *E. granulosus* cysts is extremely toxic for the host; therefore incisional biopsy and marginal excision are contraindicated (14).

CONCLUSION

Bone *Echinococcosis* is a rare (less than 4% of cases of HD) and difficult to treat parasitic infection. Cystic or lytic lesions are present in the trabecular bone and may extend to other subcortical areas. Spongy tissue constitutes a favorable ground for the spread of HCs. Over time, the parasites reach and destroy the cortex, with subsequent expansion of the disease to the surrounding tissues. Radiological diagnosis of hydatid disease may be difficult, and

the symptoms are often nonspecific. The lesion may also have a multicystic appearance or mimic a soft tissue tumor because of its solid or complex pattern. Radiography is essential for investigating the bone involvement of hydatid disease and is also useful for the study of the soft tissue localizations. MRI is the method of choice for the evaluation of the disease's extension and for the detection of morphological changes in the lesion. The rim sign is an important and useful finding for the differential diagnosis. Musculoskeletal HD goes in differential diagnosis with several pathologies. In the presence of a skeletal pathology without a conclusive diagnosis, it is important to keep a high index of suspicion for hydatid disease to ensure an early diagnosis.

REFERENCES

1. Nunnari G, Pinzone MR, Gruttadauria S, et al. Hepatic Echinococcosis: Clinical and therapeutic aspects. *World J Gastroenterol* 2012; 18(13):1448-58.
2. Eckert J, Deplazes P. Biological, Epidemiological, and Clinical Aspects of Echinococcosis, a Zoonosis of Increasing Concern. *Clin Microbiol Rev* 2004; 17(1):107-35.
3. D'Alessandro A. Polycystic Echinococcosis in tropical America: *Echinococcus vogeli* and *E. oligarthrus*. *Acta Trop* 1997; 67(1-2):43-65.
4. Çalık S, Çalık M, Yeşildağ M, Esme H. Intramuscular hydatid cyst: report of an unusual case. *J Emerg Med Case Rep* 2016; 7:61-3.
5. Polat P, Kantarci M, Alper F, Suma S, Koruyucu MB, Okur A. Hydatid Disease from Head to Toe. *RadioGraphics* 2003; 23(2):475-94.
6. Diedrich O, Kraft CN, Zhou H, Sommer T, Perlick L, Schmitt O. Orthopedic aspects of osseous Echinococcosis--radiologic diagnosis, current surgery and drug therapy aspects. *Z Orthop Ihre Grenzgeb* 2001; 139(3):261-6.
7. Morris BS, Madiwale CV, Garg A, Chavhan GB. Hydatid disease of bone: A mimic of other skeletal pathologies. *Australas Radiol* 2002; 46(4):431-4.
8. Carmena D, Benito A, Eraso E. Recent advances in the immunodiagnosis of human cystic Echinococcosis. *Enferm Infecc Microbiol Clin* 2007; 25(4):263-9.
9. Adel F, Ramia JM, Gijón L, de la Plaza-Llamas R, Arteaga-Peralta V, Ramiro-Perez C. Hidatidosis extrahepática y extrapulmonar. *Cir Cir* 2017; 85(2):121-6.
10. Marrone G, Crino F, Caruso S, et al. Multidisciplinary imaging of liver Hydatidosis. *World J Gastroenterol* 2012; 18(13):1438-47.
11. Pedrosa I, Saíz A, Arrazola J, Ferreirós J, Pedrosa CS. Hydatid Disease: Radiologic and Pathologic Features and Complications. *Radiographics* 2000; 20(3):795-817.
12. Papakonstantinou O, Athanassopoulou A, Passomenos D, et al. Recurrent vertebral hydatid disease: Spectrum of MR imaging features. *Singapore Med J* 2011; 52(6):440-5.
13. Comert RB, Aydingoz U, Ucaner A, Arıkan M. Water-lily sign on MR imaging of primary intramuscular Hydatidosis of sartorius muscle. *Skeletal Radiol* 2003; 32(7):420-3.
14. Arkun R, Mete BD. Musculoskeletal hydatid disease. *Semin Musculoskelet Radiol* 2011; 15(5):527-40.
15. Loudiye H, Aktaou S, Hassikou H, et al. Hydatid disease of bone: Review of 11 cases. *Joint Bone Spine* 2003; 70(5):352-5.

COMPARISON OF EARLY FUNCTIONAL OUTCOMES BETWEEN TWO DIFFERENT SURGICAL APPROACHES FOR TOTAL HIP ARTHROPLASTY

P. SIRTORI¹, G.M. PERETTI^{1,2}, M. ULIVI¹, R. CILIBERTO³, M.D. LOMBARDO³,
F. VERDONI¹, G. MICHIELON² and L. MANGIAVINI¹

¹IRCCS Istituto Ortopedico Galeazzi, Milan, Italy; ²Department of Biomedical Sciences for Health, University of Milan, Milan, Italy; ³Residency Program in Orthopaedics and Traumatology, University of Milan, Milan, Italy

Total Hip Arthroplasty (THA) is considered the most successful treatment for advanced hip osteoarthritis. Different surgical approaches for THA are available and they have shown excellent outcomes in the long-term follow-ups. However, few studies have analyzed the functional outcomes in the first days after a THA surgery. The purpose of this study was to compare the early functional outcomes between two different surgical techniques: a minimally invasive direct anterior approach (mini-DAA) and a postero-lateral approach (PL). Twelve patients for each group were analyzed. Pre- and postoperative (3, 10, 30 and 90 days after surgery) Patient-Reported Outcome Measures (PROMs) were administered: HOOS, HHS, VAS and SF-12-v2 scores. Moreover, comparison between surgical operation time and blood loss were examined. PROMs showed a significant improvement in the SF-12-v2 in the mini-DAA group compared to the PL group at 3 days after surgery: this difference was maintained also after 10 and 30 days. In addition, HOOS and HHS were significantly ameliorated in the mini-DAA group starting 10 days from surgery. In both groups, a physiological pain reduction was observed in the first days after surgery; comparing it to the pre-surgical VAS values, we found a significant improvement in the scores for the mini-DAA group after 30 days. Moreover, we demonstrated a significant reduction in blood loss for the mini-DAA group. Surgical operation times were similar in the two groups; however, the duration of the mini-DAA procedure was shorter compared with the known literature. In this preliminary study, we demonstrated that the minimally invasive direct anterior approach for THA may lead to benefits in the early postoperative time, as it allows for an improvement in functional outcomes, a reduction of postoperative pain, a reduction of hospitalization time and consequent reduction of postoperative complications; therefore, this surgical approach may consent an early return to work and daily activities.

Total hip arthroplasty (THA) is considered the most successful treatment for end-stage hip osteoarthritis (1, 2). Nowadays, several surgical approaches are used for THA with excellent functional outcomes (3). The indications for THA have been expanded to include progressively younger patients, and more than half of all primary THAs are expected to be under the age of 65 in the very near future.

Less pain and improved early function, faster return to normal activities of daily living, and fewer restrictions on movement are helpful goals for patients undergoing treatment and should also provide economic benefit since resource utilization will be decreased. The increased activity level of younger patients, combined with an increased life expectancy, has in turn amplified the need for greater implant longevity.

Key words: hip, arthroplasty, early recovery, functional outcomes

Corresponding author:

Laura Mangiavini,
IRCCS Istituto Ortopedico Galeazzi,
Via R. Galeazzi 4, 20161 Milan, Italy
Tel.: +39 0266214930
e-mail: laura.mangiavini@grupposandonato.it

In recent years, the direct anterior approach (DAA) has gained popularity among other approaches because it represents a tissue sparing surgery, which does not damage muscles and therefore it allows for a faster recovery (4-6).

Indeed, DAA uses an intermuscular access between the sartorius muscle and tensor of the fascia lata and allow a good exposition of the hip joint that prevents trauma to soft tissues. In addition, this surgical approach provides benefits in early reported pain and function, postoperative length of stay, less dislocations, and postoperative analgesia (7-11). Complications as dislocations and Trendelenburg gait are more frequent in the posterolateral approach (PL) because of the violation of the external rotator muscles, abductor muscles and posterior capsule of the hip joint. The DAA has become popular because of the low risk of dislocation. The integrity of the posterior capsule and the surrounding muscular structures, are the factors which reduces the risk of dislocation. In current literature, dislocation after DAA was found in 0.0-1.5% (12-15).

These features allow an early rehabilitation and better functional outcomes (16-19). Thus, the main objective of this study was to compare the functional outcomes in the early post-surgical period between mini invasive anterior approach (mini-DAA) and posterolateral approach for total hip arthroplasty.

MATERIALS AND METHODS

Patients

Twenty-four patients were selected and divided in two groups for this study. Twelve patients were enrolled for THA through a mini-DAA approach and the other 12 patients underwent THA through a posterolateral approach. All patients undergoing THA at our hospital "Istituto Ortopedico Galeazzi" had been diagnosed with hip osteoarthritis, avascular femoral head necrosis (FICAT III-IV) or developmental hip dysplasia (Crowe I and II). All patients undergoing conversion THA, revision THA, developmental hip dysplasia Crowe III-IV or another major anatomical alterations were excluded from this study. All surgeries were performed by two experienced surgeons. One surgeon with an extensive experience in PL approach and other in mini-DAA approach. In the mini-

DAA group there were 6 men (50%) and 6 women (50%) and in the PL group there were 5 men (60%) and 7 women (60%).

All the patients were well informed about the surgical procedure and risks, technique and postoperative rehabilitation protocols. Informed consent of the procedure was signed before surgery.

Surgical Procedure

All surgeries were performed under subarachnoidal or general anesthesia. In the operative phase, all patients received an antibiotic prophylaxis with cefazolin IV, dosed according to the body weight of each patient.

The mini-DAA approach was performed in supine position using a special leg positioning surgical table for mini invasive direct anterior approach (MEDACTA). A skin incision was performed 2 cm distal and lateral from the anterosuperior iliac spine. The mini-DAA approach involved exposure of tensor fascia lata. Through the interval between sartorius and tensor fascia lata, the anterior rectum was exposed and retracted. After the exposure of the hip capsule, C-shaped capsulotomy was performed. Conversely, PL approach was performed in lateral decubitus position: after the skin incision, extrarotator muscles were sectioned to reach the hip capsule, which was opened.

A cementless stem and an acetabular cup were implanted in every patient. All surgical parameters, including estimated blood loss, operative time, intraoperative complications and any other pertinent information, were recorded in detail.

Postoperative Management

Adequate deep vein thrombosis-Pulmonary embolism prophylaxis [Calcium Nadroparin 0.4-0.6 ml subcutaneously (Italfarmaco S.p.A) once a day for 30 days] was administered to all patients according to Italian guidelines. All patients received an adequate postoperative analgesia. The standard protocol included acetaminophen 1000 mg three times daily associated to celecoxib 200 mg once daily, as a first step. Ketorolac 300 mg IV and morphine were reserved for patients with moderate to severe pain according to the visual analog scale.

After the surgical procedure, all patients were transferred to the ward. Early rehabilitation was initiated 4 h after surgery or as soon as the motor block due to

spinal anesthesia was solved. The physical therapy on day zero included placing the patient in sitting and standing position, then, if possible, the patients were encouraged to begin ambulation with the help of crutches. Thereafter, physical therapy occurred twice daily until discharge from the hospital.

Functional Outcome Measurement

Pre- and post-operative completed Patient-Reported Outcome Measures (PROMs) were analyzed for each approach. With the Visual Analogue Scale (VAS) patients were asked to mark the average pain they felt in the involved hip during the previous week. The score ranges from '0' (no pain at all) to '10' (unbearable pain). The 'Hip disability and Osteoarthritis Outcome Score' (HOOS) was used to examine the opinion of the patients about their THA. The score is expressed in a scale ranging from 0 to 100 whereby a low score indicates symptoms. The Harris Hip Score (HHS) was used to determine the grade of disability due to hip disease. The quality of life was assessed with the SF-12v2 score. This score assesses 2 components; the Physical Component Summary (PCS) and the Mental Component Summary (MCS). All tests were applied on the preoperative phase at day 3, 10, 30 and 90.

Statistical analysis

Statistical analysis of categorical data was performed

using the *Chi*-square test or Fisher's exact test, where appropriate. Comparison between mini-DAA and PL groups for continuous data was analyzed, using the non-parametric "rank-sum" test of with two tails Wilcoxon's test. In case of normal distribution (Shapiro-Wilk statistics), the two tails Student's *t*-test was applied. To compare groups at different times, Student's *t*-test for paired data was used or, in case of non-normal distributions, the corresponding non-parametric Wilcoxon's signed-rank test was applied. A covariance analysis was performed to calculate post-surgical adjusted averages for the main clinical scores and the corresponding adjusted differences between mini-DAA and PL groups. The analysis was adjusted *a priori* for variables such as age, sex and preoperative scores examined. For all two-tailed tests, p-values lower than 0.05 were considered significant. Statistical analysis was conducted using the SAS statistical package v.9.4 (SAS Institute-Cary, NC, USA).

RESULTS

Twenty-four patients (24 hips) were included. Patient characteristics are shown in Table I. The groups differed in terms of age distribution, gender and Body Mass Index (BMI). The study population counted slightly more men for the mini-DAA approach (50%) compared to the PL approach group (40%). The mean age between the DAA and PL

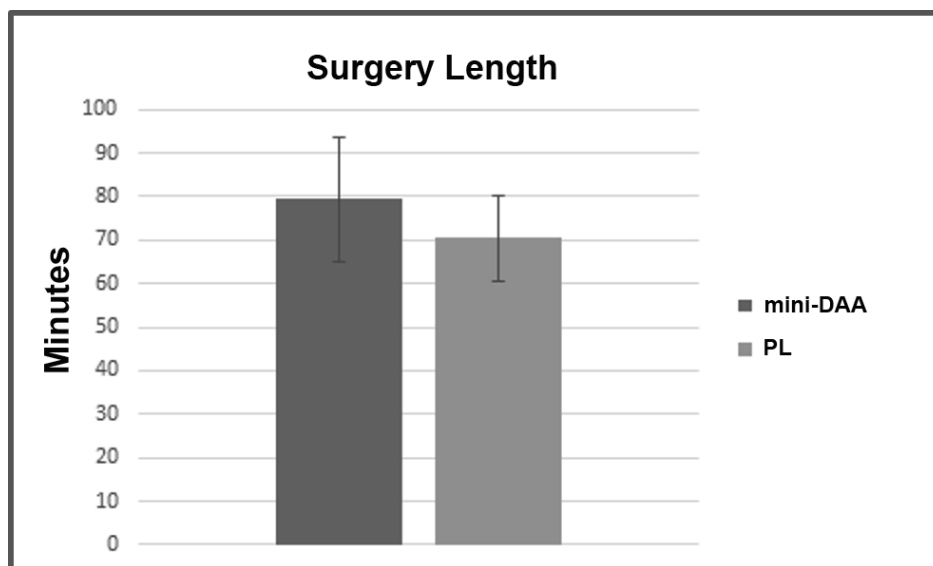


Fig. 1. Comparison of surgery timing. Surgery timing in mini-DAA and PL groups. No statistical difference was found.

group was comparable: 63.4 ± 6.4 years vs 72.2 ± 11.2 years. In the mini-DAA, the mean BMI was 26.2 ± 3.8 and 26.2 ± 3.5 for the PL group. Mean Preoperative hemoglobin was significantly different: for mini-DAA group the mean value was 11.6 ± 1.0 g/dL and 13.7 ± 0.8 g/dL for the PL group ($P < 0.001$). There were no significant differences between the mini-DAA and PL group in pre-surgery assessment of the HHS, HOOS, PCS, MCS and VAS.

Procedure time using the mini-DAA was slightly longer than the PL group, with a median duration of 79,3 minutes (min- max 56-88) for the DAA group and a mean duration of 70,4 minutes (min- max 56-88) for the PL group. Though, no significant difference was reached (Fig. 1).

The preoperative SF12 scores (both the MCS and PCS components) were comparable; however, we noticed a significant better outcome in the mini-DAA group starting from 3 days after surgery (Fig. 2). This difference was maintained until 30 days after surgery ($p\text{-value} < 0.001$) (Fig. 2).

The HOOS scores displayed a similar trend: the two groups were comparable before surgery and 3 days after surgery; whereas a significant higher score in the mini-DAA group was recorded 10 days and 30 days after surgery ($p\text{-value} < 0.001$) (Fig. 3a).

Also, the HHS score showed a significant improvement in the mini-DAA group starting 10 days after surgery. In this case, the difference between the two groups was maintained also at the

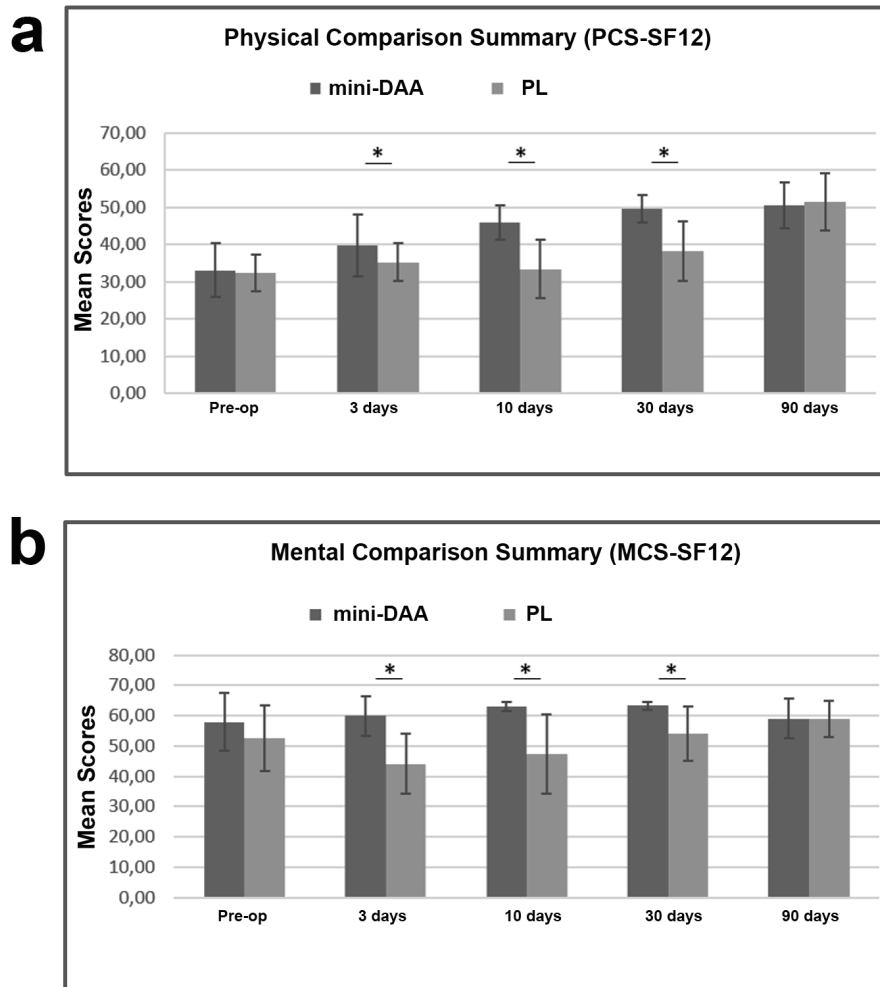


Fig. 2. Comparison of Quality of Life. SF12 PCS (a) and SF12 MCS (b) scores in mini-DAA and PL groups at different time points. Significant differences were found at 3, 10 and 30 days after surgery. $p\text{-value} < 0.001$.

longest time point (Fig. 3b).

When we analyzed the pain with the Visual Analogic Scale (VAS), we noticed a significant and physiological reduction in pain within both groups in the first days after surgery, though without a statistical significance. At 30 days from surgery, we found a significant difference between the two groups by applying a multivariate analysis, which adjusts the scores on the baseline values ($p < 0,008$) (Fig. 4a).

Finally, we studied differences in blood loss between the two groups: we found a significant decrease in hemoglobin after surgery within each group, as expected (Fig. 4b). We then compared the two groups and we performed again a multivariate

analysis: we demonstrated that blood loss in the mini-DAA groups was significantly reduced (Fig. 4b).

DISCUSSION

In this preliminary study we compared 12 patients undergoing THA through a mini invasive direct anterior approach (mini-DAA) with other 12 patients through a posterolateral approach (PL). Both surgical techniques have shown excellent outcomes in the follow-ups (typically 1 months, 3 months or later). However, few studies have analyzed the functional outcomes in the immediate post-surgical time frame. Thus, we decided to compare these outcomes 3, 10,

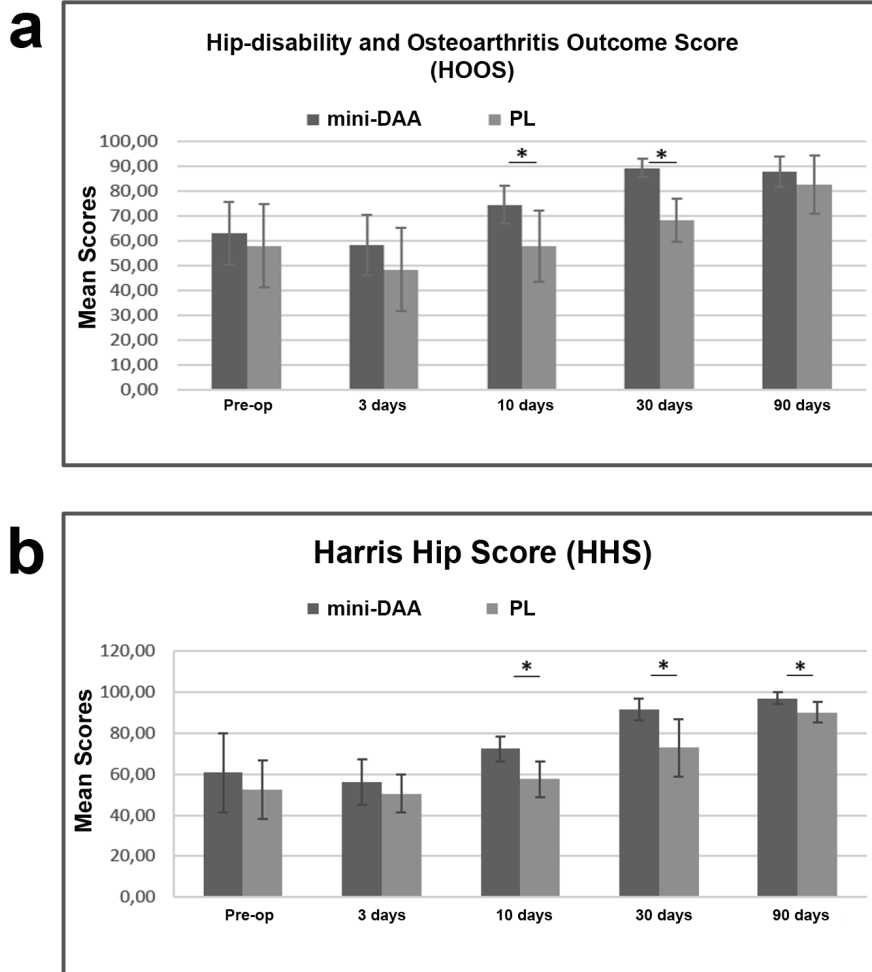


Fig. 3. Comparison of Functional Outcomes. HOOS (a) and HHS (b) scores in mini-DAA and PL groups at different time points. **HOOS scores:** significant differences were found at 10 and 30 days after surgery. p -value < 0.001 ; **HHS scores:** significant differences were found at 10, 30 and 90 days after surgery. p -value < 0.001 .

30 and 90 days after surgery.

Literature reported a longer surgical time for the DAA approach (17-19). We believe this could be due to the inherent technical difficulty to obtain an adequate exposure of the acetabulum and femoral neck. However, in our study we did not find significant differences in the duration of surgery, probably because both surgeons have an excellent expertise in performing this procedure. In addition, we did not record any intraoperative complication.

Theoretically, minimally invasive techniques are expected to reduce bleeding compared to standard approaches. This is primarily a result of sparing soft tissue and, in some cases, of the reduced invasion of the femoral canal. Indeed, in our study we demonstrated a significant reduction of perioperative blood loss in the mini-DAA group when compared with the PL group considering the differences in the

baseline values.

In our study, we observed reduction of VAS scores in both groups; however, a significant difference between the groups in favor of the mini-DAA approach was noted 30 days after surgery. A possible explanation is that mini-DAA is a soft tissue sparing technique, thus it allows for the access to the hip joint through an intermuscular plane, avoiding muscular trauma. Instead, the posterolateral approach requires the dissection and detachment of the short external rotators of the hip to reach the joint capsule, leading to increased postoperative pain.

Early functional outcomes also improved significantly in the mini-DAA group. In particular, the HOOS and HHS scores enhanced immediately after surgery. The tissue sparing and the consequent pain reduction, which allows for a faster recovery, could explain these results.

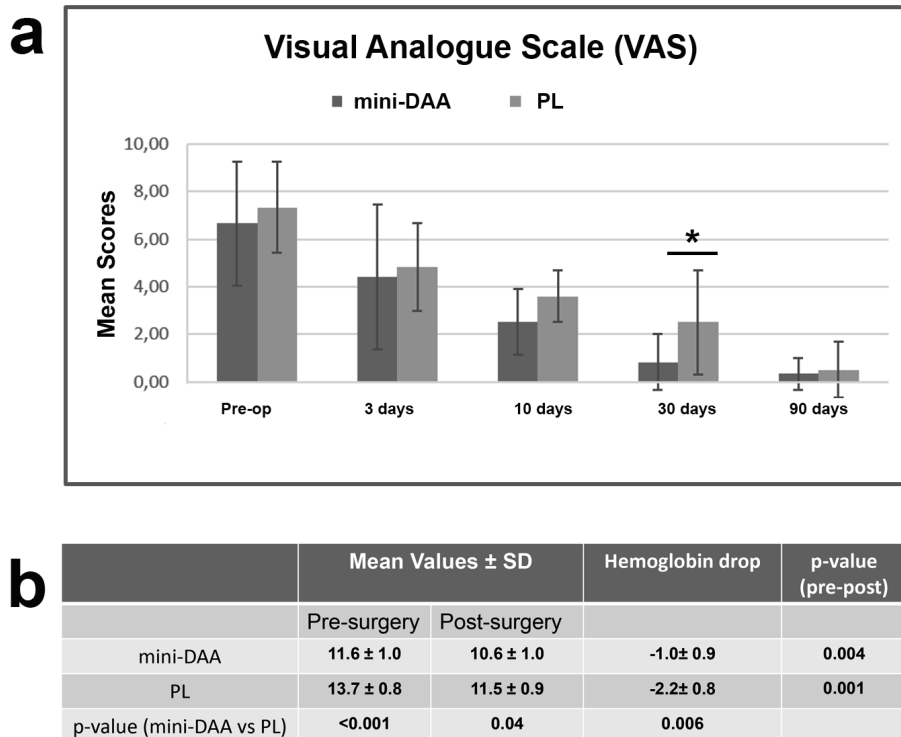


Fig. 4. Comparison of pain reduction and blood loss. **a**): VAS scores in mini-DAA and PL groups at different time points. A physiological pain reduction was noticed in both groups after surgery. A significant difference was found at 30 days after surgery. p -value <0.008; **b**): Blood loss measured by hemoglobin drop in mini-DAA and PL groups. A significant difference in hemoglobin values before and after surgery was found in each group. Comparing the two groups, a significant reduction in blood loss was measured in mini-DAA group. p -value <0.006.

Table I. *Patients' characteristics.*

	All patients (n=24)	Mini-DAA (n=12)	PL (n=12)	p-value
<i>Characeristics</i>	<i>n (%)</i>	<i>n (%)</i>	<i>n (%)</i>	
Age				0.03
<60	6 (25.0)	5 (41.7)	1 (8.3)	
60-69	8 (33.3)	5 (41.7)	3 (25.0)	
≥70	10 (41.7)	2 (16.7)	8 (66.7)	
Age Average ± SD	67.8 ± 10.0	63.4 ± 6.4	72.2 ± 11.2	0.03
Age Median (range)	68 (45- 85)	61 (55-75)	73 (45-85)	
Sex				0.68
Females	13 (54.2)	6 (50.0)	7 (58.3)	
Males	11 (45.8)	6 (50.0)	5 (41.7)	
Body Mass Index (BMI)				0.15
<25 kg/m ²	10 (41.7)	5 (41.7)	5 (41.7)	
25-<30 kg/m ²	11 (45.8)	4 (33.3)	7 (58.3)	
≥30 kg/m ²	3 (12.5)	3 (25.0)	0 (0.0)	
BMI Average± SD	26.2 ± 3.6	26.2 ± 3.8	26.2 ± 3.5	0.99
BMI Median (range)	26.4 (19.6- 32.9)	25.9 (19.9- 32.9)	27.3 (19.6- 29.8)	

Age, Sex and Body Mass Index (BMI) distribution of the analyzed patients.

Another important finding is the significant improvement in the quality of life. Indeed, SF-12 scores were significantly enhanced in the mini-DAA group even at 3 days after surgery. Thus, this mini-invasive surgical approach has a significant less impact in the quality of life, probably because it allows an immediate mobilization and therefore a faster recovery to daily activities (i.e. walking without crutches).

One of the limits of this study is that PL patients were transferred to rehabilitation centers within 7 days after discharge, whereas mini-DAA patients were discharged at home without any need of specific rehabilitation. This difference may have altered the results; in particular, it may have caused an overestimation of the outcomes in the PL group.

Another possible bias is the difference in patients' age: the mean age for the mini-DAA group was 63 years old, while it was 72 for the PL group. Thus, PL

patients were in general older and this disparity may affect the baseline data and the functional outcomes; though this bias was considered by applying a multivariate analysis.

In conclusion, our results confirm the reported literature on the medium and long-term follow-ups after THA (17-19). Indeed, both surgical approaches lead to excellent results within 1 to 3 months after surgery. On the other hands, we demonstrated important differences in the outcomes measured in the immediate postoperative period. All the scores resulted significantly improved in the mini-DAA group starting from 3 days after surgery.

This finding is crucial as it suggests that the mini-DAA approach allows for a faster mental and physical recovery. Nowadays patient's expectations and daily activities continuously intensify and the age population progressively increases: thus, people more frequently ask for a rapid rehabilitation after surgery.

Moreover, hospitalization time and costs may decrease if the mini-DAA approach is used; this will lead to a reduction in post-surgical complications (i.e. perioperative infections) and to a drop in health costs.

More data are necessary to strengthen our findings; thus, the goal is to continue data collection until a suitable sample number is reached, to allow conclusions with greater statistical power.

REFERENCES

1. Learmonth ID, Young C, Rorabeck C. The operation of the century: total hip replacement. *Lancet* 2007; 370(9597):1508-19.
2. Knight SR, Aujla R, Biswas SP. Total Hip Arthroplasty - over 100 years of operative history. *Orthop Rev* 2011; 3(2):e16.
3. Kelmanovich D, Parks ML, Sinha R, Macaulay W. Surgical approaches to total hip arthroplasty. *J South Orthop Assoc* 2003; 12(2):90-4.
4. Horne PH, Olson SA. Direct anterior approach for total hip arthroplasty using the fracture table. *Curr Rev Musculoskelet Med* 2011; 4(3):139-145.
5. Rachbauer F, Kain MSH, Leunig M. The History of the Anterior Approach to the Hip. *Orthop Clin North Am* 2009; 40(3):311-20.
6. Rachbauer F. Minimally invasive total hip arthroplasty. Anterior approach. *Orthopade* 2006; 35(7):723-4, 726-9.
7. Rachbauer F, Kain MSH, Leunig M. The History of the Anterior Approach to the Hip. *Orthop Clin North Am* 2009; 40:311-20.
8. Laude F. Arthroplastie totale de hanche par voie antérieure de Hueter mini-invasive 2004.
9. Macpherson GJ, Hank C, Schneider M, et al. The posterior approach reduces the risk of thin cement mantles with a straight femoral stem design. *Acta Orthop* 2010;81(3):292-5.
10. Kim YS, Kwon SY, Sun DH, Han SK, Maloney WJ. Modified posterior approach to total hip arthroplasty to enhance joint stability. *Clin Orthop Relat Res* 2008; 466(2):294-9.
11. Healy WL, Iorio R, Clair AJ, Pellegrini VD, Della Valle CJ, Berend KR. Complications of Total Hip Arthroplasty: Standardized List, Definitions, and Stratification Developed by The Hip Society. *Clin Orthop Relat Res* 2016; 474(2):357-64.
12. Grob K, Monahan R, Gilbey H, Yap F, Filgueira L, Kuster M. Distal Extension of the Direct Anterior Approach to the Hip Poses Risk to Neurovascular Structures. *J Bone Jt Surg* 2015; 97(2):126-32.
13. Phillips CB, Barrett JA, Losina E, et al. Incidence rates of dislocation, pulmonary embolism, and deep infection during the first six months after elective total hip replacement. *J Bone Joint Surg Am* 2003; 85-A(1):20-26.
14. Berry DJ, von Knoch M, Schleck CD, Harmsen WS. The cumulative long-term risk of dislocation after primary Charnley total hip arthroplasty. *J Bone Joint Surg Am* 2004; 86A(1):9-14.
15. Kwon MS, Kuskowski M, Mulhall KJ, Macaulay W, Brown TE, Saleh KJ. Does Surgical Approach Affect Total Hip Arthroplasty Dislocation Rates? *Clin Orthop Relat Res* 2006; 447:34-8.
16. Martin CT, Pugely AJ, Gao Y, Clark CR. A Comparison of Hospital Length of Stay and Short-term Morbidity Between the Anterior and the Posterior Approaches to Total Hip Arthroplasty. *J Arthroplasty* 2013; 28(5):849-54.
17. Pilot P, Kerens B, Draijer WF, et al. Is minimally invasive surgery less invasive in total hip replacement? A pilot study. *Injury* 2006; 37Suppl5:S17-23.
18. Cheng TE, Wallis JA, Taylor NF, et al. A Prospective Randomized Clinical Trial in Total Hip Arthroplasty-Comparing Early Results Between the Direct Anterior Approach and the Posterior Approach. *J Arthroplasty* 2017; 32(3):883-90.
19. Zhao H-Y, Kang P-D, Xia Y-Y, Shi X-J, Nie Y, Pei F-X. Comparison of Early Functional Recovery After Total Hip Arthroplasty Using a Direct Anterior or Posterolateral Approach: A Randomized Controlled Trial. *J Arthroplasty* 2017; 32(11):3421-8.

CHARACTERIZATION OF DIFFERENT *IN VITRO* CULTURE CONDITIONS TO INDUCE A FIBRO-CHONDROGENIC DIFFERENTIATION OF SWINE ADIPOSE-DERIVED STEM CELLS

I. TESSARO^{1*}, A. DI GIANCAMILLO^{2*}, E. BENASCIUTTI^{1*}, V.T. NGUYEN¹, U. POLITO²,
L. MANGIAVINI¹ and G.M. PERETTI^{1,3}

¹*IRCCS Istituto Ortopedico Galeazzi, Milano, Italy;* ²*Department of Health, Animal Science and Food Safety, University of Milan, Italy;* ³*Department of Biomedical Sciences for Health, University of Milan, Milano, Italy*

*These authors contributed equally to this work.

Meniscus repair is still a challenge for orthopaedic surgeons as this tissue has a scarce healing potential due to the limited vascularization and to the lack of progenitor cells. Lately, several tissue engineering strategies combining cells and scaffolds have been developed. Adipose-derived stem cells (ASCs) represent a novel cell source for meniscus repair as they are easy to harvest, and they possess an intrinsic chondrogenic potential. The aim of our study was to analyze and compare the chondrogenic differentiation of swine ASCs cultured *in vitro* with different supplemented media. We isolated ASCs from swine adipose tissue and we placed in pellet cultures supplemented with either Bone Morphogenetic Protein (BMP)-2 and Transforming Growth Factor (TGF)- β 3 or with Bone Morphogenetic Protein (BMP)-7 and Transforming Growth Factor (TGF)- β 1. Samples were analyzed after 7, 14 or 21 days of culture by biochemical, histological and gene expression analysis. Our preliminary results show that BMP-2 and TGF- β 3 are stronger inducers of chondrogenesis.

The menisci are fibrocartilaginous structures, which contribute to static weight bearing, to distribution of compressive forces during joint movement, to joint lubrication and stabilization, and to proprioception (1). They are mainly composed of water, then collagens, glycosaminoglycans (GAGs), DNA, and glycoproteins (2, 3). Collagen fiber arrangement is highly specialized, with the majority being circumferentially aligned (4, 5) with a different organization in the various meniscal regions. This peculiar fiber orientation creates biomechanically optimal resistance to hoop stresses. Proteoglycans are hydrophilic molecules that contribute to the large water content and to the shock absorption properties

of the meniscus (3, 6-8). In the mature tissue, cell morphology varies among different regions. The outer portion of the meniscus mostly contains fibroblast-like cells, whereas the inner part reveals more rounded cells that behave similar to chondrocytes (9, 10). The cell types distribution reflects the vascular supply: during maturation, vasculature recedes to the outer 10-30% of the tissue, leaving the majority of the tissue avascular (6, 11-14).

Based on its anatomy and vascularity, the meniscus has limited healing capacities especially in central two-thirds avascular zone (15), and thus tears are mostly treated by partial or total meniscectomy (16); however, this procedure does not prevent late onset of

Key words: swine, ASCs, fibro-chondrogenic differentiation

Corresponding author:
Giuseppe M. Peretti,
IRCCS Istituto Ortopedico Galeazzi,
Via R. Galeazzi 4,
20161 Milan, Italy
Tel: +39 0266214930
E-mail: giuseppe.peretti@unimi.it

secondary osteoarthritis (17, 18). To overcome these problems, alternative procedures to restore native tissue function may be achieved both by improving surgical techniques and by supporting cell growth and new reparative tissue formation. Tissue engineering approaches that involve the use of cells and biomaterial scaffolds have gained increasing attention as potential regenerative therapies in the field of musculoskeletal medicine (10, 19, 20). In particular, studies have reported successful repair of meniscal punch defects with a mesenchymal stem cells (MSC)-biomaterial combination on a hyaluronan-collagen base (21). Several cells sources have been tested for meniscus healing, along with meniscus fibro-chondrocytes (22, 23), chondrocytes (24, 25), MSCs, synovium-derived stem cells (26, 27), and adipose-derived stem cells (28, 29). It has not been determined which cell type is ideal for repair, however Fraser et al. (30) reported that the number of MSCs in adipose tissue is ~500-fold more than that in bone marrow.

Adipose-derived mesenchymal stem cells (ASCs) are therefore a promising candidate cell source for meniscus regeneration through the less invasive technique of intra-articular injection. The chondrogenic differentiation of ASCs may be induced by growth factors which play the most important role in this process. Different mixes of these polypeptides have been studied to evaluate the best combination for cell proliferation and chondrogenic differentiation. Rui et al. (31) observed the positive effect of Bone Morphogenetic Protein (BMP)-2 on Transforming Growth Factor (TGF)- β 3-induced chondrogenesis of synovium-derived stem cells in a pellet culture system. On the other hand, Shintani et al. (32) considered BMP-7 more potent than BMP-2 in inducing chondrogenesis, and he highlighted the need of a combined action with a member of TGF-family (particularly TGF- β 1) to trigger an alternative signaling pathway for chondrogenic differentiation.

Thus, the aim of our project was to characterize different *in vitro* culture conditions to induce a fibro-chondrogenic differentiation of swine ASCs. In particular, two combinations of growth factors were analyzed: (i) M1 differentiation medium, supplemented with TGF- β 3 and BMP-2 and (ii) M2 differentiation medium, supplemented with TGF- β 2 and BMP-7. To

establish the optimal stimulation conditions, ASCs were 3D-cultured in pellet at different time points, then the differentiated tissues were characterized by biochemistry (GAGs quantification), and by histology (GAGs presence and distribution). Moreover, the expression gene expression of cartilage-related proteins (collagen type I, collagen type II, aggrecan, versican) was quantified by Real-Time Polymerase Chain Reaction (PCR).

MATERIALS AND METHODS

If not specifically mentioned, all chemicals were purchased from Sigma-Aldrich company (St. Louis, MO, USA).

Cell purification

Adipose tissue from adult pig was sliced into small pieces. The pieces were digested at 37°C, shaken for 2 h in phosphate buffered saline (PBS; Life Technologies), amphotericin (50mM), gentamycin (50mM), penicillin and streptomycin (100 U/ml), 0.1% collagenase type I (Worthington, New Jersey, US). Collagenase was then neutralized with equal volume of DMEM.

Cells were then passed through a cell strainer, centrifuged, resuspended in DMEM, 10% v/v FBS, sodium pyruvate (1mM; Life Technologies), ultraglutamine (2mM; Lonza), HEPES (10mM; Life Technologies), amphotericin (50mM), gentamycin (50mM), penicillin and streptomycin (100 U/ml), fibroblast growth factor (FGF2; 5mg/ml) seeded on tissue culture dishes and cultured until confluence was reached.

Three-dimensional culture

Pellets of 5×10^5 cells were formed by centrifuging the cells in 1.5ml screw cap tubes in DMEM and leaving them steadily for 48 hours. Pellets were cultured into three different media: serum free medium (SFM; composed by DMEM, 2mM ultraglutamine, 10mM HEPES, 100U/ml penicillin and streptomycin, 1mM Sodium Pyruvate, 2% human serum albumin and 1X insulin-transferrin-selenium), M1 differentiating medium (composed by SFM, 10ng/ml TGF- β 3, 10ng/ml BMP-2, 10^{-7} M dexamethasone (DEX), 100uM ascorbic acid (AA) and M2 differentiating medium (composed by

SFM, 10ng/ml TGF- β 1, 10ng/ml BMP-7, 10^{-7} M DEX, 100uM AA.

Pellets were collected after 7, 14 and 21 days of differentiation in M1 medium, in M2 medium, or in SFM, as control.

Glycosaminoglycans (GAGs) quantification

Dimethylmetilene blue (DMMB) dye assay was the method chosen to measure glycosaminoglycans production by differentiated pellets. Pellets were digested overnight at 55°C with 50ug/ml proteinase K in a 100mM dipotassium phosphate solution. The enzyme action was heat-neutralized at 90°C for ten min. The lysates were centrifuged for ten min at 12000xg in a refrigerated centrifuge and the supernatant was analysed by a colorimetric assay.

A standard curve adopting chondroitin sulphate was run in parallel with all the samples in a 96 wells plate. DMMB dye was added and the plate was read at 500nm wavelength. GAGs quantification was normalized versus DNA concentration. DNA concentration was measured with CyQuant kit (Thermo Fisher Scientific, Waltham, MA, USA) following the manufacturer instructions.

RNA extraction, RT-PCR and Real Time PCR

A triplicate of each pellet was lysed in 1ml trizol (Thermo Fisher Scientific) at 56°C for three hours and vortexed. RNA was extracted following the manufacturer instructions.

cDNA for real time PCR was prepared from 1 μ g of RNA adopting High-Capacity cDNA reverse transcription kit (Thermo Fisher Scientific), following the manufacturer instructions.

Real time PCR was performed with Taqman Universal PCR Master Mix (Thermo Fisher Scientific), following the manufacturer instructions, on a Step One Plus thermocycler (Thermo Fisher Scientific). The primers were the following: Ss03373340_m1, COL1A1, Ss03373344_g1, COL2A1, Ss03374822_m1, ACAN, Ss04323141_m1, VCAN, Ss03216374_g1, YWHAZ (Thermo Fisher Scientific).

Histology

After 14 and 21 days, the pellets were fixed in 10% formalin, included in paraffin following the standard procedures and sectioned with microtome. The sections

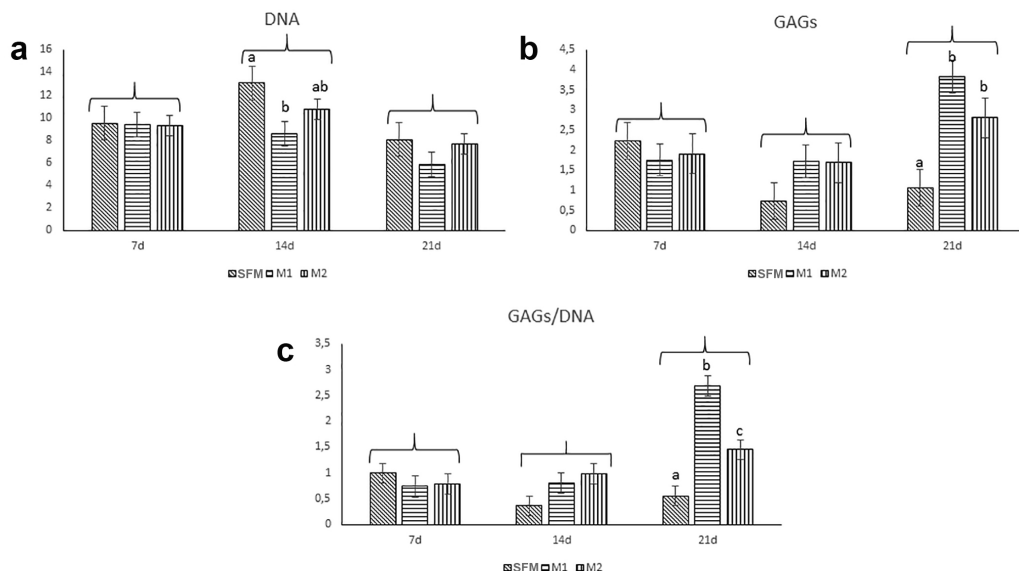


Fig. 1. DNA and Glycosaminoglycans quantification in pellet cultures. **a)** CyQuant assay to evaluate DNA content in SFM, M1 and M2 groups; **b)** DMMT analysis to evaluate GAGs content in SFM, M1 and M2 groups; **c)** GAGs/DNA ratio in SFM, M1 and M2 groups. Error bars indicate the standard error of the mean for each medium/time of differentiation; lowercase letters indicate significant differences of at least $P < 0.05$.

were stained with Safranin-O and counterstained with fast green.

RESULTS

In this study, we isolated mesenchymal cells from the adipose tissue and we evaluated the role of two different culture media (M1 and M2) in chondrogenic differentiation. Cells were cultured in pellets and evaluated at 3 different time points (7, 14 and 21 days). A control group was cultured *in vitro* with SFM (Fig. 1a).

Biochemical analysis showed an initial production of GAGs in the two different media and in the control medium after 7 days of culture (Fig. 1b). At the longest time point we noticed, a significant increase in GAGs deposition both in M1 and M2 conditions compared to the SFM group. Moreover, the GAGs/DNA ratio at 21 days was significantly increased in both groups, but particularly in M1 experimental group (Fig. 1c). Thus, we concluded that the culture medium supplemented with TGF- β 3 and BMP-2 may enhance GAGs production in ASCs.

The histological evaluation at 14 days showed a

scarce deposition of GAGs in all the experimental groups as demonstrated by the light Safranin-O stain (Fig. 2a, c, e). After 21 days of culture, SFM and M2 groups were still characterized by a low accumulation of GAGs, whereas we noticed a higher increase in Safranin-O stain in M1 group (Fig. 2b, d, f). Therefore, we concluded that the M1 culture medium may favor GAGS deposition, consistently with the biochemical analysis.

We then analyzed gene expression of selected fibro-chondrocyte markers: namely Collagen type I, collagen type II, aggrecan and versican. Cells expressed Collagen type 1 on all the three conditions starting from 7 days of culture. However, we noticed a significant upregulation of Collagen type I expression in M1 and in particular in M2 groups at 14 days (Fig. 3a). At the longest time point, both M1 and M2 displayed a downregulation of Collagen type I expression (Fig. 3a). Collagen type II resulted highly expressed in M2 group at 7 days, though this upregulation was not maintained at 14 and 21 days (Fig. 3b). Conversely, we observed a progressive increase of Collagen type II expression in M1, which reached a significant plateau at 21 days (Fig. 3b).

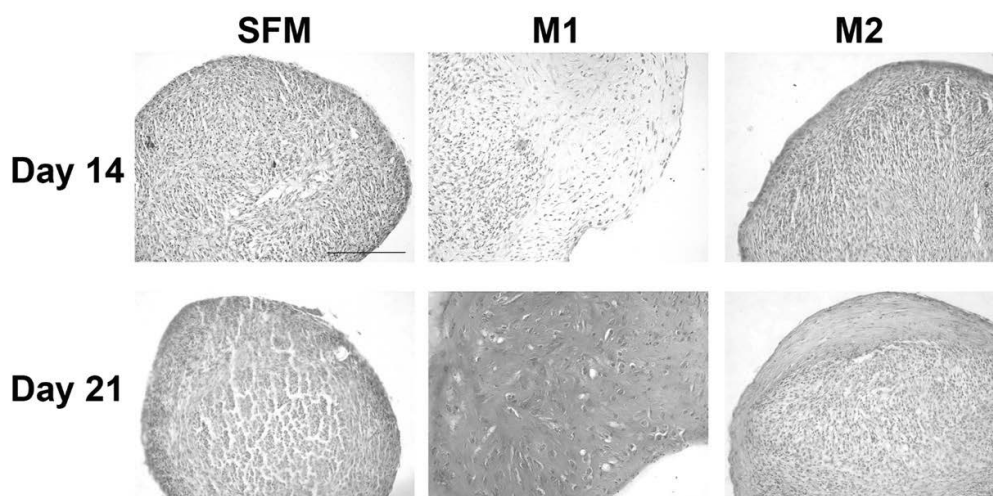


Fig. 2. Histological analysis of the pellet cultures. Safranin-O stain of SFM (a, b), M1 (c, d) and M2 (e, f) groups after 14 days (a, c, e) and 21 days (b, d, f) of culture.

Moreover, Collagen type II was scarcely detectable in control group (Fig. 3b).

Aggrecan expression displayed a similar pattern: it was barely measurable in SFM group, whereas it was progressively and significantly upregulated both in M1 and M2 (Fig. 3c). M1 showed the highest increase of Aggrecan gene expression at all time points (Fig. 3c). Finally, versican gene expression was upregulated in all three groups at 7 days (Fig. 3d). At the latter time points, we noticed a progressive increase of versican gene expression in the control group, while M1 displayed a downregulation especially at 21 days (Fig. 3d). M2 showed versican upregulation at 7 and 14 days, followed by a slight decrease at 21 days (Fig. 3d).

Therefore, gene expression analysis partially confirmed the biochemical evaluation, as the culture medium supplemented with TGF- β 3 and BMP-2 promotes the upregulation of some markers, which are expressed in the cartilaginous matrix, such as Collagen type II and Aggrecan.

In conclusion, our preliminary results demonstrated that TGF- β 3 and BMP-2 enhance

fibro-chondrogenic differentiation of ASCs in an *in vitro* setting.

DISCUSSION

The aim of this work was to compare and to analyze the role of two different culture media on chondrogenic differentiation of ASCs for future meniscus tissue engineering strategies. Meniscus repair is still an unsolved problem: its scarce vascularization in the inner part and the lack of multipotent cells contribute to the limited self-healing potential (11, 16).

In the last years, several meniscus repair strategies combining cells and scaffolds have been developed, however, the meniscus is composed by different cell types, chondrocyte-like cells in the inner part and fibroblast-like cells in the outer region, thus, regenerating the meniscus still represents a challenge. Recently, MSCs have been proposed as a potential cell source for meniscus regeneration due to their ability to differentiate into different lineages under proper stimulation. MSCs can be harvested

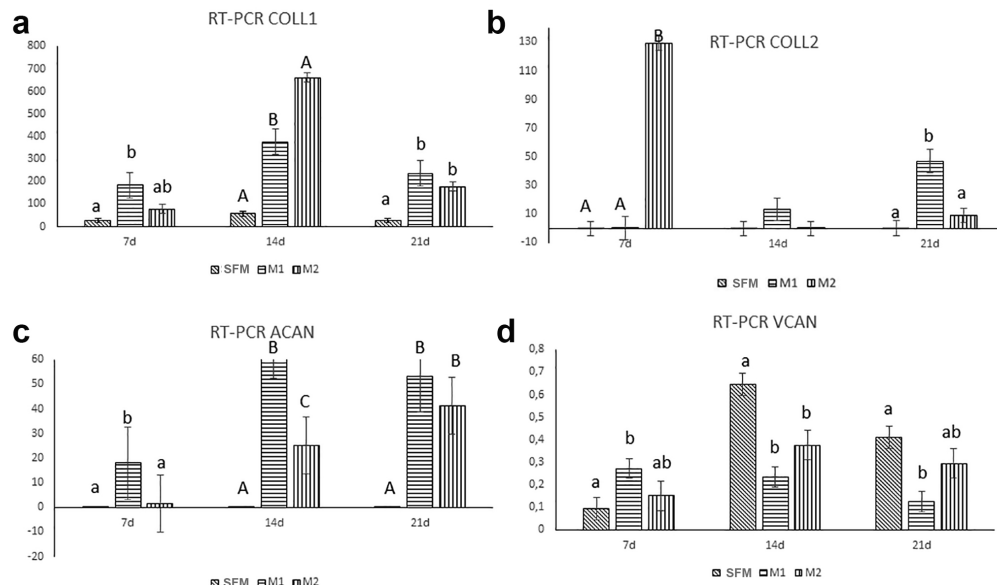


Fig. 3. Gene expression analysis of the pellet cultures. **a)**: RT-PCR analysis for Collagen type I gene expression in SFM, M1 and M2 groups; **b)**: RT-PCR analysis for Collagen type II gene expression in SFM, M1 and M2 groups; **c)**: RT-PCR analysis for aggrecan gene expression in SFM, M1 and M2 groups; **d)**: RT-PCR analysis for versican gene expression in SFM, M1 and M2 groups. Error bars indicate the standard error of the mean for each medium/time of differentiation. Lowercase letters indicate significant differences of at least $P < 0.05$; capital letters indicate significant differences of at least $P < 0.01$.

from different tissues (bone marrow, adipose tissue, umbilical cord, etc.): i.e. bone marrow-derived stem cells (BMSCs), which have been extensively characterized and used for tissue engineering purposes. ASCs represent a novel cell source, which possesses some indisputable advantages: ASCs are easier to harvest and, more importantly, their quantity is greater than BMSCs (30). Thus, we decided to analyze ASCs potential in meniscus regeneration. In previous studies, we characterized both meniscal cells and ASCs and we tested their multipotency.

Another issue is which combination of growth factors or cytokines to use in order to differentiate cells in the fibro-chondrogenic lineage. A previous study suggested that BMP-2 in combination with TGF- β 3 may induced chondrogenesis in multipotent cells derived from the synovium (31). Another work stated that BMP-7 and TGF- β 1 are stronger inducers of chondrogenesis in synovium-derived stem cells (32).

Thus, we decided to compare ASC chondrogenic differentiation process with two different supplemented media. Our results suggest that ASCs stimulated by BMP-2 and TGF- β 3 better differentiate in the chondrogenic lineage.

Our study had several limits; firstly, we analyzed ASCs behavior only in an *in vitro* setting, therefore a further *in-vivo* work is necessary to test ASCs differentiation. Moreover, we did not compare ASCs with other multipotent cells (i.e. BMSCs or synovium-derived stem cells) to test if the effect of these growth factors is cell-specific. Lastly, we should combine ASCs with a biocompatible meniscus scaffold and analyze the role of BMP-2 and TGF- β 3 in a three-dimensional environment.

In conclusion, our results may be useful to design and develop novel tissue engineering strategies for meniscal repair, as we demonstrated that adipose tissue is a possible cell source and that the combination of the above-mentioned growth factors may enhance the chondrogenic differentiation.

REFERENCES

1. Renstrom P, Johnson RJ. Anatomy and biomechanics of the menisci. Clin Sports Med 1990; 9:523-38.
2. Ghadially FN, Lalonde JM, Wedge JH. Ultrastructure of normal and torn menisci of the human knee joint. J Anat 1983; 136:773-91.
3. Herwig J, Egner E, and Buddecke E. Chemical changes of human knee joint menisci in various stages of degeneration. Ann Rheum Dis 1984; 43:635-40.
4. Bullough PG, Munuera L, Murphy J, Weinstein AM. The strength of the menisci of the knee as it relates to their fine structure. J Bone Joint Surg Br 1970; 52:564-7.
5. Petersen W, Tillmann B. Collagenous fibril texture of the human knee joint menisci. Anat Embryol 1998; 197:317-24.
6. McDevitt CA, Webber RJ. The ultrastructure and biochemistry of meniscal cartilage. Clin Orthop Relat Res 1990; 252:8-18.
7. Yanagishita M. Function of proteoglycans in the extracellular matrix. Pathol Int 1993; 43:283-93.
8. Verdonk R, Madry H, Shabshin N, et al. The role of meniscal tissue in joint protection in early osteoarthritis. Knee Surg Sports Traumatol Arthrosc 2016; 24(6):1763-74.
9. Verdonk PC, Forsyth RG, Wang J, Almqvist KF, Verdonk R, Veys EM, Verbruggen G. Characterisation of human knee meniscus cell phenotype. Osteoarthritis Cartilage 2005; 13:548-60.
10. Makris EA, Hadidi P, Athanasiou KA. The knee meniscus: Structure-function, pathophysiology, current repair techniques, and prospects for regeneration. Biomaterials 2011; 32:7411-31.
11. Danzig L, Resnick D, Gonsalves M, Akeson WH. Blood supply to the normal and abnormal menisci of the human knee. Clin Orthopaed Related Res 1983; 172:271-6.
12. Sweigart MA, Athanasiou KA. Toward tissue engineering of the knee meniscus. Tissue Eng 2001; 7:111-29.
13. Di Giancamillo A, Deponti D, Modina S, Tessaro I, Domeneghini C, Peretti GM. Age-related modulation of angiogenesis-regulating factors in the swine meniscus. J Cell Mol Med 2017; 21(11):3066-75.
14. Di Giancamillo A, Mangiavini L, Tessaro I, Marmotti A, Scurati R, Peretti GM. The meniscus vascularization: the direct correlation with tissue composition for tissue engineering purposes. J Biol Regul Homeost Agents 2016; 30(4Suppl1):85-90.
15. Woodmass JM, LaPrade RF, Sgaglione NA, Nakamura

- N, Krych AJ. Meniscal repair: reconsidering indications, techniques, and biologic augmentation. *J Bone Joint Surg Am* 2017; 99:1222-31.
16. Foad A. Self-limited healing of a radial tear of the lateral meniscus. *Knee Surg Sports Traumatol Arthrosc* 2012; 20:933-6.
17. Garrett WE Jr, Swiontkowski MF, Weinstein JN et al., American board of orthopaedic surgery practice of the orthopaedic surgeon: part-II, certification examination case mix. *J Bone Joint Surg Am* 2006; 88(3):660-7.
18. Lohmander LS, Englund PM, Dahl LL, Roos EM. The long-term consequence of anterior cruciate ligament and meniscus injuries. *Am J Sports Med* 2007; 35(10):1756-69.
19. Pereira H, Frias AM, Oliveira JM, Espregueira-Mendes J, Reis RL. Tissue engineering and regenerative medicine strategies in meniscus lesions. *Arthroscopy* 2011; 27:1706-19.
20. Moran CJ, Busilacchi A, Lee CA, Athanasiou KA, Verdonk PC. Biological augmentation and tissue engineering approaches in meniscus surgery. *Arthroscopy* 2015; 31:944-55.
21. Zellner J, et al. Role of mesenchymal stem cells in tissue engineering of meniscus. *J Biomed Mater Res A* 2010; 94:1150-61.
22. Ibarra C, Jannetta C, Vacanti CA, Cao Y, Kim TH, Upton J, Vacanti JP. Tissue engineered meniscus: a potential new alternative to allogeneic meniscus transplantation. *Transplant Proc* 1997; 29(1-2):986-8.
23. Martinek V, Ueblacker P, Bräun K, et al. Second generation of meniscus transplantation: in-vivo study with tissue engineered meniscus replacement. *Arch Orthop Trauma Surg* 2006; 126(4):228-34.
24. Peretti GM, Caruso EM, Randolph MA, Zaleske DJ. Meniscal repair using engineered tissue. *J Orthop Res* 2001; 19(2):278-85.
25. Peretti GM, Gill TJ, Xu JW, Randolph MA, Morse KR, Zaleske DJ. Cell-based therapy for meniscal repair: a large animal study. *Am J Sports Med* 2004; 32(1):146-58.
26. Marsano A, Millward-Sadler SJ, Salter DM. Differential cartilaginous tissue formation by human synovial membrane, fat pad, meniscus cells and articular chondrocytes. *Osteoarthritis Cartilage* 2007; 15(1):48-58.
27. Nakagawa Y, Muneta T, Kondo S. Synovial mesenchymal stem cells promote healing after meniscal repair in microminipigs. *Osteoarthritis Cartilage* 2015; 23(6):1007-17.
28. Pak J, Lee JH, Lee SH. Regenerative repair of damaged meniscus with autologous adipose tissue-derived stem cells. *BioMed Res Int* 2014; 2014:436029.
29. Qi Y, Yang Z, Ding Q, Zhao T, Huang Z, Feng G. Targeted transplantation of iron oxide-labeled, adipose-derived mesenchymal stem cells in promoting meniscus regeneration following a rabbit massive meniscal defect. *Exp Ther Med* 2016; 11(2):458-66.
30. Fraser JK, Wulur I, Alfonso Z, Hedrick MH. Fat tissue: An underappreciated source of stem cells for biotechnology. *Trends Biotechnol* 2006; 24:150-4.
31. Rui YF, DU L, Wang Y, Wang Y, Lui PP, Tang TT, Chan KM, Dai KR. Bone morphogenetic protein 2 promotes transforming growth factor β 3-induced chondrogenesis of human osteoarthritic synovium-derived stem cells. *Chin Med J (Engl)* 2010; 123(21):3040-8.
32. Shintani N, Hunziker EB. Chondrogenic differentiation of bovine synovium: bone morphogenetic proteins 2 and 7 and transforming growth factor β 1 induce the formation of different types of cartilaginous tissue. *Arthritis Rheum* 2007; 56(6):1869-79.

ANIMAL MODELS FOR CARTILAGE REPAIR

I. TESSARO^{1*}, V.T. NGUYEN^{1*}, A. DI GIANCAMILLO², M. AGNOLETTI¹, F. VERDONI¹, M. DOMENICUCCI³, R. SCURATI⁴, G.M. PERETTI^{1,4} and L. MANGIAVINI¹

¹IRCCS Istituto Ortopedico Galeazzi, Milano, Italy; ²Department of Health, Animal Science and Food Safety, University of Milan, Italy; ³Residency Program in Orthopaedics and Traumatology, University of Milan, Milano, Italy; ⁴Department of Biomedical Sciences for Health, University of Milan, Milano, Italy

*These authors contributed equally to this work.

Cartilage lesions still represent an unsolved problem: despite the efforts of the basic and translational research, the regeneration of this tissue is far from being reached (1-3). Articular cartilage lesions can be divided in two main groups: superficial or partial defects and full-thickness defects (4, 5). Partial lesions are not able to self-heal because multipotent cells from the bone marrow cannot reach the area leading to a progressive degeneration of the tissue (6). Conversely, full-thickness injuries possess greater chances to heal because subchondral bone involvement allows for the migration of mesenchymal cells, which fill the damaged area (7, 8). However, healing occurs through the formation of a fibrocartilaginous tissue, which has different biomechanical and biological properties (9). Native hyaline cartilage has indeed specific biomechanical properties, which confer resistance to compressive and shear stresses; the reparative fibrocartilaginous tissue lacks these abilities, therefore, the surrounding healthy cartilage progressively degenerates. In the past years, several therapeutic strategies have been developed to restore the damaged cartilage, bone marrow stimulation (chondroabrasion, drilling, micro- or nano-fractures) and more recently, tissue engineering approaches (10-14). Some of these latter procedures have already been applied in clinical practice such as matrix-induced autologous chondrocyte implantation (MACI) (15) or osteochondral scaffold implantation (16). Generally, tissue engineering approaches are based on the combination of three main elements: cells (i.e. primary chondrocytes or multipotent mesenchymal cells), biocompatible scaffolds (i.e. polymers, composites, ceramics) and signaling molecules (i.e. growth factors). Moreover, several culture conditions (i.e. static or dynamic cultures) and biomechanical stimuli can be applied during the *in vitro* culture to promote tissue maturation (17-19). However, an *in vivo* culture is mandatory to validate a new engineered construct as the *in vitro* phase lacks the essential *in vivo* environmental stimuli and because the *in vivo* culture allows for the testing of the biocompatibility and safety of a new material (18, 19). Moreover, preclinical animal models are crucial to understand the molecular mechanisms of cartilage lesions favoring the development of new regenerative strategies (20, 21). *In vivo* studies on animal models should focus on the analysis of the cellular component, analyzing the maintenance of the cellular phenotype and the tumorigenicity; on the evaluation of the biocompatibility, toxicity and degradation of the biomaterial and on the assessment of the engineered construct. In this manuscript, we will review the most common preclinical animal models, which are used to understand cartilage biology and therefore to develop new tissue engineering strategies. We will focus on both small and large animal models highlighting their peculiarities, advantages and drawbacks.

Key words: animal models, cartilage, in vivo models

Corresponding author:

Laura Mangiavini,
IRCCS Istituto Ortopedico Galeazzi,
Via R. Galeazzi 4,
20161 Milan, Italy
Tel.: +39 0266214930
e-mail: laura.mangiavini@grupposandonato.it

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Animal models

Animal models for cartilage engineered strategies can be essentially divided into 3 categories:

- 1) Animal models to analyze the biocompatibility and toxicity of the scaffolds;
- 2) Animal models to evaluate the chondrogenesis *in vivo*;
- 3) Animal models to assess the chondrogenic potentialities and mechanical properties of newly engineered constructs (Table I).

Animal models to analyze the biocompatibility and toxicity of the scaffolds

In vivo evaluation of a new material is mandatory before implantation in humans. *In vivo* testing is indeed essential to assess irritation, delayed-type hypersensitivity, systemic toxicity and local effects generated after the implant.

Irritation, delayed-type hypersensitivity

Mouse, rat or rabbit are used to test the irritation potentialities and the delayed-type hypersensitivity. The new material or the extract of it is placed in contact with the animal skin, and any adverse reaction (i.e. fur loss or abrasions) is examined and reported.

Systemic toxicity and local effects

Systemic and local toxicity tests are performed to exclude both systemic and local pathological effects due to the implant of the new biomaterial in the animals. For these studies both small- and large-size animals can be used.

In details, mice, rat or rabbit can be used to test the systemic toxicity. In this case, the biomaterial is usually implanted in subcutaneous pouches or in the muscle. The *in vivo* experimental time is usually long (24 weeks or more) to allow for the evaluation of late toxicity. During the *in vivo* time, animals are constantly monitored to check for behavior changes, multiorgan toxicity (liver, heart or kidney dysfunction). After sacrifice, histologic analyses are performed on all the tissues, urine and blood tests are completed to exclude any adverse reaction caused by the implanted material.

The local side effects are usually evaluated in rabbit or sheep model. The biomaterial is implanted in an orthotopic chondral or osteochondral defect and it is cultured *in vivo* for a long experimental time (more than 12 weeks).

At the end of the culture time, the material and the surrounding tissues are analyzed by histology.

Table I. Suitable animal models for cartilage tissue engineering studies.

	Scaffold biocompatibility and toxicity	Chondrogenesis	Biomechanics
Mouse	X	X	
Rat	X	X	
Rabbit		X	
Mini-Pig		X	X
Dog			X
Goat			X
Sheep			X
Pig			X
Horse			X

List of the available animal models based on their possible use: animal models to test scaffold biocompatibility and toxicity, chondrogenesis or biomechanics.

Some critical parameters are examined:

- Presence of fibrosis or fibrotic capsule surrounding the material
- Presence of inflammation around the implant
- Morphological changes in the surrounding tissues
- Type, number and distribution of inflammatory cells
- Presence of necrosis, material debris or granulomas
- Integration of the material with the adjacent tissues (usually evaluated by histomorphometry)

Animal models to evaluate cell phenotype in vivo

In tissue engineering and in regenerative medicine, the choice of an adequate source of cells is fundamental to achieve good results. Both differentiated and undifferentiated cells can be combined with a scaffold to obtain an engineered cartilaginous substitute and available cell sources include native articular cartilage, cartilage from the nasal septum, synovium, bone marrow and adipose tissue (22-29). Some important cellular characteristics are required: chondrogenic potential (for undifferentiated cells), proliferative potential and the ability to maintain the chondrocytic phenotype both *in vitro* and *in vivo*; for the *in vivo*, growth factors have a fundamental role as discussed later in the manuscript (6).

Terminally differentiated cells, such as articular chondrocytes, have been extensively used for the treatment of cartilage lesions combined with different scaffolds in the so-called autologous chondrocyte transplantation (ACT) technique, with satisfactory results (15, 30, 31). Primary articular chondrocytes have the obvious advantage to already possess a chondrocytic phenotype; however, they also have several disadvantages: the donor site morbidity, the limited availability, the low proliferative rate and the tendency of dedifferentiate into fibroblastic cells when cultured *in vitro* (22). Recently, the use of primary chondrocytes harvested from the nasal septum have been investigated; these cells possess an enhanced proliferation rate and a higher chondrogenic capacity compared to articular chondrocytes (25, 26).

Stem cells are the new frontier in cartilage

regeneration; they are a self-renewing cell population that exhibits an ability to undergo multilineage differentiation (32). Stem cells give rise to cell progenies, which become progressively committed to differentiate into different cell types (32). At advanced stages of development, the products are multipotent cells with a limited degree of differentiation and lower self-renewal potential (33). Many adult tissues maintain a stem cell population, which is necessary for tissue repair and remodeling. Thus, these adult stem cells can be used for tissue engineering purposes. Multipotent stem cells with a chondrogenic potential (mesenchymal stem cells) can be harvested from the bone marrow, the adipose tissue, the synovium and more recently, from the umbilical cord (28, 29, 34-37). Several *in vitro* studies have proven their efficacy to differentiate into chondrocytes (22).

The chondrogenic potential of these different cell sources needs to be assessed in an *in vivo* model, as the *in vitro* culture lacks the natural stimuli for tissue regeneration. Hence, chondrogenesis heterotopic models usually follow the *in vitro* culture to validate the cell source. Generally, small animal models (mouse or rat) are used for this purpose. Cartilage-engineered constructs are heterotopically implanted in subcutaneous or muscular pouches of immunocompromised mice (more rarely rats). The validity of this *in vivo* approach for cartilage engineering strategies was proven a few years ago (38), and nowadays the subcutaneous implant of cartilaginous substitutes is widely used. For example, our lab has extensively studied a tissue engineered construct made of primary articular chondrocytes embedded in fibrin glue and cultured first *in vitro* and then *in vivo* (39).

We have demonstrated that the *in vivo* environment contributes to the maintenance of the chondrogenic phenotype. Other research groups have also validated this method for different cell sources (40). The explanted xenografts are then usually analyzed by histology and biomechanical tests.

Moreover, the implant into immunocompromised animals is also essential to exclude tumorigenic potential of the cells.

Animal models to assess the chondrogenic potentialities and mechanical properties of new engineered constructs

The heterotopic chondrogenesis models are very useful to validate a potential cell source, but they do not provide any information on the mechanical properties and on the integration capacities of the cartilaginous substitute with the native cartilage and the subchondral bone. Thus, orthotopic models are crucial to address this issue. Different approaches for cartilage lesions are available: partial-thickness or full-thickness lesions, acute or chronic lesions, lesions with a different degree of self-healing based on animal age (41). Both small- (mice, rats), medium- (rabbit) and large-size (dog, sheep, goat, pig, horse) models are suitable (21).

Small-size animal models

Rodents are attractive models because housing costs are relatively low and because they have a short lifespan, which allows for their analysis throughout development and maturation. Moreover, several antibodies and reagents are available for rodents.

However, rodent (mice and rats) joints are small and they are characterized by a thin layer of cartilage, which hardly permits the implantation of a graft. Moreover, growth plate persists throughout the life both in mice and in rats. Thus, the presence of metaphyseal chondrocytes increases the self-healing potential of articular cartilage lesions and therefore it may confound the experimental results. On the other hand, the availability of transgenic mice is a great resource to study the molecular mechanisms of cartilage lesions; the opportunity of deleting or overexpressing genes is indeed a precious tool to unveil the role of a specific protein in cartilage degradation or repair (42, 43). For example, Zhang et al. noticed that the expression of Ccn1 in cartilage of osteoarthritic mice increased; thus, they generated a transgenic mouse model characterized by the overexpression of Ccn1 exclusively in cartilage and they confirmed that this extracellular matrix protein might play an important role in cartilage degeneration (43).

Besides, in the past years different mouse models of spontaneous (44) or induced osteoarthritis (45-48)

have been developed. These models can be used both for the mechanistic understanding of the disease and for the application of gene therapy strategies (49-51). Thus, the knowledge of the molecular basis of cartilage degradation and repair might lead to the development of novel tissue engineering strategies.

Medium-size animal models

Medium-size animal models, namely rabbits, are widely used for cartilage engineering strategies mainly because rabbit knee joint shares several characteristics with the human knee. For example, the rabbit femoral trochlea represents an appropriate site for cartilage-engineered implants: it is easily accessible and, due to the wide degree of flexion, this anatomical region is subjected to weight bearing similarly to human femoral condyles. Both partial- and full-thickness defects have been generated with rabbit models; for example, Marmotti et al. developed a novel osteochondral substitute composed by cartilage fragments, hyaluronic acid, fibrin glue and Platelet Rich Plasma and they implanted it in osteochondral lesions of the rabbit trochlea (52). They demonstrated that this novel osteochondral graft was a good cell source and it could repair the defects (52).

However, cartilage thickness in the knee is relatively thin (53) and it possesses self-healing capacities due to the migration of undifferentiated cells to the site of lesion (8). Thus, the rabbit is more suitable for partial-thickness cartilage defects.

Moreover, several rabbit models of induced osteoarthritis are available: the disease is usually triggered by partial/total meniscectomy, anterior cruciate ligament tears or the combination of the two methods; alternatively, osteoarthritis is also induced by collagenase injection, denervation or immobilization (54-57). Therefore, the rabbit model is also useful to test several gene therapy approaches with the aim to investigate the role of specific proteins in cartilage repair and regeneration (58).

Large-size animal models

Small- and medium-size animals are useful to study the chondrogenic potential of the cell sources, to explore the physiopathology of cartilage lesions,

and to test tissue-engineered constructs for small defects (particularly partial-thickness lesions). Conversely, large-size animal models are essential to assess a chondral or osteochondral substitute in an articular environment. Knee is usually the preferred site for the implantation and this joint leads to several advantages:

1. Grafting of larger scaffolds (or grafting of multiple scaffolds);
2. Analysis of the integration between the substitute and the surrounding tissues (cartilage and subchondral bone);
3. Evaluation of the role of biomechanical stresses on tissue maturation.

Different animal models are available (dog, goat, sheep, pig and horse) and each of them has specific characteristics, which need to be considered in the experimental plan. In general, knee anatomy of larger-size animals share several features with the human one, and probably the biggest benefit of these models is that a second-look arthroscopy can be performed in many of these animals.

Hereafter we will briefly describe the different large-size animal models focusing on the peculiar properties of each of them.

Dog

Dog's knee shares many characteristics with the human one; thus, it is an ideal model for cartilage repair. The canine model has been also widely used to explore osteoarthritis pathogenesis. Pond and Nuki (59) have developed an induced-osteoarthritis model by unilateral cranial cruciate ligament (CCL) transection, which has been widely exploited especially in the veterinary field to analyze osteoarthritic changes (60) and to identify new genetic pathways (61) and markers (62) in the development of the disease.

Several research groups have used the canine model (63, 64). Kazemi et al. implanted a substitute composed by Bone Marrow Mesenchymal Stem Cells (BM-MSC) embedded in a platelet rich plasma hydrogel: they demonstrated by macroscopy and histology a good degree of repair in the experimental groups compared to empty defects (63).

The main advantages of the canine model are the

size of the knee joint and the possibility to generate osteochondral defects. Moreover, canines are very docile and therefore they can tolerate immobilization (or external fixation) and a second-look arthroscopy. On the other hand, cartilage thickness is significantly thinner than the human one and the invasion of canine bone marrow cells into the scaffold may generate an immune response if cells from another species are used (65).

The main limitation for the use of canine model is however ethical, dogs are considered companion animals, thus, the general population does not easily accept their application in experimental studies. Moreover, dogs are quite expensive compared with other animals and it is quite difficult to find skeletally mature dog models (21).

Goat

Goats are also a good model due to the similarities with human anatomy and because cartilage is thicker when compared to rabbits or dogs; though it is thinner than sheep or pig's cartilage (65). Goats' housing and handling are relatively inexpensive, moreover, a second look arthroscopy is feasible on these animals and this perhaps represents the greatest advantage. Thus, in the last years there has been an increase in the use of this species. For example, Mumme et al. chose the goat to test the chondrogenic potential and the regenerative capacity of chondrocytes harvested from the nasal septum in an osteochondral lesion (25). They demonstrated the superiority of nasal chondrocytes on articular chondrocytes: this result lead to the development of a clinical trial (66). Hence, the goat model allows for the grafting of large substitutes although the maximum lesion size is not considered critical for humans.

The main disadvantages of the goat are the anesthesiologic difficulties and the risk of immune response if the subchondral bone is involved. In addition, goats are natural browsers and consequently, the control of the pre-operative phase and the post-operative recovery might be challenging.

Sheep

Sheep knees also share several features with the human joint; therefore, also this animal model

is suitable for cartilage repair studies. Advantages of the sheep include the low costs of housing and size of the joints, which ease the grafting of large substitute. Indeed, our lab and other research groups have chosen this animal model for their studies (67-70). Recently, we published an original paper on the comparison of 3 cell-free scaffolds for one-stage treatment of osteochondral lesions (71). In this work, we have been able to generate big defects (11 mm width and 9 mm depth) in the medial femoral condyle of 18 skeletally mature sheep; we demonstrated the superiority of one of the 3 scaffolds when compared to the other ones after 3 months of *in vivo* culture.

Another benefit of the sheep model is the possibility to perform an arthroscopic evaluation to test the graft macroscopically. However, a great variability in cartilage thickness has been reported, which may interfere with the results (20). The subchondral bone may also be particularly thick and compact in older sheep; so, it can inhibit the migration of multipotent cells from the bone marrow and it may delay the integration of the graft (20).

Pig

Swine model is the most suitable animal model, as its anatomy closely resembles humans, not only for joints but also for heart and other organs (72). The affinity between humans and pigs also prevent host immune reactions thus, xenogeneic cells might potentially be implanted in a pig model. Swine are also appropriate for second-look arthroscopy.

Our lab has extensive experience on pig models for tissue engineering strategies both for *in vitro* and *in vivo* studies (73-80). In our last paper, we implanted a novel biphasic composite (with or without autologous cells) in the trochlea of skeletally mature pigs. This study proved that the novel scaffold was biocompatible and that it could integrate with the surrounding cartilage and bone with the formation of a cartilaginous tissue. Other labs have also used the swine model (81-83) especially for osteochondral defects, but some features of this animal are detrimental for experimental purposes. First, the size of the animal: pigs are characterized by a rapid skeletal growth and they gain weight rapidly; this leads to an increase in housing costs.

Secondly, pigs are extremely noisy and they may easily become aggressive, thus affecting the housing conditions and the handling of the animals (84). Finally, anesthesiologic procedures for two-step surgeries might be difficult.

For all these reasons, smaller swine models (minipig) are preferred and they are indeed largely used (36, 85-87). Minipigs are also easier to handle and they possess a sufficient cartilage thickness. Different minipig strains are available (i.e. Yucatan or Göttingen) (84, 88, 89) and they are all suitable for cartilage repair studies.

More recently, transgenic minipigs have been developed with the aim to reduce the immunogenicity of porcine chondrocytes and therefore to allow xenotransplantation of porcine chondrocytes in primates (90, 91). However, the costs of these novel techniques are extremely high and there are concerns on the possible effects of the transgene in humans; thus, these approaches are still exclusively experimental.

Horse

Horses are the largest animal models available for cartilage repair studies; the size represents a remarkable benefit as more defects can be generated and treated in the same joint. Cartilage thickness is also comparable to the human tissue (65). Moreover, horses are subjected to intense and aggressive loading conditions that can be useful to investigate the response of a cartilage-engineered scaffold to extreme biomechanical stresses. (92-94)

The first studies on horses have been performed in the carpus (95); however, the anatomy of this joint lead to a great variability in the results (96) and as a consequence, later studies focused on the knee joint, which possesses a thicker cartilage layer and weight loading conditions similar to the human joint. For tissue engineering purposes, both the patellofemoral and the tibiofemoral joints are used: Several studies have demonstrated that the critical defect size is 9 mm. They have indeed shown that smaller defects have a self-healing potential, which interferes with the evaluation of the results (97). Horses represent a powerful model for cartilage repair studies because they allow the analysis of big and multiple lesions

with load bearing conditions comparable to humans. Moreover, arthroscopic treatments and second-look surgeries can be performed. Specific rehabilitation protocols mimicking the human ones have been developed. On the other hand, these animals require specific facilities and diagnostic tools, i.e. a CT scan for large animals; thus, handling of the experiments is difficult and very expensive. Besides, horses are considered companion pets and this arouses serious ethical concerns in many countries such as Europe.

DISCUSSION

In this paper, we have reviewed the most important animal models for cartilage repair studies. The specific biomechanics of articular cartilage and its poor self-healing properties indeed require *in vivo* models to test novel engineered constructs. First, animal models are essential to test biocompatibility and toxicity of a new material; second, *in vivo* studies allow the assessment of the chondrogenic potential of different cell sources combined with different scaffolds. Last, the *in vivo* environment is crucial to evaluate the biomechanical properties of a cartilage-engineered substitute.

Several animal models are available and have been extensively used in the last decades, from small- to medium- and large-size animals (Table I). Which is the most appropriate animal model for cartilage repair then? The perfect animal model does not exist and the correct choice depends on the aim of the study: small animals, such as mice or rats, are useful to test different cell sources both in heterotopic and orthotopic models. Moreover, genetically modified mice are also essential to deepen the knowledge on the molecular mechanisms of cartilage degeneration and repair. Rabbits are better models for orthotopic studies due to the bigger joints; moreover, housing and handling of these medium-size animals does not involve specific facilities. However, self-healing potential of cartilage has been reported in the rabbit model (53), also, cartilage thickness is not comparable with the human one. Thus, large animals (such as pigs, goats, horses) are more suitable for orthotopic implants as their size allows the generation of critical and multiple defects; besides,

cartilage biomechanical behavior and thickness between human and these animals is similar.

Housing and handling of big animals is however challenging, as it requires appropriate facilities and equipment. Moreover, any of the above-mentioned large-size models has its peculiarities, such as joint anatomy or cartilage thickness, that need to be considered in the experimental planning.

The efficacy of a novel cartilage engineered strategy should be proven in more than one animal model, thus, new materials should be first tested in small animals and subsequently, preliminary and short-term proof of concept experiments should be performed in rabbits and then confirmed in a large animal. Ideally, the efficacy of every novel cartilage substitute should be tested in horses, as their knee more closely resembles the human anatomy both in the tissue thickness and in the biomechanical behavior. However, none of the above described animals possesses the same molecular, anatomical or biomechanical characteristics of the human knee hence, the efficacy of tissue engineering strategies can be ultimately confirmed only in controlled clinical trials.

REFERENCES

1. Lories RJ and Luyten FP. The bone-cartilage unit in osteoarthritis. *Nat Rev Rheumatol* 2011; 7(1):43-49.
2. Makris EA, Gomoll AH, Malizos KN, Hu JC and Athanasiou KA. Repair and tissue engineering techniques for articular cartilage. *Nat Rev Rheumatol* 2015; 11(1):21-34.
3. Farr J, Cole B, Dhawan A, Kercher J and Sherman S. Clinical cartilage restoration: evolution and overview. *Clin Orthop Relat Res* 2011; 469(10):2696-705.
4. Madry H, Kon E, Condello V, et al. Early osteoarthritis of the knee. *Knee Surg Sports Traumatol Arthrosc* 2016; 24(6):1753-62.
5. Moskowitz RW. Osteoarthritis cartilage histopathology: grading and staging. *Osteoarthritis Cartilage* 2006; 14(1):1-2.
6. Hunziker EB. The elusive path to cartilage regeneration. *Adv Mater* 2009; 21(32-33):3419-24.
7. Madry H, van Dijk CN and Mueller-Gerbl M. The basic science of the subchondral bone. *Knee Surg*

- Sports Traumatol Arthrosc 2010; 18(4):419-33.
7. Shapiro F, Koide S and Glimcher MJ. Cell origin and differentiation in the repair of full-thickness defects of articular cartilage. *J Bone Joint Surg Am* 1993; 75(4):532-53.
 8. Gomoll AH, Madry H, Knutsen G, van Dijk N, Seil R, Brittberg M and Kon E. The subchondral bone in articular cartilage repair: current problems in the surgical management. *Knee Surg Sports Traumatol Arthrosc* 2010; 18(4):434-47.
 9. Bedi A, Feeley BT and Williams RJ, 3rd. Management of articular cartilage defects of the knee. *J Bone Joint Surg Am* 2010; 92(4):994-1009.
 10. Gomoll AH, Farr J, Gillogly SD, Kercher J and Minas T. Surgical management of articular cartilage defects of the knee. *J Bone Joint Surg Am* 2010; 92(14):2470-90.
 11. Huang BJ, Hu JC and Athanasiou KA. Cell-based tissue engineering strategies used in the clinical repair of articular cartilage. *Biomaterials* 2016; 98:1-22.
 12. Temenoff JS and Mikos AG. Review: tissue engineering for regeneration of articular cartilage. *Biomaterials* 2000; 21(5):431-40.
 13. Redman SN, Oldfield SF and Archer CW. Current strategies for articular cartilage repair. *Eur Cell Mater* 2005; 9:23-32; discussion 23-32.
 14. Brittberg M, Lindahl A, Nilsson A, Ohlsson C, Isaksson O and Peterson L. Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. *N Engl J Med* 1994; 331(14):889-95.
 15. Seo SJ, Mahapatra C, Singh RK, Knowles JC and Kim HW. Strategies for osteochondral repair: Focus on scaffolds. *J Tissue Eng* 2014; 5:2041731414541850.
 16. Boushell MK, Hung CT, Hunziker EB, Strauss EJ and Lu HH. Current strategies for integrative cartilage repair. *Connect Tissue Res* 2016:1-14.
 17. Ahmed TA and Hincke MT. Strategies for articular cartilage lesion repair and functional restoration. *Tissue Eng Part B Rev* 2010; 16(3):305-29.
 18. Chiang H and Jiang CC. Repair of articular cartilage defects: review and perspectives. *J Formos Med Assoc* 2009; 108(2):87-101.
 19. Ahern BJ, Parvizi J, Boston R and Schaer TP. Preclinical animal models in single site cartilage defect testing: a systematic review. *Osteoarthritis Cartilage* 2009; 17(6):705-13.
 20. Chu CR, Szczodry M and Bruno S. Animal models for cartilage regeneration and repair. *Tissue Eng Part B Rev* 2010; 16(1):105-15.
 21. Vonk LA, de Windt TS, Slaper-Cortenbach IC and Saris DB. Autologous, allogeneic, induced pluripotent stem cell or a combination stem cell therapy? Where are we headed in cartilage repair and why: a concise review. *Stem Cell Res Ther* 2015; 6:94.
 22. Grassel S and Lorenz J. Tissue-engineering strategies to repair chondral and osteochondral tissue in osteoarthritis: use of mesenchymal stem cells. *Curr Rheumatol Rep* 2014; 16(10):452.
 23. Tsumaki N, Okada M and Yamashita A. iPS cell technologies and cartilage regeneration. *Bone* 2015; 70:48-54.
 24. Mumme M, Steinitz A, Nuss KM, et al. Regenerative Potential of Tissue-Engineered Nasal Chondrocytes in Goat Articular Cartilage Defects. *Tissue Eng Part A* 2016; 22(21-22):1286-95.
 25. Pelttari K, Pippenger B, Mumme M, et al. Adult human neural crest-derived cells for articular cartilage repair. *Sci Transl Med* 2014; 6(251):251ra119.
 26. Zuk PA, Zhu M, Mizuno H, et al. Multilineage cells from human adipose tissue: implications for cell-based therapies. *Tissue Eng* 2001; 7(2):211-28.
 27. De Girolamo L, Sartori MF, Arrigoni E, Rimondini L, Albisetti W, Weinstein RL and Brini AT. Human adipose-derived stem cells as future tools in tissue regeneration: osteogenic differentiation and cell-scaffold interaction. *Int J Artif Organs* 2008; 31(6):467-79.
 28. De Bari C, Dell'Accio F, Tylzanowski P and Luyten FP. Multipotent mesenchymal stem cells from adult human synovial membrane. *Arthritis Rheum* 2001; 44(8):1928-42.
 29. Loken S, Ludvigsen TC, Hoysveen T, Holm I, Engebretsen L and Reinholt FP. Autologous chondrocyte implantation to repair knee cartilage injury: ultrastructural evaluation at 2 years and long-term follow-up including muscle strength measurements. *Knee Surg Sports Traumatol Arthrosc* 2009; 17(11):1278-88.
 30. Brittberg M, Peterson L, Sjogren-Jansson E, Tallheden T and Lindahl A. Articular cartilage engineering with

- autologous chondrocyte transplantation. A review of recent developments. *J Bone Joint Surg Am* 2003; 85(A Suppl 3):109-115.
31. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS and Jones JM. Embryonic stem cell lines derived from human blastocysts. *Science* 1998; 282(5391):1145-7.
 32. Pittenger MF, Mackay AM, Beck SC, et al. Multilineage potential of adult human mesenchymal stem cells. *Science* 1999; 284(5411):143-7.
 33. Bielli A, Scioli MG, Gentile P, Cervelli V and Orlandi A. Adipose-derived stem cells in cartilage regeneration: current perspectives. *Regen Med* 2016; 11(7):693-703.
 34. Erices A, Conget P and Minguell JJ. Mesenchymal progenitor cells in human umbilical cord blood. *Br J Haematol* 2000; 109(1):235-42.
 35. Ha CW, Park YB, Chung JY and Park YG. Cartilage Repair Using Composites of Human Umbilical Cord Blood-Derived Mesenchymal Stem Cells and Hyaluronic Acid Hydrogel in a Minipig Model. *Stem Cells Transl Med* 2015; 4(9):1044-51.
 36. Marmotti A, Peretti GM, Mattia S, et al. A future in our past: the umbilical cord for orthopaedic tissue engineering. *Joints* 2014; 2(1):20-5.
 37. Vacanti CA, Langer R, Schloo B and Vacanti JP. Synthetic polymers seeded with chondrocytes provide a template for new cartilage formation. *Plast Reconstr Surg* 1991; 88(5):753-9.
 38. Deponti D, Di Giancamillo A, Mangiavini L, et al. Fibrin-based model for cartilage regeneration: tissue maturation from in vitro to in vivo. *Tissue Eng Part A* 2012; 18(11-12):1109-22.
 39. Wei JP, Nawata M, Wakitani S, et al. Human amniotic mesenchymal cells differentiate into chondrocytes. *Cloning Stem Cells* 2009; 11(1):19-26.
 40. Reinholz GG, Lu L, Saris DB, Yaszemski MJ and O'Driscoll SW. Animal models for cartilage reconstruction. *Biomaterials* 2004; 25(9):1511-21.
 41. Ohta Y, Okabe T, Larmour C, et al. Articular cartilage endurance and resistance to osteoarthritic changes require transcription factor Erg. *Arthritis Rheumatol* 2015; 67(10):2679-90.
 42. Zhang Y, Sheu TJ, Hoak D, et al. Ccn1 Regulates Chondrocyte Maturation and Cartilage Development. *J Bone Miner Res* 2016; 31(3):549-59.
 43. Bendele A, McComb J, Gould T, McAbee T, Sennello G, Chlipala E and Guy M. Animal models of arthritis: relevance to human disease. *Toxicol Pathol* 1999; 27(1):134-42.
 44. Glasson SS, Blanchet TJ and Morris EA. The surgical destabilization of the medial meniscus (DMM) model of osteoarthritis in the 129/SvEv mouse. *Osteoarthritis Cartilage* 2007; 15(9):1061-9.
 45. Moodie JP, Stok KS, Muller R, Vincent TL and Shefelbine SJ. Multimodal imaging demonstrates concomitant changes in bone and cartilage after destabilisation of the medial meniscus and increased joint laxity. *Osteoarthritis Cartilage* 2011; 19(2):163-70.
 46. Christiansen BA, Anderson MJ, Lee CA, Williams JC, Yik JH and Haudenschild DR. Musculoskeletal changes following non-invasive knee injury using a novel mouse model of post-traumatic osteoarthritis. *Osteoarthritis Cartilage* 2012; 20(7):773-82.
 47. Khorasani MS, Diko S, Hsia AW, Anderson MJ, Genetos DC, Haudenschild DR and Christiansen BA. Effect of alendronate on post-traumatic osteoarthritis induced by anterior cruciate ligament rupture in mice. *Arthritis Res Ther* 2015; 17:30.
 48. Trippel SB, Ghivizzani SC and Nixon AJ. Gene-based approaches for the repair of articular cartilage. *Gene Ther* 2004; 11(4):351-9.
 49. Cucchiari M, Henrionnet C, Mainard D, Pinzano A and Madry H. New trends in articular cartilage repair. *J Exp Orthop* 2015; 2(1):8.
 50. Rey-Rico A, Frisch J, Venkatesan JK, Schmitt G, Madry H and Cucchiari M. Determination of effective rAAV-mediated gene transfer conditions to support chondrogenic differentiation processes in human primary bone marrow aspirates. *Gene Ther* 2015; 22(1):50-7.
 51. Marmotti A, Bruzzone M, Bonasia DE, et al. One-step osteochondral repair with cartilage fragments in a composite scaffold. *Knee Surg Sports Traumatol Arthrosc* 2012; 20(12):2590-601.
 52. Hunziker EB. Biologic repair of articular cartilage. Defect models in experimental animals and matrix requirements. *Clin Orthop Relat Res* 1999; (367 Suppl):S135-46.
 53. Li Y, Zhang Y, Chen C, Zhang H, Ma C and Xia Y. Establishment of a rabbit model to study the

- influence of advanced glycation end products accumulation on osteoarthritis and the protective effect of pioglitazone. *Osteoarthritis Cartilage* 2016; 24(2):307-14.
54. Zhou Q, Wei B, Liu S, et al. Cartilage matrix changes in contralateral mobile knees in a rabbit model of osteoarthritis induced by immobilization. *BMC Musculoskelet Disord* 2015; 16:224.
 55. Liu Z, Hu X, Man Z, Zhang J, Jiang Y and Ao Y. A novel rabbit model of early osteoarthritis exhibits gradual cartilage degeneration after medial collateral ligament transection outside the joint capsule. *Sci Rep* 2016; 6:34423.
 56. Hermeto LC, DeRossi R, Oliveira RJ, et al. Effects of intra-articular injection of mesenchymal stem cells associated with platelet-rich plasma in a rabbit model of osteoarthritis. *Genet Mol Res* 2016; 15:3.
 57. Madry H, Kaul G, Zurakowski D, Vunjak-Novakovic G and Cucchiariini M. Cartilage constructs engineered from chondrocytes overexpressing IGF-I improve the repair of osteochondral defects in a rabbit model. *Eur Cell Mater* 2013; 25:229-47.
 58. Pond MJ and Nuki G. Experimentally-induced osteoarthritis in the dog. *Ann Rheum Dis* 1973; 32(4):387-8.
 59. Pozzi A, Kim SE, Conrad BP, Horodyski M and Banks SA. Ex vivo pathomechanics of the canine Pond-Nuki model. *PLoS One* 2013; 8(12):e81383.
 60. Stoker AM, Cook JL, Kuroki K and Fox DB. Site-specific analysis of gene expression in early osteoarthritis using the Pond-Nuki model in dogs. *J Orthop Surg Res* 2006; 1:8.
 61. Goranov NV. Serum markers of lipid peroxidation, antioxidant enzymatic defense, and collagen degradation in an experimental (Pond-Nuki) canine model of osteoarthritis. *Vet Clin Pathol* 2007; 36(2):192-5.
 62. Kazemi D, Shams Asenjan K, Dehdilani N and Parsa H. Canine articular cartilage regeneration using mesenchymal stem cells seeded on platelet rich fibrin: Macroscopic and histological assessments. *Bone Joint Res* 2017; 6(2):98-107.
 63. Lv YM and Yu QS. Repair of articular osteochondral defects of the knee joint using a composite lamellar scaffold. *Bone Joint Res* 2015; 4(4):56-64.
 64. Frisbie DD, Cross MW and McIlwraith CW. A comparative study of articular cartilage thickness in the stifle of animal species used in human pre-clinical studies compared to articular cartilage thickness in the human knee. *Vet Comp Orthop Traumatol* 2006; 19(3):142-6.
 65. Mumme M, Barbero A, Miot S, et al. Nasal chondrocyte-based engineered autologous cartilage tissue for repair of articular cartilage defects: an observational first-in-human trial. *Lancet* 2016; 388(10055):1985-94.
 66. Beck A, Murphy DJ, Carey-Smith R, Wood DJ and Zheng MH. Treatment of Articular Cartilage Defects With Microfracture and Autologous Matrix-Induced Chondrogenesis Leads to Extensive Subchondral Bone Cyst Formation in a Sheep Model. *Am J Sports Med* 2016; 44(10):2629-43.
 67. Hindle P, Baily J, Khan N, Biant LC, Simpson AH and Peault B. Perivascular Mesenchymal Stem Cells in Sheep: Characterization and Autologous Transplantation in a Model of Articular Cartilage Repair. *Stem Cells Dev* 2016; 25(21):1659-69.
 68. Kitamura N, Yokota M, Kurokawa T, Gong JP and Yasuda K. In vivo cartilage regeneration induced by a double-network hydrogel: Evaluation of a novel therapeutic strategy for femoral articular cartilage defects in a sheep model. *J Biomed Mater Res A* 2016; 104(9):2159-65.
 69. Mrosek EH, Chung HW, Fitzsimmons JS, O'Driscoll SW, Reinholz GG and Schagemann JC. Porous tantalum biocomposites for osteochondral defect repair: A follow-up study in a sheep model. *Bone Joint Res* 2016; 5(9):403-11.
 70. Gervaso F, Mangiavini L, Di Giancamillo A, et al. Comparison of three novel biphasic scaffolds for one-stage treatment of osteochondral defects in a sheep model. *J Biol Regul Homeost Agents* 2016; 30(4 Suppl 1):24-31.
 71. Swindle MM, Smith AC and Hepburn BJ. Swine as models in experimental surgery. *J Invest Surg* 1988; 1(1):65-79.
 72. Peretti GM, Gill TJ, Xu JW, Randolph MA, Morse KR and Zaleske DJ. Cell-based therapy for meniscal repair: a large animal study. *Am J Sports Med* 2004; 32(1):146-58.
 73. Peretti GM, Xu JW, Bonassar LJ, Kirchhoff CH, Yaremchuk MJ and Randolph MA. Review of injectable cartilage engineering using fibrin gel

- in mice and swine models. *Tissue Eng* 2006; 12(5):1151-68.
74. Scotti C, Buragas MS, Mangiavini L, et al. A tissue engineered osteochondral plug: an in vitro morphological evaluation. *Knee Surg Sports Traumatol Arthrosc* 2007; 15(11):1363-69.
 75. Scotti C, Mangiavini L, Boschetti F, Vitari F, Domeneghini C, Fraschini G and Peretti GM. Effect of in vitro culture on a chondrocyte-fibrin glue hydrogel for cartilage repair. *Knee Surg Sports Traumatol Arthrosc* 2010; 18(10):1400-6.
 76. Scotti C, Pozzi A, Mangiavini L, et al. Healing of meniscal tissue by cellular fibrin glue: an in vivo study. *Knee Surg Sports Traumatol Arthrosc* 2009; 17(6):645-51.
 77. Sosio C, Boschetti F, Bevilacqua C, et al. Effect of blood on the morphological, biochemical and biomechanical properties of engineered cartilage. *Knee Surg Sports Traumatol Arthrosc* 2007; 15(10):1251-7.
 78. Sosio C, Boschetti F, Mangiavini L, et al. Blood exposure has a negative effect on engineered cartilage. *Knee Surg Sports Traumatol Arthrosc* 2011; 19(6):1035-42.
 79. Sosio C, Di Giancamillo A, Deponti D, et al. Osteochondral repair by a novel interconnecting collagen-hydroxyapatite substitute: a large-animal study. *Tissue Eng Part A* 2015; 21(3-4):704-15.
 80. He A, Liu L, Luo X, et al. Repair of osteochondral defects with in vitro engineered cartilage based on autologous bone marrow stromal cells in a swine model. *Sci Rep* 2017; 7:40489.
 81. Meppelink AM, Zhao X, Griffin DJ, et al. Hyaline Articular Matrix Formed by Dynamic Self-Regenerating Cartilage and Hydrogels. *Tissue Eng Part A* 2016; 22(13-14): 962-70.
 82. Chiang H, Kuo TF, Tsai CC, et al. Repair of porcine articular cartilage defect with autologous chondrocyte transplantation. *J Orthop Res* 2005; 23(3):584-93.
 83. Muller C, Marzahn U, Kohl B, et al. Hybrid pig versus Gottingen minipig-derived cartilage and chondrocytes show pig line-dependent differences. *Exp Biol Med (Maywood)* 2013; 238(11):1210-22.
 84. Fisher MB, Belkin NS, Milby AH, et al. Cartilage repair and subchondral bone remodeling in response to focal lesions in a mini-pig model: implications for tissue engineering. *Tissue Eng Part A* 2015; 21(3-4):850-60.
 85. Kim IL, Pfeifer CG, Fisher MB, et al. Fibrous Scaffolds with Varied Fiber Chemistry and Growth Factor Delivery Promote Repair in a Porcine Cartilage Defect Model. *Tissue Eng Part A* 2015; 21(21-22):2680-90.
 86. de Girolamo L, Niada S, Arrigoni E, et al. Repair of osteochondral defects in the minipig model by OPF hydrogel loaded with adipose-derived mesenchymal stem cells. *Regen Med* 2015; 10(2):135-51.
 87. Ebihara G, Sato M, Yamato M, et al. Cartilage repair in transplanted scaffold-free chondrocyte sheets using a minipig model. *Biomaterials* 2012; 33(15):3846-51.
 88. Chang CH, Kuo TF, Lin FH, et al. Tissue engineering-based cartilage repair with mesenchymal stem cells in a porcine model. *J Orthop Res* 2011; 29(12):1874-80.
 89. Costa C, Brokaw JL and Fodor WL. Characterization of cartilage from H-transferase transgenic pigs. *Transplant Proc* 2008; 40(2):554-6.
 90. Sommaggio R, Bello-Gil D, Perez-Cruz M, Brokaw JL, Manez R and Costa C. Genetic engineering strategies to prevent the effects of antibody and complement on xenogeneic chondrocytes. *Eur Cell Mater* 2015; 30:258-70.
 91. Goodrich LR, Chen AC, Werpny NM, et al. Addition of Mesenchymal Stem Cells to Autologous Platelet-Enhanced Fibrin Scaffolds in Chondral Defects: Does It Enhance Repair? *J Bone Joint Surg Am* 2016; 98(1):23-34.
 92. Vindas Bolanos RA, Cokelaere SM, Estrada McDermott JM, et al. The use of a cartilage decellularized matrix scaffold for the repair of osteochondral defects: the importance of long-term studies in a large animal model. *Osteoarthritis Cartilage* 2017; 25(3):413-20.
 93. Griffin DJ, Bonnevie ED, Lachowsky DJ, et al. Mechanical characterization of matrix-induced autologous chondrocyte implantation (MACI(R)) grafts in an equine model at 53 weeks. *J Biomech* 2015; 48(10):1944-9.
 94. Riddle WE, Jr. Healing of articular cartilage in the horse. *J Am Vet Med Assoc* 1970; 157(11):1471-9.
 95. McIlwraith CW, Fortier LA, Frisbie DD and Nixon AJ. Equine Models of Articular Cartilage Repair.

Cartilage 2011; 2(4):317-26.

96. Hurtig MB, Fretz PB, Doige CE and Schnurr DL.

Effects of lesion size and location on equine articular cartilage repair. Can J Vet Res 1988; 52(1):137-46.

EXTERNAL FIXATION VERSUS TITANIUM ENDOMEDULLARY NAIL IN THE TREATMENT OF DIAPHYSEAL FRACTURES OF THE LOWER LIMB IN PEDIATRIC AGE: OUR EXPERIENCE

F. VERDONI¹, D. CURCI¹, F. GRASSO², M. AGNOLETTI¹, R. SCURATI³,
G.M. PERETTI^{1,3} and L. MANGIAVINI¹

¹IRCCS Istituto Ortopedico Galeazzi, Milan, Italy; ²Residency Program in Orthopaedics and Traumatology, University of Milan, Milan, Italy; ³Department of Biomedical Sciences for Health, University of Milan, Italy

In the past, the use of external fixation technique (FE) in children was limited, as the patient's compliance to the treatment was scarce due to an important visual impact for the little patient. With the evolution of the surgical approach and the technology of fixators, we have been able to considerably implement the treatment possibilities, thus allowing for an immediate load bearing of the operated limb and for the early mobilization of the joints. The FE technique does not represent an overcoming of classical synthesis techniques by internal fixation with elastic intramedullary nails, but it simply offers a valid treatment alternative to selected cases. In this work, we radiologically and clinically evaluated pediatric patients treated with FE for diaphyseal fractures of the lower limb and we compared them with patients treated with standard Titanium Endomedullary Nail (TEN) techniques. Our results confirmed that FE is a valid alternative treatment for these types of fractures.

Fractures of the lower limb in children are common (30-40% of all fractures) and in most cases they are treated conservatively (1).

Different treatments are available: temporary traction (2), conservative treatment with a plaster cast (3), external fixation (4), open reduction and internal fixation (5) or intramedullary stabilization by TEN (Titanium Endomedullary Nail) (6). The treatment choice is determined by factors such as patient age, location and type of fracture (7, 8). Furthermore, some surgical approaches require a prolonged hospital stay, an extended period of inactivity and aesthetically unsatisfactory scars.

Stabilization and synthesis with TEN have gained popularity and it is now a well-recognized method for the treatment of pediatric fractures of long bones as it achieves excellent results by a minimally invasive surgical technique.

However, the synthesis by external fixation offers an alternative and valid treatment opportunity and it allows for some undoubtable advantages such as the early postoperative mobilization, a shorter hospital stay, an early recovery of joint movement and a prompt return to physical activity (9, 10). Thus, in the last years, surgeons showed an increasing interest in this technique because of its advantageous characteristics especially in children with outcomes that are comparable to those obtained with the treatment with TEN. The Monolateral Micrometric Fixator (MMF) consists of two units: the reduction unit, which is used in the first phase; and the fixation unit, which is used in the final part of the surgery. The surgical technique is intuitive and indications for treatment by external fixator are the standard ones: exposed fractures, comminuted fractures,

Key words: children, fracture, external fixation

Corresponding author:

Laura Mangiavini, M.D.,
IRCCS Istituto Ortopedico Galeazzi,
Via R. Galeazzi 4,
20161, Milan, Italy
Tel.: +39 02 6621 4930
E-mail: laura.mangiavini@grupposandonato.it

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fractures previously treated by internal synthesis and not adequately reduced (11).

The aim of the study is to compare the clinical results of pediatric diaphyseal fractures treated with TEN with similar fractures synthetized with FE. The radiological parameters, the bone callus time formation, the pain control, the incidence of vascular-nervous complications and the time to return to sport activities were considered.

MATERIALS AND METHODS

Twenty-eight patients affected by diaphyseal lower limb fractures were retrospectively evaluated from 2016 to 2017. Seventeen patients were males, 11 patients were females with an average age of 8 years (range 6-10 years). All fractures were localized at the lower limbs, of which 15 affecting the tibia and 13 the femur. Eleven tibial fractures

were treated by means of TEN and 4 by MMF external fixator. Eight patients with fractures of the femur were treated by means of TEN and 5 by FE. All treated fractures were closed, presenting different types of displacement.

In the TEN group, the surgical procedure was performed under intraoperative radiographic control; in the first phase, the reduction was obtained, while in the second phase, the nails were inserted into the intramedullary site. Exposure to X-rays was prolonged in this case compared to treatment with FE, where the insertion of the distal and proximal fiche was radiographically guided and undoubtedly faster.

Patients were evaluated clinically and radiologically. In detail, the Visual Analogic Scale (VAS) was used in order to quantify pain while X-rays assessed the time of callus formation. Radiographic controls were planned 1-2 months after surgery with the last check at 3 months before the device removal.

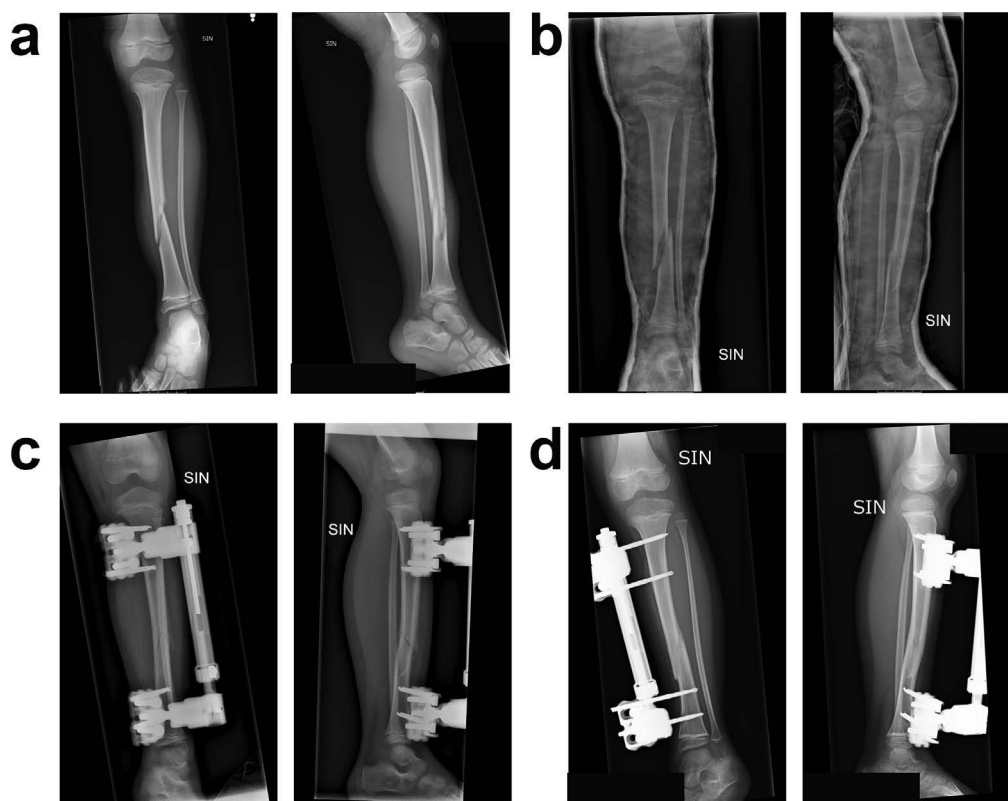


Fig. 1. *a):* Anterior and lateral view of a diaphyseal tibial fracture at time 0; *b):* Conservative treatment attempt with cast. Bony segments displacement is evident in the anterior view; *c):* Anterior and lateral view of the above-described fracture immediately after FE application; *d):* Anterior and lateral view 3 months after surgery.

RESULTS

All patients were clinically evaluated and checked, up to 6 months after surgery. No intraoperative complications have been observed. The average time to perform the TEN reduction and surgery was 45 min in case of femoral fractures (range 43 to 59 min) and 37 minutes (range of 34 to 45 min) in case of tibial fractures. The timing for the FE group was slightly lower: average time for tibial fractures was 33 minutes (range from 28 to 40) and for femoral fractures was 43 minutes (range from 40 to 50). However, these parameters are surgeon-dependent, thus they may present considerable variability.

In 15 patients treated for tibia fracture, the same results were obtained in both groups, satisfying bone healing with bone callus formation and remodeling after 3 months in the FE group (Fig. 1) and in the

TEN group (Fig. 2). Moreover, we recorded good pain control in both treatments with average VAS values of 3 (range 2-5). In both series, an initial attempt of closed reduction with cast was made; however, the failure of conservative treatment and the persistence of bone fragments displacement imposed a surgical treatment.

In the group of patients with diaphyseal femoral fracture, a complete bone healing after 6 months from surgery was obtained both in the TEN and in the FE group. Postoperative pain control was satisfactory in both treatments with mean VAS values of 2 (range 1-4). On the other hand, we noticed a difference in the time to return to sport: patients treated with FE were able to return to their usual sport activity earlier, probably because of their earlier weight bearing. In 2 patients, a superficial infection occurred around the fiches, treated and resolved by antibiotic therapy. No revision surgery was required.



Fig. 2. *a):* Anterior and lateral view of a diaphyseal tibial fracture at time 0; *b):* Conservative treatment attempt with cast. Bony segments displacement is evident in the lateral view; *c):* Anterior and lateral view of the above-described fracture immediately TEN application; *d):* Anterior and lateral view 3 months after surgery.

DISCUSSION

In our study, no significant differences were found between FE and TEN treatment. Indeed, we have achieved an excellent radiological results, with formation of a consistent bone callus at 1 month from the beginning of the treatment. Moreover, we obtained good pain control in both groups. We observed an earlier return to sports activity, probably linked to the immediate weight-bearing allowed by the FE.

The two surgical techniques have advantages and disadvantages; thus, the choice should be evaluated on a case-by-case basis (1, 4, 6). In particular, FE treatment has some unquestionable disadvantages and limitations: a long learning curve of the surgical technique, the patient's compliance, which is not optimal in patients under the age of 5 and the psychological impact linked to the external fixator (9, 10).

On the other hand, FE advantages are numerous and among these, we should consider the early load bearing and the easy removal of the device, which requires a much shorter second surgical time than the TENs. Generally, we can state that the use of FE is not destined only to adolescent patients, as it was in the past, but also to younger patients.

These are tested and safe surgical techniques that have led to constant healing of the fracture. Given the overlap of the two techniques in terms of effectiveness, the choice is mainly based on the patient's compliance level and on the surgeon's experience.

REFERENCES

1. El Hayek T, Abou-Daher A, Meouchy W, Ley P, Chammas N, Griffet J. External fixation in the treatment of fractures in children. *J Pediatr Orthop B* 2004; 13:103-9.
2. Pearce MS. Calcaneal pin traction in the management of unstable tibial fractures. *Aust N Z J Surg* 1993; 63:279-83.
3. Pollak AN, Cooperman DR, Thompson GH. Spica cast treatment of femoral shaft fractures in children e the prognostic value of the mechanism of injury. *J Trauma* 1994; 37:223-9.
4. Blasier RD, Aronson J, Tursky EA. External fixation of pediatric femur fractures. *J Pediatr Orthop* 1997; 17:342-6.
5. Caird MS, Mueller KA, Puryear A, Farley FA. Compression plating of pediatric femoral shaft fractures. *J Pediatr Orthop* 2003; 23:448-52.
6. Buford Jr D, Christensen K, Weatherall P. Intramedullary nailing of femoral fractures in adolescents. *Clin Orthop Relat Res* 1998; 350:85-9.
7. Skaggs DL, Leet AI, Money MD, Shaw BA, Hale JM, Tolo VT. Secondary fractures associated with external fixation in pediatric femur fractures. *J Pediatr Orthop* 1999; 19:582-6.
8. Gonz lez-Herranz P, Burgos-Flores J, Rapariz JM, Lopez-Mondejar JA, Ocete JG, Amaya S. Intramedullary nailing of the femur in children. Effects on its proximal end. *J Bone Joint Surg Br* 1995; 77:262-6.
9. Hedin H, Larsson S. Technique and considerations when using external fixation as a standard treatment of femoral fractures in children. *Injury* 2004; 35:1255-63.
10. Kuremsky MA, Frick SL. Advances in the surgical management of pediatric femoral shaft fractures. *Curr Opin Pediatr* 2007; 19:51-7.
11. Slongo T, Audige' L, AO Pediatric Classification Group (2007). *AO Pediatric Comprehensive Classification of Long-Bone Fractures (PCCF)*. Davos, Switzerland: AO Foundation 2010.

MEDICAL MANAGEMENT OF ACUTE NON-SPECIFIC LOW BACK PAIN: COMPARISON OF DIFFERENT MEDICAL TREATMENTS, ONE CENTER'S RETROSPECTIVE ANALYSIS

A. PERNA^{1,2}, LUCA RICCIARDI^{1,3,4}, GIUSEPPE BARONE⁵, F. C. TAMBURRELLI^{1,2},
L. PROIETTI^{1,2} and ENRICO POLA^{1,2}

¹Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Rome, Italy; ²Division of Spinal Surgery, Catholic University of Sacred Heart, Rome, Italy; ³Departments of Neurosurgery Catholic University of Sacred Heart, Rome, Italy; ⁴Department of Neurosurgery, Mayo Clinic, Jacksonville, FL, United States; ⁵Spinal Division I, IRCCS Orthopedic Institute Galeazzi, Milan, Italy

The first two authors contributed equally to this study

Within the developed countries, low back pain and related ailments represent one of the most relevant conditions, affecting both health and socio-economic systems. Non-specific acute low back pain (nsALBP) could represent either an isolated event or the presenting symptom of an underlying condition. Its management is usually medical and radiological investigations should be avoided. However, a general agreement on the best pharmacological treatment is still missing. In the present study, we retrospectively collected patients' data from the institutional database. Two different medical treatments were recognized and groups consequently set. Outcomes were the clinical status, measured using the visual analogue scale (VAS) score for back pain, the use rate of rescue drugs, side effects related to medical treatment, compliance to the treatment, and global satisfaction. The pharma blending of Tramadol 75 mg and Dexketoprofen 25 mg (available blended in a single tablet), resulted effective in the nsALBP management, with less side effects and rescue-drug use along with a higher compliance. Furthermore, patient satisfaction in this treatment group was significantly higher. Many different medical treatments have been investigated and reported in nsALBP management during the last decades. Associations of different drugs are the most common protocols, even though there is no general agreement. Tramadol and Dexketoprofen provide a good clinical result, fewer side effects and a long-lasting and more effective painkiller action, reducing the use of rescue drugs. Moreover, our results suggest that a single tab may provide a higher compliance rate.

Non-specific acute low back pain (ALBP) is a painful condition, which usually lasts 4 weeks, resulting in spontaneous regression, and its lifetime prevalence ranges from 50% to 80% (1). Daily-life activities, quality of life (QoL) and social interactions may result compromised in those patients (2). Furthermore, sick leave days and

patient care represent an important social cost (1, 7). The diagnosis is based on the clinical and anamnestic evaluation, thus radiological examinations should be at first avoided (4-26). However, recurrent episodes can figure out underlying conditions (24).

Etiological diagnosis is often challenging, especially when a non-specific or concerning

Key words: low back pain, opioids, emergency medicine, pain management, medical management

Corresponding author:

Dr Enrico Pola,
Fondazione Policlinico Universitario Agostino
Gemelli IRCCS,
Largo Agostino Gemelli 8,
00168 Rome, Italy
e-mail: enricopola@hotmail.com

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background is reported. Although acute low back pain can be sustained by multiple factors, both inflammation and mechanics disorders are widely accepted as the main effectors, which may also support each other (3). However, there is no agreement on a gold standard medical treatment; thus, symptomatic therapy is administered, according to scientific evidence (5). Many drug classes have been investigated for this purpose, such as nonsteroidal anti-inflammatory drugs (NSAIDs), skeletal muscle relaxants (SMR), oral opioid analgesics (OOA), benzodiazepines (BDZ), systemic corticosteroids (SCS), antidepressant tricyclic (TCA), and antiepileptic drugs (AED) (3,5).

The association between NSAIDs and SMR usually provide an effective pain control, especially in association with a rescue drug as acetaminophen, so administering three different drugs (5,15). However, a single drug administration could improve patients' compliance if similar clinical outcomes are reported.

The aim of the present study was to retrospective investigate the role a pharma blending, consisting in Dexketoprofen and Tramadol, in non-specific ALBP.

MATERIALS AND METHODS

Setting and institutional database

As the standard of care at our Emergency Department, Fondazione Policlinico Universitario A. Gemelli IRCCS in Rome, patients trialed for non-specific ALBP are managed using a standardized data collection system. From these patients, we systematically collect demographic data (age, sex, BMI, anamnestic record, medical history, chronic therapies, smoke addiction, and alcoholic status), measurement of back pain intensity using a ten itemized point visual analogue scale (VAS), clinical, radiological, and laboratory hospital-reports, treatments received and prescriptions. Every patient is provided by a pain diary where to daily collect VAS rate, drug assumption, side effects. Standard follow-up visits are scheduled at 7 and 15 days after discharge. During the last follow-up visit, patients are asked to express an overall satisfaction rate of our management, in a range from 0 to 10, which is attached to their report.

Study design and its aim

The present investigation consists of a retrospective

database analysis with a set time range was from April 2017 to April 2018. The aim of the present study is to evaluate and compare the effectiveness of two different medical conservative treatments in non-specific ALBP management. The study matches institutional review board (IRB), national ethics criteria, and is in accordance to the Helsinki agreement.

Inclusion and exclusion criteria

According to the diagnosis-related group (DRG), the admitted patient reports for ALBP during the set time range were selected and considered eligible for our review. Study inclusion criteria were age between 18 to 40 years, back pain level from moderate to severe intensity ($VAS \geq 4$), first reported admission for non-specific ALBP. We set as exclusion criteria for pregnancy or women breastfeeding, neoplastic pathologies, story of chronic low back pain (LBP), past lumbar surgery, metabolic bone disease, neuromuscular disease, drugs allergies, moderate to severe renal dysfunction (creatinine clearance <90 ml/min), hepatopathy (MELD Score >9) asthma, epilepsy and history of drugs or alcohol abuse.

Data collection and measurements

From enrolled patients' records, we collected the VAS score at the admission (t_0); medical treatment administered at emergency department, and the prescription for the domiciliary medical therapy; daily VAS score, drugs assumption rate, use of the prescribed rescue drugs and side effects from the pain diaries during the 5 following days; VAS score during scheduled (7-15 days) follow-up visits. Data were reported as the mean $\pm$ standard deviation (SD). During the last follow-up check, patients expressed their satisfaction with the treatment, in a range from 1 to 10, and such evaluation was collected.

Groups' definition and patients' assignment

Patients were stratified by medical treatment and two groups were set:

- Group A: Diclofenac 75 mg and Thiocolchicoside 4 mg at the emergency department, thereafter twice a day for 5 days.
- Group B: Tramadol 75 mg and Dexketoprofen 25 mg (available as blended in a single tablet) at the emergency department, thereafter twice a day for 5 days.

Acetaminophen 1000 mg for 2/die was the suggested rescue drug in each study groups. Concomitant use different drugs or variations in dose administration were restricted at discharge. Any report of drug self-administration other than the rescue prescribed one determined patient' exclusion from the present study.

Outcomes

The primary outcome was identified in pain reduction rate, measured by VAS score. Secondary outcomes were side effects, rescue drug use, and patient satisfaction.

Statistical analysis

Data were statistically analyzed using the *t*-Student

Table I. Table showing the major patient characteristics of the two groups.

Characteristics	Patient data
GROUP A	
Age (yr)	35.8 years (DS 6.33)
Sex (M: F)	26 F, 19 M
BMI	24.8 (DS 3.34)
Comorbidities	
Arterial hypertension	3 patients
Gastric reflux	11 patients
Smoker	17 patients
Obesity	6 patients
Depression	3 patients
GROUP B	
Age (yr)	36.1 years (DS 6.19)
Sex (M: F)	22 F, 20 M
BMI	25.2 (DS 2.87)
Comorbidities	
Arterial hypertension	2 patients
Gastric reflux	13 patients
Smoker	14 patients
Obesity	7 patients
Depression	2 patients

test to compare quantitative variables. The significance was established for a value of $p < 0.05$. Dedicated SPSS statistical calculation software (SOSS Inc, Chicago, IL) was employed.

RESULTS

One-hundred-and-seventy-four patient reports were collected in our retrospective analysis. Only 87 of those [46 female (F), 41 male (M)] matched inclusion and exclusion criteria and were finally included in the present investigation. Forty-five patients (26 F, 19 M) were treated with Diclofenac and Thiocolchicoside (Group A - 51.7%), 42 (22 F, 20 M) with tramadol and dextetoprofen (Group B-48.3%). The two groups were homogeneous for demographic characteristics, as reported in Table I. Fifteen (17.2%) patients did not comply with the treatment protocol due to side effects and were excluded from the study. Among those, 9 patients (5 F, 4 M) were into group A, and 4 reported stomach ache, 2 nausea, 3 diarrhea; 6 patients (4 F, 2 M) were in group B, 4 reported nausea and 2 severe constipation. In Group A the mean VAS score was 7.2 (SD 1.29) at t0, 2.8 (DS 1.43) at 7 days, and 1.3 (DS 1.12) at 15 days.

In Group B the mean VAS score was 7.3 (DS 1.33) at t0, 2.2 (DS 1.12) at 7 days, and 1.1 (DS 0.97) at 15 days (Table II).

In Group A, 35 (77.7%) patients reported the used of the rescue drug in the first day, 31 (68.8%) in the second day, 27 (60%) in the third day, 21 (46.6%) in the fourth day and 15 (33.3%) in the last day. In Group B, 11 (26.2%) patients used the rescue drug on the first day, 10 on the second day (23.8%), 7 (17.3%) on the third day, 5 (11.9%) on the fourth day and 4 (10.2%) on the last day (Table III).

In Group A, 12 patients (5.4%) reported they forgot to take one of the two drugs at least once during the duration of the protocol. In group B, no patient missed any administration. We collected and reported side effects in the two groups in Fig. 1.

DISCUSSION

Background

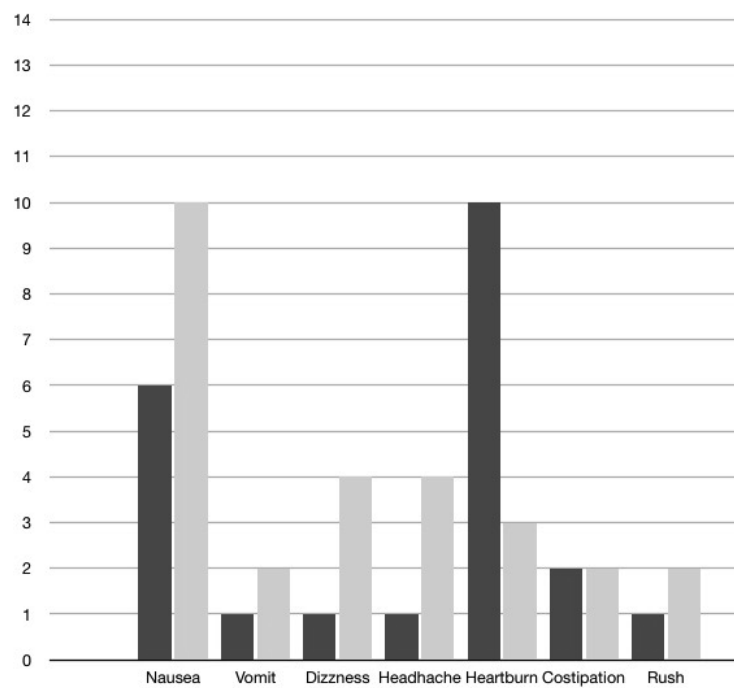
Acute low back pain is one of the most relevant

Table II. Table showing main results.

VAS t0	VAS 7 days	VAS 15 days
GROUP A		
7.2 (SD 1.29)	2.8 (DS 1.43) (P =0.01)	1.3 (DS 1.12) (p= 0.02)
GROUP B		
7.3 (DS 1.33)	2.2 (DS 1.12) (P=0.001)	1.1 (DS 0.97) (p= 0.01)

Table III. Table showing the use of rescue drug.

	GROUP A	GROUP B
DAY 1	35 pt (77.7%)	11 pt (26.2%)
DAY 2	31 pt (68.8%)	10 pt (23.8%)
DAY 3	27 pt (60%)	7 pt (17.3%)
DAY 4	21 pt (46.6%)	5 pt (11.9%)

**Fig. 1.** Most common side effects in the two groups (on the left **group A**, on the right **group B**); Day 1.

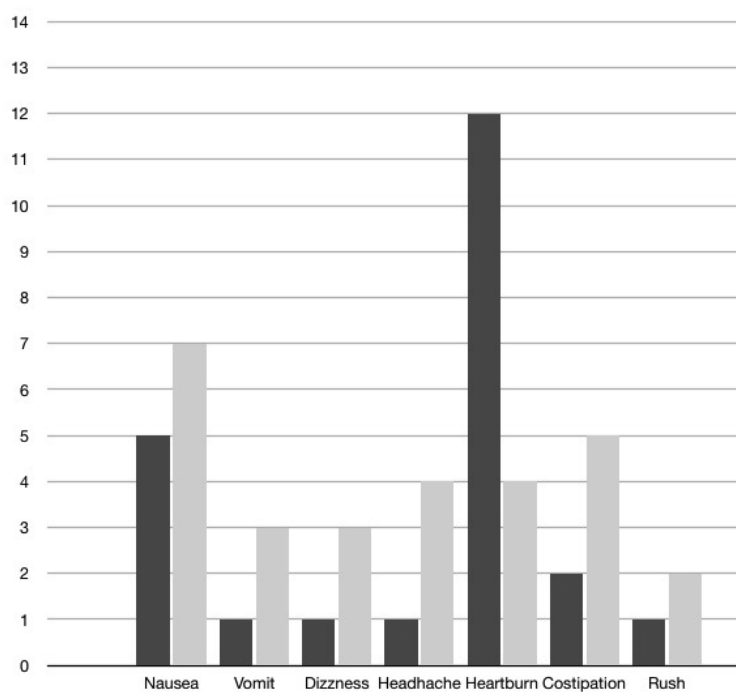


Fig. 2. Most common side effects in the two groups (on the left **group A**, on the right **group B**); Day 2.

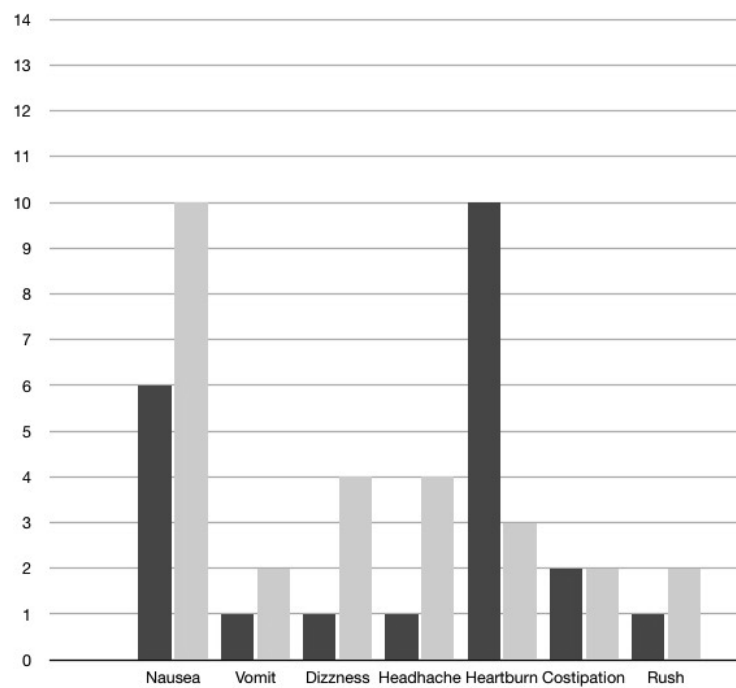


Fig. 3. Most common side effects in the two groups (on the left **group A**, on the right **group B**); Day 3.

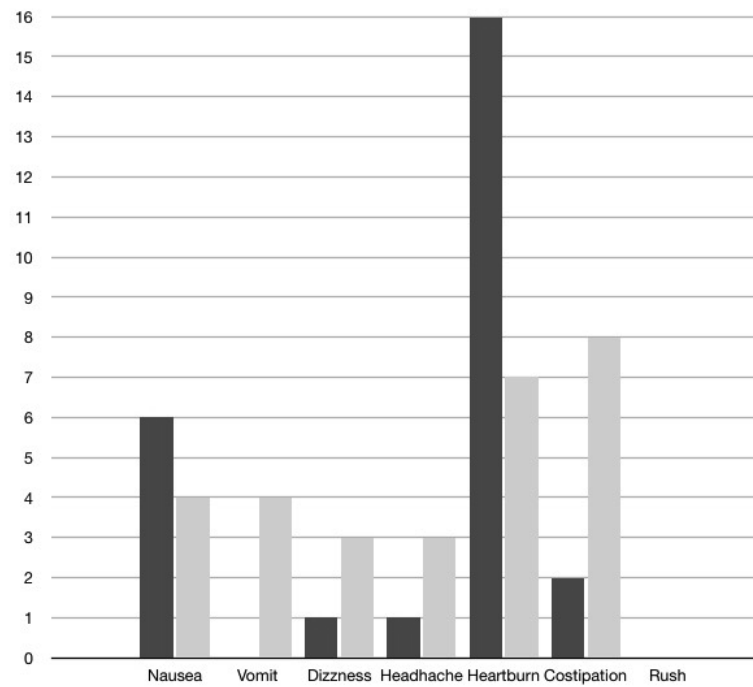


Fig. 4. Most common side effects in the two groups (on the left **group A**, on the right **group B**); Day 4.

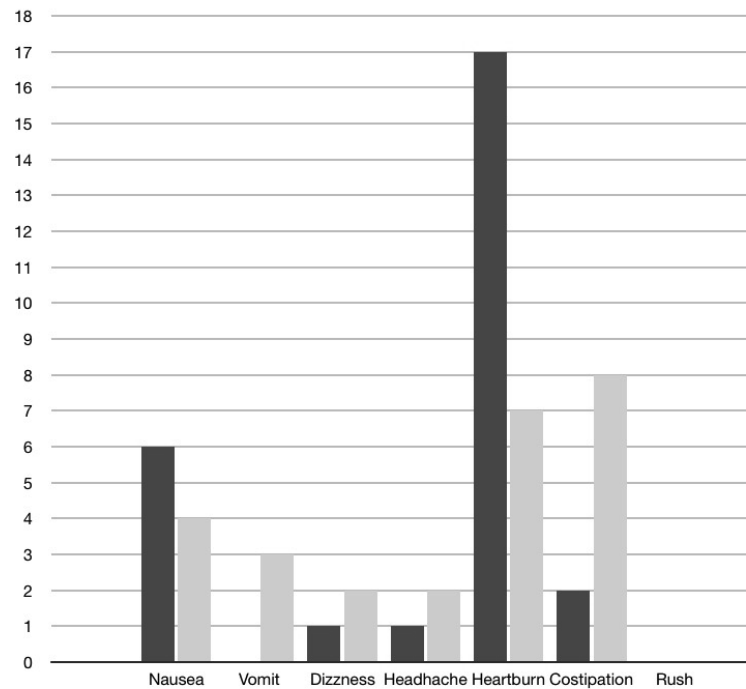


Fig. 5. Most common side effects in the two groups (on the left **group A**, on the right **group B**); Day 5.

health problems in industrialized countries, resulting in a non-negligible cost to society (19). Pain relief, QoL and functional restoration and social cost reduction represent the main goals in ALBP management (15). When not sustained by underlying conditions, ALBP finds its etiology in mechanical and/or inflammatory causes (3). Although a wide array of drugs and medical protocols are available, there is no consensus on a gold standard (16). However, NSAIDs' role in inflammation and pain control has been widely demonstrated, thus they are commonly administered in ALBP management (11). A recent systematic review conducted by Van Tulder MW et al (20) suggests that NSAIDs are poorly effective for short-term improvement in patients with ALBP. Furthermore, no specific NSAID was identified as the most effective, and oral administration demonstrated to be as effective as either intravenous or intramuscular. Moreover, they did not find that the combination with muscles relaxant (MR) improve clinical outcomes.

Muscle spasms represent the main mechanical cause sustaining painful conditions in non-specific ALBP; furthermore, they determine a derived inflammation that sustains them and increases related-pain, determining a self-sustaining condition. Accordingly, MR has been widely administered in ALBP management. However, many related side effects, such as headache, blurred vision, drowsiness, nausea and vomiting, provide concerns about their use (17, 18). Van Tulder MW et al (18) systematically reviewed the literature and found that MR other than BDZ result effective in the reduction of short-term pain in patients with ALBP; however, they should be used with caution having many reported side effects. Recent clinical guidelines restrict the use of MR in those patients NSAIDs non-responders. TCA and SSRIs are effective in controlling neuropathic pain. However, in their meta-analysis Staiger et al (23) have found no evidence supporting TCA usefulness in non-specific ALBP management. OOA result extremely effective in pain relief in many medical conditions. They are commonly administered in non-specific ALBP, but their long-term effects are very limited (21). Furthermore, there is no evidence of better clinical outcomes using OOA when compared

to NSAIDs, which result safer having a lower side-effects rate.

Several studies have shown that the combination of two or more analgesics allow the increase in efficacy and reduce the doses of the individual drugs, consequently the side effects. (9, 10, 21). Accordingly, a combination of Dexketoprofen and Tramadol has been designed to provide central and peripheral analgesia administering lower doses than those used in single drug protocols.

Present investigation

In our series, we observed that VAS and ODI scores significantly decreased within the first week and at the last follow-up measurement in both study groups, without significant differences in their trends. However, pain diaries showed that pain relief following the first drug administration was higher in group B. Furthermore, we found a higher self-administration rate of the rescue drug during the domiciliary treatment in Group A, due to the drug combination features in group B, which have dexketoprofen as quick-acting agent and tramadol which provides long-term effects (25).

In group A, both higher side effects and non-compliant patient rates were reported while those reported in group B were more tolerant and compliant. The most influencing side effect was found to be heartburn, reported in 17 cases in group A and 3 in group B.

Compliance is higher when a single drug is administered rather than two or more. A single tablet administered once per day containing a blending of Tramadol and Dexketoprofen could be more effective in terms of patient compliance. In agreement with this hypothesis, in Group B there was no missed dose administration, whereas in group A, 12 patients forgot, at least once, to take either the SMR or the NSAID.

Moreover, patient satisfaction is often not investigated. As the standard of care, at our department we administer five points itemized scale to patients during their last follow-up check, to estimate the overall satisfaction about the treatment they received. Thus, this retrospective investigation allowed us to collect a patient-oriented satisfaction score and to compare the two groups. This analysis

showed that those patients included in group B reported a higher mean satisfaction rate compared to group A (mean 2.9 SD 1.2 vs 4.2 SD 0.9, $p=0.02$). Accordingly, the single tablet blending of tramadol and dextetoprofen once per day may also provide a higher satisfaction rate.

Country-restricted cost analysis

We also conducted a cost analysis comparing the two groups. In Italy (26) the cost of the entire treatment (including 10 administrations), was €7.11 (\$8.24) in Group A and €6.45 (\$7.84) in Group B. Furthermore, the rescue drug had a cost of €7.40 (\$8.47) for 16 tabs and, having its mean usage data in investigated treatments groups, the cost estimation is €2.65 (\$3.03) and €0.65 (\$0.74) in two weeks. Therefore, the whole treatment cost is estimated to be higher in Group A.

Limitations

Among the limitations of the present investigation, we report as most relevant: the study design as retrospective investigation, small patients sample, only two treatments compared. Further and better-designed studies are mandatory to better investigate this topic.

DISCUSSION

Even though many treatments have been investigated, there is no agreement on a gold standard treatment in non-specific ALBP management. According to our results, dextetoprofen and tramadol, blended in a single tablet, may provide good clinical outcomes and increase patient satisfaction and compliance. Furthermore, the cost of this treatment is significantly lower.

REFERENCES

1. Deyo RA, Mirza SK, Martin BI. Back pain prevalence and visit rates. *Spine* 2006; 31(23):2724-7.
2. Pengel LHM, Herbert RD, Maher CG, et al. Acute low back pain: systematic review of its prognosis. *BMJ* 2003; 327(7410):323-7.
3. Cherkin DC, Wheeler KJ, Barlow W, Deyo RA. Medication use for low back pain in primary care. *Spine* 1998; 23:607-14.
4. Van Tulder MW, Assendelft WJ, Koes BW, et al. Spinal radiographic findings and nonspecific low back pain: a systematic review of observational studies. *Spine* 1997; 22(4):427-34.
5. Chou R, Huffman LH, American Pain Society, et al. Medications for acute and chronic low back pain: a review of the evidence for an American Pain Society/American College of Physicians clinical practice guideline. *Ann Intern Med* 2007; 147(7):505-14.
6. Barden J, Edwards JE, McQuay HJ, Moore RA. Pain and analgesic response after third molar extraction and other postsurgical pain. *Pain* 2004; 107:86-90.
7. Steven P. Cohen, Charles E. Argoff, Eugene J. Carragee. Clinical Review: Management of low back pain. *BMJ* 2008; 337:a2718.
8. Andersson GB. Epidemiology of low back pain. *Acta Orthop Scand Suppl* 1998; 281:28-31.
9. McQuay HJ, Derry S, Eccleston C, Wiffen PJ, Moore RA. Evidence for analgesic effect in acute pain - 50 years on. *Pain* 2012; 153(7):1364-7.
10. Moore RA, Derry S, McQuay HJ, Wiffen PJ. Single dose oral analgesics for acute postoperative pain in adults. *Cochrane Database Syst Rev* 2011; 7(9):CD008659
11. Moore RA, Edwards J, Barden J, McQuay HJ. *Bandolier's Little Book of Pain*. Oxford: Oxford University Press 2003.
12. Barden J, Derry S, McQuay HJ, Moore RA. Single dose oral ketoprofen and dextetoprofen for acute postoperative pain in adults. *Cochrane Database Syst Rev* 2009; 7(4):CD007355.
13. Hernández-Díaz S, García Rodríguez LA. Association between nonsteroidal anti-inflammatory drugs and upper gastrointestinal tract bleeding/perforation: An overview of epidemiologic studies published in the 1990s. *Archives of Internal Medicine* 2000; 160:2093-9.
14. Barbanoj MJ, Antonijon RM, Gich I. Clinical pharmacokinetics of dextetoprofen. *Clinical Pharmacokinetics* 2001; 40(4):245-62.
15. Schnitzer TJ, Ferraro A, Hunsche E, Kong SX. A comprehensive review of clinical trials on the efficacy and safety of drugs for the treatment of low back pain. *J Pain Symptom Manage* 2004; 28:72-95.

16. Borenstein DG. A clinician's approach to acute low back pain. *Amer J Med* 1997; 102(1A):16S-22S.
17. Cherkin DC, Wheeler KJ, Barlow W, Deyo RA. Medication use for low back pain in primary care. *Spine* 1998; 23(5): 607-14.
18. van Tulder MW, Touray T, Furlan AD, Solway S, Bouter LM. Muscle relaxants for non-specific low back pain. *Cochrane Database Syst Rev* 2003; CD004252.
19. Deyo RA, Cherkin D, Conrad D, et al. Cost, controversy, crisis: Low back pain and the health of the public. *Annu Rev Public Health* 1991; 12:141-56.
20. van Tulder MW, Scholten RJ, Koes BW, Deyo RA. Nonsteroidal anti-inflammatory drugs for low back pain: a systematic review within the framework of the Cochrane Collaboration Back Review Group. *Spine* 2000; 25:2501-13.
21. Bigos S, Bowyer O, Braen G, et al. Acute Low Back Problems in Adults: Clinical Practice Guideline No. 14. AHCPR Publication No. 95-0642. Rockville, MD: Agency for Health Care Policy and Research, Public Health Service, U.S. Department of Health and Human Services, December 1994.
22. Moore RA, Derry CJ, Derry S, Straube S, McQuay HJ. A conservative method of testing whether combination analgesics produce additive or synergistic effects using evidence from acute pain and migraine. *Eur J Pain* 2012; 16(4):585-91.
23. Staiger TO, Gaster B, Sullivan MD, Deyo RA. Systematic review of anti-depressants in the treatment of chronic low back pain. *Spine* 2003; 28:2540-5.
24. Tannor AY. Lumbar Spine X-Ray as a Standard Investigation for all Low back Pain in Ghana: Is It Evidence Based? *Ghana Med J* 2017; 51(1):24-29.
25. Varrassi G, Hanna M, Macheras G, Montero A, Montes Perez A, Meissner W, Perrot S, Scarpignato C. Multimodal analgesia in moderate-to-severe pain: a role for a new fixed combination of dexketoprofen and tramadol. *Curr Med Res Opin* 2017; 33(6):1165-73
26. <https://www.federfarma.it/Farmacie-e-farmacie/Cerca-un-farmaco.aspx>.

REINFUSION-DRAINS REDUCE BLOOD TRANSFUSIONS IN TOTAL JOINT ARTHROPLASTY

O. GALASSO¹, D. CASTIONI¹, M. MERCURIO¹, M. STICOZZI³, E. PORCO¹,
M. DE GORI² and G. GASPARINI¹

¹*Department of Orthopaedic and Trauma Surgery, "Magna Graecia" University, Catanzaro, Italy;*

²*Department of Orthopaedic and Trauma Surgery, "S. Francesco di Paola" hospital, Paola, Italy;*

³*Department of Orthopaedic and Trauma Surgery, Vigevano civil hospital, Vigevano, Italy*

This study aims to evaluate the effect of postoperative blood recovery with reinfusion drains on hematologic parameters and blood transfusion rate in patients undergoing total joint arthroplasty. Three-hundred-and-forty-four patient records were reviewed and 271 patients were included in the study; 56.8% of patients were treated with postoperative cell salvage procedure using reinfusion drains (PCS) and 43.2% had closed-suction drain (CSD) postoperatively. In comparison to the CSD group, the PCS group showed higher hemoglobin (Hb) levels on the first and second days postoperatively but no statistical differences were noted at the day of discharge. 75.2% and 37.7% of patients required blood transfusions in the CSD and PCS groups, respectively. The PCS group had a lower number of blood transfusions than the CSD group. At multivariate analysis, Hb loss rate was related to preoperative Hb values, total amount of drained blood and chronic antiplatelet therapy. The number of blood transfusions was related to preoperative Hb values, closed-suction drains, preoperative platelet count, TKA surgery and BMI. This study supports the use of PCS with reinfusion drains after THA and TKA at least for the short-term.

Total hip and knee arthroplasties (THAs and TKAs) have proven to be effective surgical treatments for degenerative conditions of the joints. These procedures are becoming more frequently used and approximately 7 million Americans are living with a hip or knee replacement (1). Important cost elements in total joint arthroplasty (TJA) are blood management and the transfusion strategy (2).

Blood loss strongly influences the time of recovery after surgery and the quality of the convalescence. Higher pre- and postoperative hemoglobin (Hb) levels are correlated with early functional recovery, higher patient satisfaction and a shorter hospital stay with a reduced red cell transfusion rate (3). Bleeding during surgical procedures depends on the

technique and the duration of the surgical procedure, hemostatic accuracy, the patient's comorbidities and the use of some drugs (2). Many strategies to reduce blood loss, such as diathermy coagulation, the use of tourniquets, patient positioning, hypotensive anesthesia, clamping drains, norepinephrine (4), and saline and tranexamic acid infiltrations have been used (2).

In recent decades, a variety of approaches have been developed to reduce the use of allogenic blood transfusions. Preoperative autologous blood donation (PABD) and postoperative cell salvage (PCS) were proposed in TJAs but the real effect is unclear. Some studies (5, 6) have reported that PABD and PCS can reduce allogenic blood usage and restore basal Hb

Key words: Total joint arthroplasty, closed-suction drains, postoperative cell salvage, reinfusion drain, blood loss, blood transfusion

Corresponding author:

Dr Michele Mercurio,

Department of Orthopaedic and Trauma Surgery,

"Magna Graecia" University, Catanzaro,

Viale Europa,

88100, Catanzaro, Italy

e-mail: mercuriomi@gmail.com

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concentrations; Cushner et al. (7) criticized PABD for being associated with a higher risk of anemia in the postoperative period while Horstmann et al. (8) showed the opposite effect of PCS restoring Hb concentrations. Despite these inconsistent data, many surgeons continue to use drains combined with a collection and reinfusion system after TJAs. The aim of this study was to evaluate the effect of postoperative blood recovery with reinfusion drains on hematologic parameters and blood transfusion rates in patients undergoing THA or TKA.

MATERIALS AND METHODS

Data collection

We reviewed the surgery reports of 344 patients who underwent primary and elective THA or TKA at our institution between January 2009 and May 2016. The exclusion criteria were as follows: coagulation disorders; deep venous thrombosis; pulmonary embolisms; malignancy; ongoing or previous infections; HIV; myocardial infarctions within the past 12 months; intake of anticoagulant therapy. THA was performed using either a modified posterolateral or an anterolateral approach with a non-cemented implant. In TKA, a high tourniquet was applied with a midline skin incision, a medial parapatellar approach, and all components were cemented (9).

Two-hundred-and-seventy-one patients were included in the study: 154 underwent PCS with a reinfusion drain (ATS Bulb set Redax SpA, Poggio Rusco, MN, Italy) and 117 had a closed-suction drain (CSD) and served as a control. For both groups of patients, the drain was placed intra-articularly before closure and was removed 24h postoperatively. All the patients were treated with 4,000 IU low-molecular-weight enoxaparin sodium (Clexane®, Sanofi-Aventis, Milan, Italy) for venous thromboembolism prophylaxis 12h prior to surgery, which was continued for 30 or 35 days (TKA or THA, respectively) after surgery.

Preoperative variables included the patient's age, body mass index (BMI), and the presence of diabetes mellitus, kidney and cardiovascular disease. Hb, hematocrit, platelets, sodium, potassium and sideremia were analyzed preoperatively and at 6, 24, 48, 72 and 96 h after surgery and at the day of discharge. INR and aPTT were evaluated preoperatively, at 6h after surgery and at the day of discharge. The type of anesthesia, total drainage volume,

amount of retransfused blood and units of allogenic red cells transfused were also analyzed. The Hb loss rate (HbLR) was computed using the following formula to evaluate the blood loss of patients at hospital discharge: $HbLR = (\text{preoperative Hb} - \text{Hb at discharge}) / \text{preoperative Hb} \times 100$.

Reinfusion and transfusion criteria

Both drain systems were activated 30 min after surgery. Blood collected was reinfused within 6h postoperatively in PCS group and, successively, the device functioned as a closed-suction drain. A minimum drainage volume of 150 ml was required to reinfuse, and reinfusion of more than 1,500 ml was not allowed. Postoperatively, the indication for an allogenic packed red cell transfusion was influenced by the clinical signs of anemia, a history of cardiovascular disease and an Hb level below 8.0 g/dL.

Statistical analysis

All data were collected, measured and reported within one-decimal accuracy. Mean and standard deviation comparison of the PCS and CSD groups was performed using an unpaired *t*-test or the Mann-Whitney *U* test when appropriate; a 2x2 contingency table was used to compare categorical variables. Uni- and multi-variate linear regressions were performed on the whole population to test possible predictors of outcomes as previously done (10). Explanatory and confounding variables included in the analysis were as follows: gender (categorical), age (continuous), BMI (continuous), hypertension (categorical), diabetes (categorical), kidney disease (categorical), cardiovascular disease (categorical), preoperative Hb (continuous), preoperative hematocrit (continuous), preoperative platelet (continuous), preoperative natremia (continuous), preoperative kalemia (continuous), preoperative sideremia (continuous), preoperative aPTT (continuous), preoperative INR (continuous), preoperative antiplatelet therapy (categorical), preoperative iron supplementation (categorical), preoperative folate supplementation (categorical), preoperative autologous blood donation (categorical), reinfusion (categorical), number of transfusion (continuous), anesthesia (categorical), surgery (categorical), drained blood (continuous), and reinfused blood (continuous). Hb loss rate (continuous) and number of transfusions (continuous) were treated as outcomes,

and the effect of possible predictors was checked for their value. Only explanatory and confounding variables that showed a trend toward an association with the outcome of interest (e.g., $P < 0.10$) in the univariate analysis were inserted in multiple regression analysis (11). A p value of less than 0.05 was considered significant. IBM SPSS Statistics (version 21.0.0.1, IBM Corp., Armonk, NY, USA) and PRISMA GraphPad software were used for database construction and statistical analysis.

RESULTS

Two-hundred-and-seventy-one patients were evaluated and demographics are reported in Table I. The PCS and CSD groups consisted of 154 (56.8%) and 117 (43.2%) patients, respectively. As shown in Table II, there was a similar distribution between groups considering the number of patients, gender, the type of surgery (i.e., THA and TKA), comorbidities and preoperative levels of Hb and platelets. The PCS group showed a significantly higher preoperative hematocrit and BMI ($P = 0.0001$ and $P = 0.001$, respectively) in comparison to the CSD group.

The PCS group showed higher Hb levels on the first and second days postoperatively in comparison to CSD ($P = 0.001$ and $P < 0.0001$, respectively) but no significant differences were noted at the day of discharge ($P = 0.240$). The PCS group showed a higher HbLR than the CSD group ($P = 0.001$); however, 37.7% and 75.2% of the patients required blood transfusions in the PCS and CSD groups, respectively. The PCS group had a lower number of blood transfusions than the CSD group ($P < 0.0001$). Tables III and IV show the relationship found with the univariate analysis between the pre- or postoperative variables and HbLR or the number of transfusions, respectively.

Using the multivariate analysis, HbLR was related to preoperative Hb values ($\beta = 0.533$; $P < 0.0001$), total amount of drained blood ($\beta = 0.163$; $P = 0.035$) and chronic antiplatelet therapy ($\beta = 0.169$; $P = 0.028$). This model accounted for 25.6% of the total variance of HbLR. Using the multivariate analysis, the number of blood transfusions was related to preoperative Hb values ($\beta = -0.307$; $P = 0.002$), closed-

suction drains ($\beta = 0.254$; $P < 0.0001$), preoperative platelet values ($\beta = -0.204$; $P = 0.001$), TKA surgery ($\beta = 0.138$; $P = 0.028$) and BMI ($\beta = -0.307$; $P = 0.002$).

Table I. Demographics of the study population.

N. of patients (%)	271 (100)
Age (years)	66 $\pm$ 9
Male- n. (%)	94 (34.7)
Female - n. (%)	177 (65.3)
BMI	30.2 $\pm$ 5.3
Cardiovascular disease - n. (%)	170 (62.7)
Diabetes - n. (%)	52 (19.2)
Kidney disease - n. (%)	22 (8.1)
THA - n. (%)	123 (45.4)
TKA - n. (%)	148 (54.6)
Preop. Hb (g/dl)	13.5 $\pm$ 1.5
Preop. Ht (%)	40.4 $\pm$ 5.4
Preop. Plt ($\times 10^3/\mu\text{l}$)	268.6 $\pm$ 72.6
Preop. sideremia ($\mu\text{g/dl}$)	71.5 $\pm$ 22.8
Preop. natremia (mEq/l)	141.6 $\pm$ 2.9
Preop. kalemia (mEq/l)	4.5 $\pm$ 0.4
Preop. aPTT (sec.)	27.5 $\pm$ 3.9
Preop. INR	1.0 $\pm$ 0.2
Preop. antiplatelet therapy - n. (%)	65 (24)
Preop. iron supplementation - n. (%)	12 (4.4)
Preop. folate supplementation - n. (%)	10 (3.7)
PABD - n. (%)	105 (38.7)
Blood transfusions	1 $\pm$ 1
General anesthesia - n. (%)	195 (72)
Spinal anesthesia - n. (%)	76 (28)
Amount of drained blood	492.7 $\pm$ 311.2
Amount of reinfused blood	270.8 $\pm$ 146.4

Plus-minus values are mean $\pm$ standard deviation. BMI: body mass index; THA: total hip arthroplasty; TKA: total knee arthroplasty; Preop: preoperative; Hb: hemoglobin; Ht: hematocrit; Plt: platelets; Aptt: activated partial thromboplastin time; INR: international normalized ratio; PABD: Preoperative autologous blood donation.

This model accounted for 29.1% of the total variance of the number of blood transfusions.

DISCUSSION

Inconsistent data have been reported previously for the use of PCS or CSD in TJA. In the current study, PCS had higher Hb levels at 24 and 48 h postoperatively than in the CSD group. Similarly, a recent meta-analysis (12) demonstrated higher Hb levels at 24 hours after THA in the PCS group than in the CSD group. There were no statistically significant differences in Hb levels from the third postoperative day to discharge, confirming previous data for TKA (13) and THA (12). Jones et al. (14) found no difference between the two groups, while Kirkos et al. (15) showed an opposite result with higher Hb levels at discharge in the PCS group.

Al-Zahid et al. (16) compared PCS, CSD and a no-drain group and failed to find statistically significant differences between the three groups.

In the current study, the PCS group had lower units of allogenic blood transfusions in comparison to the CSD group. Fewer units of allogenic blood transfusions allow reduction of the risk involved in this procedure: transmission of blood borne infections, transfusion-related reactions, blood group transfusion errors and high related costs. In agreement with these data, we next demonstrated a statistically significant association between a higher number of transfusions and the use of CSD. There are many studies with controversial results on the use of drainage with closed aspiration after TKA (17). In a recent study, Huang et al. evaluated patient- and surgery-related factors associated with risk of allogenic blood transfusion and showed that

Table II. Characteristics of the PCS and CSD group.

	PCS group	CSD group	<i>P</i>
N. of patients (%)	154 (56.8)	117 (43.2)	0.074
Age (years)	65.6 ± 10.1	67.4 ± 7.9	0.253
Male - n. (%)	63 (40.9)	31 (26.5)	0.089
Female - n. (%)	91 (59.1)	86 (73.5)	0.286
BMI	31.1 ± 5.7	28.8 ± 4.4	0.001
Cardiovascular disease - n. (%)	142 (92.2)	78 (66.7)	0.095
Diabetes - n. (%)	37 (24)	15 (12.8)	0.065
Kidney disease - n. (%)	15 (9.7)	7 (6)	0.372
THA - n. (%)	71 (46.1)	52 (44.4)	0.913
TKA - n. (%)	83 (53.9)	65 (55.6)	0.918
Preop. Hb (g/dl)	13.7 ± 1.5	13.3 ± 1.5	0.068
Preop. Ht (%)	41.7 ± 4.7	38.7 ± 5.8	< 0.0001
Preop. Plt (x 10 ³ /μl)	265.5 ± 74.7	272.6 ± 69.9	0.333

PCS: post-operative cell salvage; *CSD*: closed-suction drain; Plus-minus values are mean ± standard deviation; *BMI*: body mass index; *THA*: total hip arthroplasty; *TKA*: total knee arthroplasty; *Preop.*: preoperative; *Hb*: hemoglobin; *Ht*: hematocrit; *Plt*: platelets; bold indicates statistically significant parameters.

Table III. Univariate linear regression analysis of predictors of hemoglobin loss rate (HbLR).

	β	P
Gender (F/M)	0.197	0.004
Age	0.011	0.870
BMI	0.778	0.437
Diabetes mellitus	0.035	0.620
Kidney disease	0.048	0.491
Cardiovascular disease	0.039	0.576
Preoperative Hb	0.473	< 0.0001
Preoperative Ht	0.353	< 0.0001
Preoperative Plt	-0.078	0.264
Preoperative natremia	-0.227	0.019
Preoperative kalemia	0.040	0.685
Preoperative sideremia	0.275	0.001
Preoperative aPTT	0.128	0.120
Preoperative INR	-0.005	0.950
Chronic antiplatelet therapy	0.185	0.018
Preoperative iron supplementation	0.032	0.701
Preoperative folate supplementation	-0.009	0.915
PABD	-0.740	0.289
Number of transfusions	-0.029	0.678
General/spinal anesthesia	0.014	0.848
Hip/knee replacement	-0.730	0.294
Amount of drained blood	0.109	0.117
Amount of reinfused blood	0.208	0.031
CSD/PCS	0.222	0.001

F: female; M: male; PCS: post-operative cell salvage; CSD: closed-suction drain; BMI: body mass index; Hb: hemoglobin; Ht: haematocrit; Plt: platelets; aPTT: activated partial thromboplastin time; INR: international normalized ratio; PABD: Preoperative autologous blood donation; bold indicates statistically significant parameters.

Table IV. Univariate linear regression analysis of predictors of the number of transfusions.

	β	P
Gender (F/M)	-0.256	< 0.0001
Age	0.206	0.001
BMI	-0.156	0.020
Diabetes mellitus	-0.007	0.910
Kidney disease	-0.047	0.442
Cardiovascular disease	-0.390	0.517
Preoperative Hb	-0.355	< 0.0001
Preoperative Ht	-0.342	< 0.0001
Preoperative Plt	-0.143	0.019
Preoperative natremia	0.264	0.001
Preoperative kalemia	0.033	0.680
Preoperative sideremia	-0.156	0.029
Preoperative aPTT	-0.006	0.937
Preoperative INR	0.017	0.794
Chronic antiplatelet therapy	-0.095	0.171
Preoperative iron supplementation	0.165	0.044
Preoperative folate supplementation	0.060	0.465
PABD	0.048	0.432
HbLR	-0.290	0.678
General/spinal anesthesia	-0.073	0.252
Hip/knee replacement	0.154	0.011
Amount of drained blood	0.061	0.320
Amount of reinfused blood	0.069	0.371
CSD/PCS	-0.342	< 0.0001

F: female; M: male; PCS: post-operative cell salvage; CSD: closed-suction drain; BMI: body mass index; Hb: haemoglobin; Ht: haematocrit; Plt: platelets; aPTT: activated partial thromboplastin time; INR: international normalized ratio; PABD: Preoperative autologous blood donation; HbLR: Hb loss rate; bold indicates statistically significant parameters

drain use was associated with a higher number of transfusions (18). In a prospective study, Simpson et al. compared the reinfusion discharges with CSD discharges and reported a significantly lower rate of transfusion increases in TKA-treated patients treated with reinfusion (19). Recent meta-analyses showed that an autologous blood transfusion drainage system was more efficacious than CSD in reducing the blood transfusion rate after TKA (13, 20) and THA (12). In contrast, Martin et al. demonstrated that the use of a reinfusion system did not reduce the homologous transfusion requirements and blood loss was significantly higher in patients who underwent CSD compared to those who were reinfused (21). Perazzo et al. (2) found that the PCS group had much greater blood loss than the CSD group, but no differences were observed in terms of decreases in the Hb levels and transfusion rate.

The current study showed that BMI is inversely correlated with the number of blood transfusions. However, this finding should be interpreted with caution because the PCS group showed a statistically significant higher BMI than the CSD group. Pola et al. demonstrated that the risk of perioperative blood transfusion in patients undergoing elective THA was associated with a synergistic effect of factors such as age, sex, hypertension and BMI (22). A systematic examination (23) on the relationship between BMI and perioperative blood transfusion during THA and TKA found that patients with increased BMIs had lower transfusion rates and lost a significantly smaller percentage of estimated blood volume.

We next demonstrated that the preoperative Hb levels were inversely related to the number of blood transfusions and directly related to the HbLR. A recent analysis of a large dataset of 15,187 patients undergoing primary THA or TKA showed that higher preoperative Hb was associated with decreased risk for allogenic blood transfusion (18). Similarly, Gordon et al. (17) determined that in patients treated with PCS, lower preoperative Hb levels carried a significant risk for transfusion after TKA. Considering THA, Aderinto et al. demonstrated that only preoperative Hb and BMI were significantly independent factors increasing the need for transfusion (24).

In the current study, a lower preoperative platelet count value was related to a higher number of transfusions, and as expected, HbLR was related to chronic preoperative antiplatelet therapy. In a multivariate analysis of blood loss during primary THA or TKA the preoperative platelet count was one of the characteristics that affected the amount of blood loss in TKA but not in THA (25).

Interestingly, we noted that TKAs were associated with a higher number of blood transfusions than THAs. Jones et al. (14) found that the type of surgery (THA or TKA) and the use of a tourniquet (in TKA) did not alter the requirements for allogenic blood transfusions. In contrast, Perazzo et al. (2) found that the transfusion rate was higher in the THA patients in comparison to the TKA group.

The limits of this study are the retrospective nature, the non-randomized design and the relatively small number of patients involved. The evaluation of patients up to their discharge from the hospital represents a further weakness of the study. Indeed, we could not report the effect of the PCS on a longer follow-up time. Despite the limitations mentioned above, this study supports the use of PCS with reinfusion drains after THA and TKA at least for the short-term. A larger, randomized and prospective study is needed to draw firm conclusions for the routine use of PCS in patients undergoing TJA.

REFERENCES

1. Maradit Kremers H, Larson DR, Crowson CS, et al. Prevalence of Total Hip and Knee Replacement in the United States. *J Bone Joint Surg Am* 2015; 97:1386-97.
2. Perazzo P, Viganò M, De Girolamo L, Verde F, Vinci A, Banfi G, Romagnoli S. Blood management and transfusion strategies in 600 patients undergoing total joint arthroplasty: an analysis of pre-operative autologous blood donation. *Blood Transfus* 2013; 11:370-6.
3. Husted H, Holm G, Jacobsen S. Predictors of length of stay and patient satisfaction after hip and knee replacement surgery: fast-track experience in 712 patients. *Acta Orthop* 2008; 79:168-73.
4. Gasparini G, Papaleo P, Pola P, Cerciello S, Pola

- E, Fabbriani C. Local infusion of norepinephrine reduces blood losses and need of transfusion in total knee arthroplasty. *Int Orthop* 2006; 30:253-6.
5. Wong CJ, Vandervoort MK, Vandervoort SL, et al. A cluster-randomized controlled trial of a blood conservation algorithm in patients undergoing total hip joint arthroplasty. *Transfusion* 2007; 47:832-41.
 6. Muñoz M, Ariza D, Campos A, Martín-Montañez E, Pavia J. The cost of post-operative shed blood salvage after total knee arthroplasty: an analysis of 1,093 consecutive procedures. *Blood Transfus* 2013; 11:260-71.
 7. Cushner FD, Hawes T, Kessler D, Hill K, Scuderi GR. Orthopaedic-induced anemia: the fallacy of autologous donation programs. *Clin Orthop* 2005; 431:145-9.
 8. Horstmann WG, Kuipers BM, Slappendel R, Castelein RM, Kollen BJ, Verheyen CC. Postoperative autologous blood transfusion drain or no drain in primary total hip arthroplasty? A randomised controlled trial. *Int Orthop* 2012; 36:2033-9.
 9. Gasparini G, De Gori M, Calonego G, Della Bora T, Caroleo B, Galasso O. Drug elution from high-dose antibiotic-loaded acrylic cement: a comparative, in vitro study. *Orthopedics* 2014; 37:e999-1005.
 10. Mariconda M, Galasso O, Secondulfo V, Cozzolino A, Milano C. The functional relevance of neurological recovery after lumbar discectomy: a follow-up of more than 20 years. *J Bone Joint Surg Br* 2008; 90:622-8.
 11. Galasso O, Mariconda M, Donato G, et al. Histopathological, clinical, and electrophysiological features influencing postoperative outcomes in carpal tunnel syndrome. *J Orthop Res* 2011; 29:1298-304.
 12. Xie H, Pan JK, Hong KH, Guo D, Fang J, Yang WY, Liu J. Postoperative autotransfusion drain after total hip arthroplasty: a meta-analysis of randomized controlled trials. *Sci Rep* 2016; 6:27461.
 13. Hong KH, Pan JK, Yang WY, Luo MH, Xu SC, Liu J. Comparison between autologous blood transfusion drainage and closed-suction drainage/no drainage in total knee arthroplasty: a meta-analysis. *BMC Musculoskelet Disord* 2016; 17:142.
 14. Jones HW, Savage L, White C, Goddard R, Lumley H, Kashif F, Gurusamy K. Postoperative autologous blood salvage drains--are they useful in primary uncemented hip and knee arthroplasty? A prospective study of 186 cases. *Acta Orthop Belg* 2004; 70:466-73.
 15. Kirkos JM, Krystallis CT, Konstantinidis PA, Papavasiliou KA, Kyrkos MJ, Ikonomidis LG. Postoperative re-perfusion of drained blood in patients undergoing total knee arthroplasty: is it effective and cost-efficient?. *Acta Orthop Belg* 2006; 72:18-23.
 16. Al-Zahid S, Davies A. Closed suction drains, reinfusion drains or no drains in primary total knee replacement? *Ann R Coll Surg Engl* 2012; 94:347-50.
 17. Gordon AC, Pulido P, Copp SN, Ezzet KA, Walker RH, Colwell CW Jr. The Use of Reinfusion Drains after Total Knee Arthroplasty in Patients Treated with Low Molecular Weight Heparin for Thromboembolic Prophylaxis. *HSS Journal* 2005; 1:19-24.
 18. Huang Z, Huang C, Xie J, et al. Analysis of a large data set to identify predictors of blood transfusion in primary total hip and knee arthroplasty. *Transfusion* 2018; 58:1855-62.
 19. Simpson MB, Murphy KP, Chambers HG, Bucknell AL. The effect of postoperative wound drainage reinfusion in reducing the need for blood transfusions in elective total joint arthroplasty: a prospective, randomized study. *Orthopedics* 1994; 17:133-7.
 20. Pan JK, Hong KH, Xie H, Luo MH, Guo D, Liu J. The efficacy and safety of autologous blood transfusion drainage in patients undergoing total knee arthroplasty: a meta-analysis of 16 randomized controlled trials. *BMC Musculoskelet Disord* 2016; 17:452.
 21. Martin A, von Strempel A. Transfusion of autologous blood from reinfusion systems in total knee arthroplasty. *International Orthopaedics* 2006; 30:541-4.
 22. Pola E, Papaleo P, Santoliquido A, Gasparini G, Aulisa L, De Santis E. Clinical factors associated with an increased risk of perioperative blood transfusion in nonanemic patients undergoing total hip arthroplasty. *J Bone Joint Surg Am* 2004; 86A:57-61.
 23. Frisch N, Wessell NM, Charters M, Peterson E, Cann B, Greenstein A, Silverton CD. Effect of Body Mass Index on Blood Transfusion in Total Hip and Knee

- Arthroplasty. *Orthopedics* 2016; 39:e844-9.
24. Aderinto J, Brenkel IJ. Pre-operative predictors of the requirement for blood transfusion following total hip replacement. *J Bone Joint Surg Br* 2004; 86:970-3.
25. Lošťák J, Gallo J, Mlčůchová D. Multivariate analysis of blood loss during primary total hip or knee arthroplasty. *Acta Chir Orthop Traumatol Cech* 2013; 80:219-2.

AGGRESSIVE HEMANGIOMA STARTING FROM L5 BODY, TREATED WITH MASS EMBOLIZATION AND RADIATION THERAPY WITH PROTONS: A CASE REPORT

R. VANELLI¹, M. GHIARA¹, E. JANNELLI^{1,3}, M.C. RIVELLINO^{1,3},
E. FERRANTI CALDERONI^{1,3}, M.R. FIORE², F. BENAZZO^{1,3} and F. CUZZOCREA¹

¹*Clinica di Ortopedia e Traumatologia, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy;*

²*Radiotherapy Unit, National Center of Oncological Hadrontherapy (CNAO) Pavia, Italy;*

³*Università degli Studi di Pavia, Pavia, Italy*

A hemangioma is a benign tumor of endothelial cells, multiplying into the medullary spaces of the cancellous bone. This tumor is in most cases not symptomatic, and in this case, they do not require any specific treatment. Pain and neurological symptoms derived from hemangiomas as shown in 2-11% of the cases. The 55% of these cases presents low back pain, while the 45% presents neurological deficit from compression of the spinal cord, peripheral nerves or both. We present a clinical case report of a young woman, affected by aggressive L5 hemangioma causing a spinal canal stenosis with associated sciatalgic symptoms. We performed a review of the current literature on the treatment options, giving the rationale of our treatment choice (mass embolization and radiation therapy with protons).

A hemangioma is a benign tumor of endothelial cells, multiplying into the medullary spaces of the cancellous bone. Virchow (1), in 1864, was the first to describe it, while Perman in 1926 described its radiological features. It is the most common benign tumor of the vertebral column, with an incidence of 10-12% in the general population (2), more common in the fourth and fifth decade of life (3) and more frequent in the thoracic and lumbar traits of the vertebral column.

This tumor in most cases is not symptomatic. They are stage 1 benign tumors according to Enneking's classification (4) and do not require any specific treatment. Pain and neurological symptoms derived from hemangiomas as shown in 2-11% of the cases (5) and 55% of these cases present low back pain, while 45% present neurological deficit from compression of the spinal cord, peripheral nerves or both (6). These symptoms, varying from a neuropathy to a complete peripheral palsy of the affected side or

limb, are due to an epidermal expansion of the mass that can cause medullary compression (spinal canal stenosis or epidural haemorrhages) or, more rarely, stress fractures.

Imaging in this kind of tumor is peculiar. The MRI shows a signal of hyperintensity in T1 and T2 sequences. CT-scan shows a reduction of bone trabeculae that gives greater radiopacity. Angiograph allows the differential diagnosis of a non-evolutive hemangioma from a more aggressive histological type, based on the evaluation of the hypervascularization. These aspects are related to the histological composition of the neoplasia, characterized by adipocytes, vessels and interstitial edema (7).

The differential diagnosis derives from pathologies with similar clinical and imaging features, such as vertebral metastasis, vascular-originated malignant tumors (angiosarcoma and epithelioid hemangioendotheliomas) and multiple myeloma

Key words: hemangioma, lumbar column, radiation therapy, protons, embolization

Corresponding author:

Dr Eugenio Jannelli,
Clinica di Ortopedia e Traumatologia,
Fondazione IRCCS Policlinico San Matteo,
Viale Camillo Golgi 19, 27100 Pavia, PV, Italy
Tel.: +393331620346
e-mail: eugenio.jannelli@libero.it

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(2). The clinical and radiological features that lead to a certain diagnosis are still debated, because there is a very small percentage of aggressive and symptomatic hemangiomas. The standard treatment protocol is also still debated.

We present a clinical case report of a young woman in fertile age, affected by aggressive L5 hemangioma causing a spinal canal stenosis with associated sciatalgic symptoms. The discussion will be completed with a review of the current literature on the treatment options, giving the rationale of our treatment choice.

Case report

A 43-year-old woman with no relevant pathologies in the anamnesis came to our attention in April 2016. She presented gluteal right pain with sciatalgic irradiation, associated to a 20 mt conditioned claudication. In May 2016, she took a lumbo-sacral 1.5 T MRI, reporting an L4-L5 space right paramedian herniation, with intraforaminal expansion and conflicts with the L5 nerve.

The patient had a cycle of rehabilitation therapy and pharmacological treatment with NSAIDs and

steroids, without any clinical benefit. After 10 months, the symptoms became chronic and the patient was accepted in our division for further investigation. At clinical examination, the patient presented low back pain with right sciatalgic irradiation, a positive Lasègue maneuver, hypoesthesia in the dermatomes corresponding to the irradiation of L5 and S1 nerves, without any sign of muscular weakness and symmetrical osteotendinous reflexes.

A new 1.5 T MRI and a CT-scan with contrast medium were performed. The MRI showed suggestive findings of an aggressive hemangioma starting from the body of L5 associated with an expansion in the epidural space and a mass effect in the lateral processes, especially in the right foramen. There was not any deformity of the body of L5. (Fig. 1).

The CT-scan performed with the contrast medium confirmed the diagnosis of L5 hemangioma with an increment of the antero-posterior diameter of the vertebral body at that level.

Based on the clinical and radiological findings, a surgical biopsy was planned. The results of the biopsy confirmed the diagnosis of aggressive

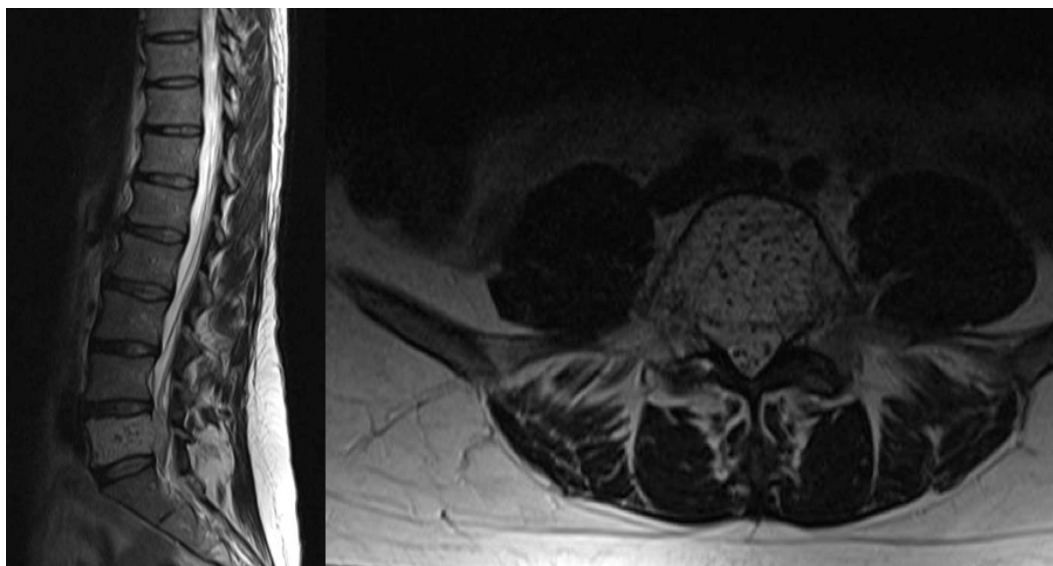


FIG. 1. *The first MRI (sagittal view and axial view) showed findings suggestive for an aggressive hemangioma starting from the body of L5, with associated expansion in the epidural space and a mass effect in the lateral processes, especially in the right foramen.*

hemangioma. According to the literature (8), a preventive angiographic embolization of the mass was performed. (Fig. 2). During the angiography, there was hypervascularization of the body of L5, in the site of the mass, with arterial afference from the sacral median artery. A microcatheter Progreat® (produced by Terumo) was inserted into the arterial afference, from the more distal trait near the body of L5, with a Transend 0.014" guide (Stryker). PVA embospheres of 500-700µm was then injected until complete devascularization of the lesion was reached.

Further clinical controls and a new MRI after 6 and 12 months were programmed. After 5 weeks from the procedure the patient reported a net improvement of the symptoms, the Lasègue maneuver was negative and the myeloradicular deficits were almost resolved. There was also a net improvement of the claudication. After 2 months, the patient reported recurrence of the low back pain and initial presence of sciatalgic irradiation to the right lower limb, poorly improved with steroid therapy. The MRI was anticipated and it showed a progression of the lesion in L5, with extracompartmental expansion

in the epidural space and bilateral mass effect with foraminal conflict.

The absence of deformity in the vertebral body and the absence of neat myeloradicular deficits contraindicated a surgical procedure as a first therapeutic approach. After collegial discussion with the oncologists, in light of the young age and the intention of the patient to procreate, the radiation therapy in September 2017 with protons started. An active scanning system with a total dose of 50 Gy (RBE)/fraction, 25 fractions, 5 fractions per week in an interval of 39 days was used. IMPT technique was used (Intensity Modulated Particle Therapy). A new clinical evaluation and a new control MRI after 3 months from the last radiation dose (November 2017) was then programmed.

Before starting the radiation protocol, the patient took the "Oswestry Low Back Pain Disability Questionnaire" (9) to evaluate the clinical and functional state at the beginning and the progresses obtained after the procedure. The questionnaire presented a value of 70% (crippled) with clinical features that were limiting the basic life activities. During the clinical control in February 2018, the

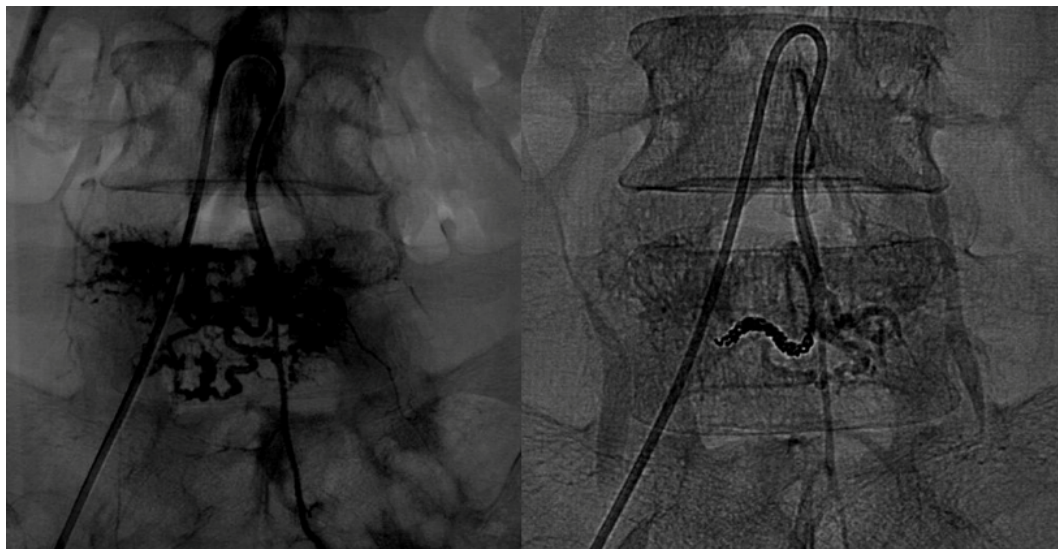


FIG. 2. Arteriography: pre and post embolizations images.

patient reported a state of relative well-being, with the disappearance of sciatalgic symptoms. She had no myeloradicular deficits. Although the low back pain was still present, but with a progressive improvement, appearing as an early and transient complication after the procedure. The “Oswestry Low Back Pain Disability” score was 30%, with a moderate disability and a net profit of the 40%. After 3 months the MRI showed radiological stability of the lesion, with the presence of an edema in the paravertebral tissues (due to the radiant therapy) and a persistency of the extracompartmental component into the anterior epidural space. During the clinical control in September 2018, the “Oswestry Low Back Pain Disability” score was 10% with minimal disability and a net profit of 60% compared to the first questionnaire. The patient reported that the pain prevented her from walking more than a mile. The clinical examination was negative. In September 2018, a new MRI was performed showing a reduction of the extracompartmental component and a reduction of the post-radiation edema in the paravertebral tissues (Fig. 3). Other findings were the same as that one obtained before.

DISCUSSION

In mild forms, an annual radiological follow up with associated symptomatic therapy is the most effective protocol (10). There are several therapeutic options described in literature to treat aggressive hemangiomas. It is well known that the most appropriate treatment depends on the severity of the symptoms and on the general conditions of the patient.

The treatment is based on the dimensions of the mass and on the possible presence of the mass in the medullary space. Surgery is indicated if there is a fast and progressive worsening of the neurologic symptoms (11). Major surgery, varying from laminectomy and stabilization to an *en bloc* resection of the tumor, it is still the gold standard today. More attention is now payed to isolated or combined percutaneous techniques *e.g.* the introduction of PMMA into the vertebral body. This procedure induces sclerosis of the venous vessels of the hemangioma and consolidation of the micro fractures into the vertebral body (caused by the hemangioma itself). This technique has a lower complication rate

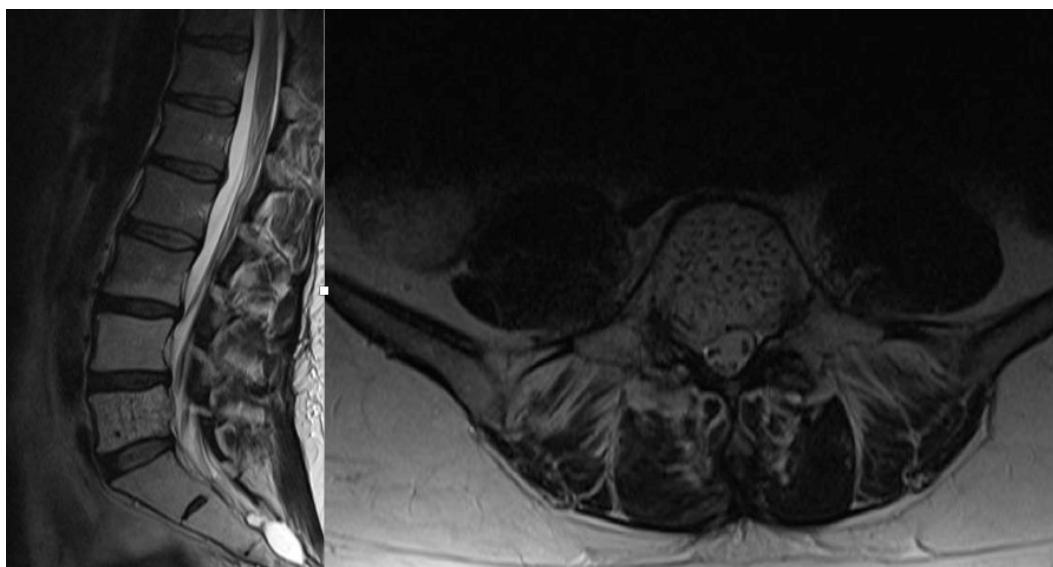


FIG. 3. The last MRI (*sagittal view and axial view*) showed a reduction of the extracompartmental component and a reduction of the post-radiation edema in the paravertebral tissues.

and an immediate pain-relief effect, allowing early revalidation and results that are comparable to the ones obtained with a surgical vertebral stabilization or arthrodesis (12).

Embolization of the vessels was used for many years with discording results, often transient in the long period. Today it is considered the gold standard as a preoperative procedure to diminish the risk of bleeding -in 1951 Manning found a mortality rate of 20-25% correlated to intraoperative blood loss-. Less bleeding permits a better view in the operatory field if there is need of major surgery (13).

Endoarterial embolization has also been proposed as definitive treatment. Raco et al. (14) reported an improvement of the clinical state and the absence of recurrence at 18 and 36 months in 2 patients treated only with embolization of the mass. The contraindications of this procedure are artero-venous malformations that can lead to the migration of the embolization material into arterial collaterals like the intercostal arteries (15).

Isolated radiant therapy on the lesion was the gold standard for a long period of time (16). It was especially recommended if there are few symptoms and if they develop over a long period. However, the therapeutic effective dose to administer was not defined (17-18).

The role of radiant therapy has been revaluated as adjuvant treatment after surgery in the case reported by Bremmes et al (19). In this case report, the authors performed a decompressive laminectomy associated with a biopsy of the lesion, confirming the diagnostic suspect of hemangioma. They report successful clinical and imaging results due to the use of radiant therapy post-surgery, with complete remission of the clinical symptoms associated with radiological stability of the lesion. Intensity modulated proton therapy (IMPT) uses the electromagnetic spatial control of circumscribed "pencil beams" of protons. These particles have variable energy and intensity. The beams take advantage of the Bragg peak that is the peak of dose at the end of range. The Bragg peak is combined with the modulation of the pencil beam's variables, resulting in a target-localized modulation in the dose. IMPT improves on X-ray intensity modulated beams with dose modulation

along the beam axis, as well as lateral, in-field, dose modulation (20). Using the IMPT causes a difference in the dose, resulting in a higher dose in target tissue and a lower dose in healthy ones, in comparison with normal X-rays. In addition, IMT causes a modulation of biological effects that is in an interesting way associated with the tissue targeting. The clinical use of IMPT is becoming of crescent interest.

However, there are some technical, physical and clinical questions not yet solved. Technical questions consist in the control of the process to manipulate the pencil beams from the creation of the proton beam to delivery within the patient with the required accuracy. The physical question remains about the variability in the planned proton penetration and the patient's anatomical features that lead to intrinsic uncertainty in tissue stopping powers (the measure of energy loss per unit distance). The clinical question concerns the impact and management of the possible risks associated with the procedure, the actual clinical benefit of IMPT and the differential biological response compared with X-rays. In any case, IMPT will probably replace other models of proton field delivery.

There are multiple risks associated with this procedure, as Obana *et al.* described in their clinical case. They reported the case of a hemangiosarcoma developed from a hemangioma subjected to multiple cycles of radiant therapy (21).

A hemangioma is a benign tumor of endothelial cells, multiplying into the medullary spaces of the cancellous bone. It is the most common benign tumor of the vertebral column. In this paper, an alternative treatment method with IMPT is proposed and it showed a clinical improvement and radiological reduction of the lesion. This treatment method can be used in young patients in fertile age.

REFERENCE

1. Baker ND, Klein MJ, Greenspan A, Neuwirth M. Symptomatic vertebral hemangiomas: a report of four cases. *Skeletal Radiol* 1986; 15(6):458-63.
2. Jiang L, Liu XG, et al. Diagnosis and treatment of vertebral hemangiomas with neurologic deficit: a report of 29 cases and literature review. *Spine J*

- 2014; 14(6):944-54.
3. Bartels RH, Grotenhuis JA, Van der Spek JA. Symptomatic vertebral hemangiomas. *J Neurosurg Sci* 1991; 35(4):187-92.
 4. Enneking WF, Spanier SS, Goodman MA. A system for the surgical staging of musculoskeletal sarcoma. *Clin Orthop Relat Res* 1980; (153):106-20.
 5. Pastushyn AI, Slin'ko EI, Mirzoyeva GM. Vertebral hemangiomas: diagnosis, management, natural history and clinicopathological correlates in 86 patients. *Surg Neurol* 1998; 50(6):535-47.
 6. Gaudino S, Martucci M, Colantonio R, Lozupone E, Visconti E, Leone A, Colosimo C. A systematic approach to vertebral hemangioma. *Skeletal Radiol* 2015; 44(1):25-36. Baudrez V, Galant C, Vande Berg BC. Benign vertebral hemangioma: MR-histological correlation. *Skeletal Radiol* 2001; 30(8):442-6.
 7. Kawahara N, Tomita K, Murakami H, Demura S, Yoshioka K, Kato S. Total en bloc spondylectomy of the lower lumbar spine: a surgical techniques of combined posterior-anterior approach. *Spine (Phila Pa 1976)* 2011; 36(1):74-82. Fairbank JC. Oswestry disability index. *J Neurosurg Spine* 2014; 20(2):239-41.
 8. Bandiera S, Gasbarrini A, De Iure F, Cappuccio M, Picci P, Boriani S. Symptomatic vertebral hemangioma: the treatment of 23 cases and a review of the literature. *Chir Organi Mov* 2002; 87(1):1-15.
 9. Kato S, Kawahara N, Murakami H, Demura S, Yoshioka K, Okayama T, Fujita T, Tomita K. Surgical management of aggressive vertebral hemangiomas causing spinal cord compression: long-term clinical follow-up of five cases. *J Orthop Sci* 2010; 15(3):350-6.
 10. Guarnieri G, Ambrosanio G, Vassallo P, Pezzullo MG, Galasso R, Lavanga A, Izzo R, Muto M. Vertebroplasty as treatment of aggressive and symptomatic vertebral hemangiomas: up to 4 years of follow-up. *Neuroradiology* 2009; 51(7):471-6.
 11. Cotten A, Deramond H, Cortet B, Lejeune JP, Leclerc X, Chastanet P, Clarisse J. Preoperative percutaneous injection of methyl methacrylate and N-butyl cyanoacrylate in vertebral hemangiomas. *AJNR Am J Neuroradiol* 1996; 17(1):137-42.
 12. Raco A, Ciappetta P, Artico M, Salvati M, Guidetti G, Guglielmi G. Vertebral hemangiomas with cord compression: the role of embolization in five cases. *Surg Neurol* 1990; 34(3):164-8.
 13. Goyal M, Mishra NK, Sharma A, Gaikwad SB, Mohanty BK, Sharma S. Alcohol ablation of symptomatic vertebral hemangiomas. *AJNR Am J Neuroradiol* 1999; 20(6):1091-6.
 14. Nattrass FJ, Ramage D. Hæmangioma of vertebra: a cause of compression of the cord. *J Neurol Psychopathol* 1932; 12(47):231-7.
 15. Rades D, Bajrovic A, Alberti W, Rudat V. Is there a dose-effect relationship for the treatment of symptomatic vertebral hemangioma? *Int J Radiat Oncol Biol Phys* 2003; 55(1):178-81.
 16. Miszczyk L, Ficek K, Trela K, Spindel J. The efficacy of radiotherapy for vertebral hemangiomas. *Neoplasma* 2001; 48(1):82-4.
 17. Bremnes RM, Hauge HN, Sagsveen R. Radiotherapy in the treatment of symptomatic vertebral hemangiomas: technical case report. *Neurosurgery* 1996; 39(5):1054-8.
 18. Kooy HM, Grassberger C. Intensity modulated proton therapy. *Br J Radiol* 2015; 88(1051):20150195.
 19. Obana Y, Tanji K, Furuta I, Yamazumi T, Hashimoto S, Kikuchi H, Tanaka S, Ohba Y. A case of malignant transformation in thoracic vertebral hemangioma following repetitive irradiation and extraction. *Pathol Int* 1996; 46(1):71-8.

PRIMARY TOTAL KNEE REPLACEMENT IN HEMOPHILIACS: EXPERIENCE OF A SINGLE INSTITUTION OVER FOURTEEN YEARS OF SURGICAL PROCEDURES

G. PASTA¹, R. VANELLI¹, E. JANNELLI^{1,4}, A. CASTELLI¹, M. MOSCONI^{1,4}, P.S. PRETI²,
C. AMBAGLIO³ and F. BENAZZO^{1,4}

¹*Clinica di Ortopedia e Traumatologia, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy;*

²*Internal Medicine Department; Hemophilia Center and bleeding congenital disorders, Medicina Generale 2, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy;* ³*Hemophilia Center and bleeding congenital disorders, Medicina Generale 2, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy;*

⁴*Università degli Studi di Pavia, Pavia, Italy*

This retrospective study reports clinical and functional orthopedic outcomes and complications after 14 primary total knee replacement (TKR) performed between 2000 and 2014. The mean age at surgery was 42 years (range 26-59), with a removal-free survival of 100% at the end of follow-up (months 109.85). The KSS score was 49.64 pre-operatively (range 31-63) and 78.14 at final follow-up (range 45-90), the KSS function score was 64.64 pre-operatively (range 35-80) and 84.57 at final follow-up (range 45-100). According to this study, there are three main factors that can influence long-term and early surgical outcomes: post-operative fibrosis, a previous synovectomy and presence of inhibitors. Even if our results are slightly suboptimal compared to those obtained in non-hemophilic patients, this study shows that TKR is an effective surgical procedure in hemophiliacs.

Hemophilia is a congenital X-linked disorder characterized by a defective function or synthesis of clotting factors VIII (Hemophilia A) or IX (Hemophilia B). Arthropathy is an important complication of hemophilia and the knee is the most frequently affected joint (1). This disease evolves into three stages: the early stage is characterized by recurrent hemarthroses, the second by a chronic synovitis and the end-stage by a degenerative arthritis with various deformities, including axial deviation, stiffness and chronic pain. Deformities are similar to those observed in the inflammatory and degenerative arthropathies, but the development and evolution are earlier than those observed in non-hemophilic patients (1). Furthermore, clinical symptoms do not always correlate with the severity of radiological signs (2). Although synovectomy is effective in order to reduce the number of bleeding,

it is not able to stop the progression of articular damages (3) and primary total knee replacement represents the gold standard of treatment in patients with end-stage arthropathy, unresponsive to non-surgical therapies.

MATERIALS AND METHODS

Between 2000 and 2014, 14 total knee replacements (TKRs) were performed in 10 patients affected by moderate (F VIII < 5U/dl) and by severe Hemophilia A (FVIII < 1U/dl) with end-stage arthropathy. The subjects had been regularly followed at "Hemophilia Treatment Centre" and at Orthopedic Division of IRCCS Foundation Policlinico San Matteo, Pavia, Italy. We collected data on age at surgery, side of primary TKR, presence of inhibitors against FVIII, previous synovectomy, type of clotting factor

Key words: hemophilia, hemophilic arthropathy, synovectomy, total knee replacement, inhibitors, surgery

Corresponding author:

Dr Gianluigi Pasta,
Clinica di Ortopedia e Traumatologia,
Fondazione IRCCS Policlinico San Matteo,
Viale Camillo Golgi 19,
27100, Pavia, Italy
e-mail: gianluigipasta@yahoo.it

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concentrate used and positivity to some viral agents (HIV, HBV, HCV). (Table I).

Patients have been followed by means of a periodic outpatient check-up (at 1, 3, 6 and every 12 months after surgery) to accurately monitor the TKR's clinical and functional results, until the recovery of normal walking. The KSS score and KSS function (4) are used for pre-operative and post-operative evaluation for each patient. We performed moreover a radiographic examination (at 3, 6 and every 12 months after surgery), which consisted in standard X-rays (AP and lateral view).

During the post-operative hospitalization (first, third e fifth post-operative days) we evaluated the blood loss in all patients. All subjects without

inhibitors were infused with the clotting factor concentrate every day of hospitalization.

We used 4 different types of cemented implant: Zimmer Persona® Posterior stabilized (3 implants), Zimmer NexGen LPS-Flex® (4 implants), Zimmer NexGen Legacy Constrained Condylar Knee ® (4 implants) and LINK Endo-Model® (3 implants). The degree of prosthetic constraint was chosen according to pre-operative joint assessment. Particular attention was paid to joint instability degree, evaluated in clinical examination, and to bone loss, evaluated in pre-operative radiological study.

All TKRs were performed by a single primary surgeon at our Orthopaedic Division, in a standard operating room. Patients were placed in the supine

Table I. *Pre-operative clinical details.*

Patient	1	2	2	3	4	5	6	6	7	8	8	9	9	10
Age at surgery	53	42	34	26	39	34	44	39	31	54	59	50	49	34
Side	R	R	L	R	R	R	L	R	L	R	L	L	R	L
Positivity to HCV, HBV, HIV	hcv	hcv	hcv	-	hcv- hiv	Hcv- hiv- hcv	hcv	hcv	Hcv- hiv- hcv	Hcv- hiv	Hcv- hiv	Hcv- hiv	Hcv- hiv	hcv
Inhibitors	-	-	-	-	-	-	yes	yes	-	-	-	-	-	-
Previous synovectomy	no	yes	yes	no	no	no	yes	no	no	no	yes	no	yes	no



Fig. 1. *a): Twenty-six-year-old patient, right knee arthropathy before TKR; b): Twenty-six-year-old patient, right knee after TKR (Zimmer Persona® Posterior stabilized).*

decubitus, with general anesthesia and all underwent antibiotic prophylaxis to reduce the risk of infection and a custom amount of clotting factor concentrate was infused (range: 3000-8000 U) depending on body weight.

Whenever possible, patients underwent a minitrivector surgical approach (5). In 3 cases it was necessary to perform TTA osteotomy because of an anchilotic joint, one of which was caused by a previous diaphyseal fracture. In 9 cases, we used an

Table II. *KSS score and KSS function score pre-operative and post-operative evaluation for each patient.*

Patients	KSS score (pre-operative)	KSS score (post-operative)	KSS function score (pre-operative)	KSS function score (post-operative)
1	43	88	70	100
2	54	81	70	90
2	42	81	65	90
3	48	82	70	100
4	52	81	70	90
5	63	90	80	100
6	31	45	35	45
6	41	55	35	45
7	57	87	75	90
8	52	85	60	85
8	42	85	60	83
9	57	78	70	83
9	52	69	65	83
10	61	87	80	100

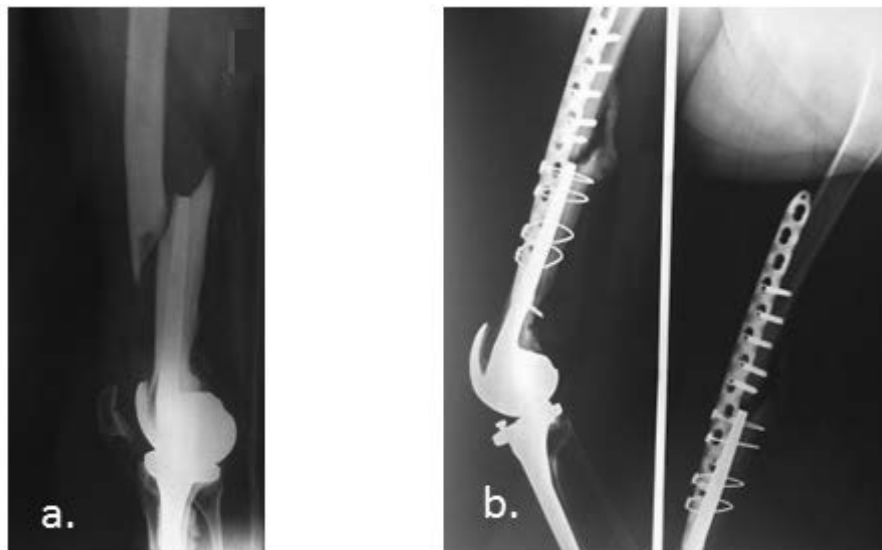


Fig. 2. *a): peri-prosthesis fracture, type C (UCS), in a 31-year-old patient; b): the fracture synthesized with plaque and screws in the same patient. The TKR did not mobilize even after re-intervention.*

hemorrhagic loop, located at the root of the limb, to limit bleeding; prior to placement of the prosthesis, the tourniquet was released and a second infusion of clotting factor concentrate was performed (range: 1000-2000 U). The TKRs were cemented up in 100% of the cases and a careful hemostasis were performed prior to suture. (Fig. 1a, b).

During the post-operative hospitalization, a red blood cell concentrate transfusion was required in 64.3% of cases (6 patients). The mean amount of replacement therapy used was 6000 U (2000 U every eight h) for the first three days, while in the last two days the dosage was reduced to 4000 U (2000 U every twelve h); in only one case the dose was unchanged for the entire duration of the hospitalization.

Discharge recommendations were similar to those usually given to patients who are not affected by hemophilia. A load compatible with the degree of pain and the use of crutches in the first month was imposed. No particular indication was given from the point of view of physiotherapy and rehabilitation. Attention was paid to early mobilization of the joint IN ORDER to recover the maximum degree of range of motion as early as possible (ROM) and to minimize the risk of fibrosis. In five cases with high risk of thrombosis (for a positive family history or presence of major risk factor), enoxaparin 2000 U aXa subcutaneously was administered every 24 h until the recovery of walk without crutches.

RESULTS

The mean follow-up was 109, 85 months and approximately 9 years (range 2.5-16.9 years). The mean age at surgery was 42 (range 26-59). Four patients underwent to two stage bilateral TKR. The TKRs had a survival of 100%, with no additional surgery in 10 cases, while in 21% of cases post-operative complications occurred. In two cases, it was necessary to evacuate the hemarthrosis, which appeared on the fourth day after surgery and treated with two different techniques. In case 6, on the left side by arthroscopy, while on the right side by arthrotomic approach because of the occurrence of hemorrhagic shock. An adhesion lysis was practiced one month after the procedure in patients 4 and 7

due to a reduced ROM. In case 9, a peri-prosthetic fracture (UCS: type C) (fig. 2a) was highlighted, synthesized with plate, screws and cables. Despite the re-intervention, an aseptic loosening does not occur (Fig. 2 b).

The KSS score and KSS function score are used for pre-operative and post-operative evaluation for each patient: the pre-operative mean score is, respectively 49.64 (range 31-63) and 64.64 (range: 35-80), while the average score at the final follow-up is, respectively 78.14 (range 45-90) and 84.57 (range 45-100). (Table II).

In one patient with previous history of inhibitors and high responders, a sudden rise of inhibitors was detected in post-surgical period associated with appearance of prolonged bleeding. In 36% of cases (four patients), a previous synovectomy had already been performed. The average period between the synovectomy and TKR was 31 years (range 25-35).

The patients were treated with different types of clotting factor concentrates and in case of inhibitors, with bypassing agents.

DISCUSSION

Total joint replacement is a mandatory choice for patients who do not respond optimally to conservative therapies. Our results are similar to the one reported in previous studies about TKR in hemophilic arthropathy (6). The removal-free survival is 100% at the last years of follow-up. The average results reported in literature are widely variable, starting from 81% (7) up to 98% (8) [other results: 90% (9, 10), 92% (11), 95% (12), 97% (1, 2, 3)].

The outcomes remain slightly suboptimal compared to the TKR observed in non-hemophilic patients who underwent the same type of surgery, because the occurrence of complications is higher. Due to the bleeding tendency and the consequent damage of the musculoskeletal system, the hemophilic patient is more exposed to post-operative complications than the general population. Although two cases of hemarthrosis, surgically evacuated, and one case of peri-prosthetic fracture (type C) did not influence the survival of the TKR.

These complications can be considered a frequent post-surgical event of the hemophilic patient as reported in many other studies (1, 13, 14, 15, 16). In the third day after surgery, one patient presented high-titer inhibitors, affecting the successful outcome of a prosthesis intervention as already reported (1, 7, 17). This case showed the worst results regarding to the KSS/KSS function score, with an increase of only 15 points and 10 points respectively.

No patient developed infections during this study. Although the infections are almost considered a frequent complication after invasive interventions, the rate generally reported in the hemophilic patient ranges from 0 to 17% (6, 9, 18, 19, 20) but one study shows a larger range from 0 to 25% with an average of 9% (7). The three HIV positive patients had outcomes similar to those of HIV negative ones, confirming the data that HIV infection well controlled by antiviral drugs, is not to be held responsible for a possible unfavorable outcome of prosthesis (21) or for the onset of subsequent infections (22).

The aim of the total joint replacement surgery in the hemophilic patients is to recover, as much as possible, the joint function removing pain. In our study, we obtained an increase in the KSS score of 32 points and a improvement of 20 points in the KSS function score. These results are inferior to those in literature (23), which has an increase interval ranging from 37 to 64 points (2) regarding the KSS score, and from 21 to 35 points (2, 13) for the KSS function score.

The best results were found in patients with younger age at surgery, where the anatomical alterations were still contained and in which a good ligament balance was present. Furthermore, previous studies show that the pre-operative correction of flexion contracture and pain control before TKR surgical procedure (14), may improve the post-surgical functional level. Synovectomy could play a role on postponing TKR procedure (9). In our study, the four patients with a previous synovectomy had a mean age higher than the others at the time of the total joint replacement.

The indication of total knee replacement in patients with an end-stage hemophilic arthropathy develops earlier and can be considered as a compulsory choice

when articular disease is unresponsive to others surgical and non-surgical therapies.

The average survival of the implants was 100% at the end of the follow-up, despite the important articular degeneration before surgery. Three were the main factors that influenced the outcomes: post-operative fibrosis that led to the need of a re-intervention, the presence of inhibitors that has resulted in an higher rate of complications and a previous synovectomy. The HIV infection controlled by antiretroviral therapy does not affect the clinical and functional outcomes of a total joint replacement surgery. Even if our results are slightly suboptimal compared to those of a non-haemophilic patients, this study shows that TKR is an effective surgical procedure in hemophiliacs.

REFERENCES

1. Chevalier Y, Dargaud Y, Lienhart A, Chamouard V, Negrier C. Seventy-two total knee arthroplasties performed in patients with haemophilia using continuous infusion. *Vox Sang* 2013; 104(2):135-43.
2. Zingg PO1, Fucntese SF, Lutz W, Brand B, Mamisch N, Koch PP. Haemophilic knee arthropathy: long-term outcome after total knee replacement. *Knee Surg Sports Traumatol Arthrosc* 2012; 20(12):2465-70.
3. Ernstbrunner L, Hingsammer A, Catanzaro S, Sutter R, Brand B, Wieser K, Fucntese SF. Long-term results of total knee arthroplasty in haemophilic: an 18-year follow-up. *Knee Surg Sports Traumatol Arthrosc* 2017; 25(11):3431-8.
4. Insall JN, Dorr LD, Scott RD, Scott WN. Rationale of the Knee Society clinical rating system. *Clin Orthop Relat Res* 1989; (248):13-4.
5. Benazzo F, Rossi SMP. The trivector approach for minimally invasive total knee arthroplasty: a technical note. *J Orthop Traumatol* 2012; 13(3):159-62.
6. Sikkema T, Boerboom AL, Meijer K. A comparison between the complications and long-term outcome of hip and knee replacement therapy in patients with and without haemophilia; a controlled retrospective cohort study. *Haemophilia* 2011; 17(2):300-3.
7. Solimeno LP, Mancuso ME, Pasta G, Santagostino E, Perfetto S, Mannucci PM. Factors influencing the long-term outcome of primary total knee replacement

- in haemophiliacs: a review of 116 procedures at a single institution. *Br J Haematol* 2009; 145(2):227-34.
8. Strauss AC, Goldmann G, Schmolders J, et al. Total knee replacement in patients with end-stage haemophilic arthropathy. 25-years results. *Z Orthop Unfall* 2015; 153(5):526-32.
 9. Heeg M, Meyer K, Smid WM, Van Horn JR, Van der Meer J. Total knee and hip arthroplasty in haemophilic patients. *Haemophilia* 1998; 4(5):747-51.
 10. Norian JM, Ries MD, Karp S, Hambleton J. Total knee arthroplasty in hemophilic arthropathy. *J Bone Joint Surg Am* 2002; 84-A(7):1138-41.
 11. Westberg M, Paus AC, Holme PA, Tjønnfjord GE. Haemophilic arthropathy: long-term outcomes in 107 primary total knee arthroplasties. *Knee* 2014; 21(1):147-50.
 12. Jenkins PJ, Ekrol I, Lawson GM. Total knee replacement in patients with haemophilia: the Scottish experience. *Scott Med J* 2013; 58(4):223-7.
 13. Karthaus RP, Novakova IR. Total knee replacement in haemophilic arthropathy. *J Bone Joint Surg Br* 1988; 70(3):382-5.
 14. Beeton K, Rodriguez-Merchan EC, Alltree J. Total joint arthroplasty in haemophilia. *Haemophilia* 2000; 6(5):474-81.
 15. Legroux-Gérot I, Strouk G, Parquet A, Goodemand J, Gougeon F, Duquesnoy B. Total knee arthroplasty in haemophilic arthropathy. *Joint Bone Spine* 2003; 70(1):22-32.
 16. Sheth D. S., Oldfield D., Ambrose C., Clyburn T. Total knee arthroplasty in haemophilic arthropathy. "The journal of Arthroplasty" 2004, vol. 19 No. 1
 17. Mortazavi S. M. J., Haghpanah B., Ebrahimi nasab M. M., Baghdadi T., Toogeh G. Functional outcome of total knee arthroplasty in patients with haemophilia. "Haemophilia" 2016, 22, 919-924.
 18. Cohen I., Heim M., Martinowitz U., Chechick A. Orthopaedic outcome of total knee replacement in Haemophilia A. "Haemophilia" 2000, 6, 104-109
 19. Silva M., Luck J. V. jr. Long-term results of primary total knee replacement in patients with haemophilia. "The journal of bone nd joint surgery" 2005, 87-A, 85-91
 20. Figgie M. P., Goldberg V. M., Figgie H. E. 3rd, Heiple K. G., Sahel M. Total knee arthroplasty for the tratment of chronic hemophilia arthropathy. "Clrn Orthop" 1989, 248, 98-107
 21. Unger A. S., Kessler C. M., Lewis R. J. Total knee arthroplasty in human immunodeficiency virus-infected hemophiliacs. "The journal of Arthroplasty" 1995, 10, 448-512
 22. Wang K., Street A., Dowrick A., Liew S. Clinical outcomes and patient satisfaction following total joint replacement in haemophilia- 23-years experience in knees, hips and elbows
 23. Moore M. F., Tobase P., Allen D. D. Meta-analysis: outcomes of total knee arthroplasty in the haemophilia population. "Haemophilia" 2016, 22, e275-e285.

SECONDARY FEMUR FRACTURE FOLLOWING TREATMENT WITH ANTEROGRADE NAILING: THE STATE OF THE ART

G. CAZZATO, G. MASCI, F. LIUZZA, L. CAPASSO, M. FLORIO, C. PERISANO, R. VITIELLO, G. CIOLLI and G. MACCAURO

Ortopedia e Traumatologia, Istituto di Clinica Ortopedica, Dipartimento di Geriatria, Neuroscienze ed Ortopedia, Fondazione Policlinico Universitario A. Gemelli IRRCCS, Università Cattolica del Sacro Cuore, Roma, Italy

Cephalomedullary nailing (CMN) currently represents the best surgical technique for the treatment of intertrochanteric hip fractures. Although the success of CMN in terms of functional recovery and fracture healing, in clinical practice there are many complications. Later femur fracture following treatment of trochanteric fracture with CMN is not a very frequent complication but, when it occurs, its treatment is the most complex, because of the increase of peri-operative mortality. There are studies in literature, which have demonstrated that the incidence of this complication is about 0.5-3%. Diagnosis and classification are made with standard radiographs, using the AO classification and the modified Vancouver classification. In the actual literature, to determinate the predisposing factor to the secondary fractures, the authors focused their attention on patient-related and surgical related risk factors. The treatment is variable and it depends on the type and characteristics of fracture and device. Outcomes analyzed in literature were mortality and bone healing. The aim of this manuscript is to provide an overview of this topic and to describe the state of the art of the secondary fracture after surgical treatment with intramedullary nailing.

Cephalomedullary nailing (CMN) currently represents the best surgical technique for the treatment of intertrochanteric hip fracture. Simplicity of this surgical method and the increase in performance in basicervical and subtrocantneric fractures, made this technique increasingly widespread. Although the success of CMN in terms of functional recovery and fracture healing, in clinical practice there are many complications such as: superficial and deep infection, malalignment, non-unions of the fracture, intra operative fractures and breaking of fixation devices. Later femur fracture following treatment of trochanteric fracture with CMN is not a very frequent complication but, when it occurs, its treatment is the most complex, because of the increase of peri-

operative mortality (1-4) Even if many of these complications have been extensively described, there are very few studies about secondary fracture around intramedullary nails.

Because of the continuous rise of the average age and the almost immediate stabilization of the fracture, consequentially increasing the average survival rate after surgery, the number of patients that undergo secondary fracture around CMN is increasing.

The aim of this manuscript is to provide an overview of this topic and to describe the state of the art of the secondary fracture after surgical treatment with intramedullary nailing.

All the studies present in the literature regarding this argument were analyzed until June 2018. Studies

Key words: secondary femur fracture, femur nailing, antegrad nailing, peri-implant femur fracture

Corresponding author:

Gianpiero Cazzato, MD,
UOC Ortopedia e Traumatologia,
Istituto di Clinica Ortopedica, Dipartimento di Geriatria,
Neuroscienze ed Ortopedia, Fondazione Policlinico
Universitario A. Gemelli IRRCCS,
Università Cattolica del Sacro Cuore, Roma
L.go Agostino Gemelli 8 - 00168 Roma, RM, Italy
e-mail: g.cazzato@hotmail.com

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regarding secondary fracture around intramedullary retrograde nailing and intra operative fractures were excluded, focusing only on secondary fractures around intramedullary anterograde nailing after surgery.

There are studies in the actual literature that have demonstrated that the incidence of this complication is about 0.5-3% (1). In particular, in a recent retrospective study the rate is 1.2% (1). In another study, made on an Asian population of 931 patients treated with cephalomedullary anterograde nail, the secondary fracture rate was 1.7% (2). In a review of 89 studies, for 13568 patients, the incidence was approximately 1.7 % (4).

The risk of a second femur fracture after any fixation is 40 times in the general population (5). Similar to intertrochanteric fracture, even in this kind of injury, there is a bimodal age of occurrence: in younger patients, they are the result of high-energy trauma, in the elderly patients they are instead caused by a lower energy trauma.

Generally, the average time to peri-implant fracture described in literature is around 7.5-9 months after the index procedure, often when the primary fracture is already healed (5). Lang et al demonstrate that the average time to secondary fracture following initial surgery for trochanteric fracture was 122.7 days+/-32 weeks (1).

Diagnosis

Diagnosis is typically made through the clinical history of falls and/or pain and radiographs. The patient complains of pain and at the physical examination, presents functional limitation and limb rotational defect.

Conventional radiographs are the gold standard for diagnosis of peri-implant fractures (Fig 1). High-quality radiographs are essential to find radiolucent lines in the femur shaft or around the nail. A comparison to older radiographs is essential to detect any changes. In some cases, to avoid unrecognized or underestimated lesion, computed tomography with 3D reconstruction or MRI may allow further visualization and classification of fracture lines and they supply a better view of the line, which represents the extension of the fracture around and behind the nail.

Classification

Peri-implant fracture classification is simpler than the peri-prosthetic fracture classification, also because of the choice of surgical treatment. Classification is made with standard radiographs, using the AO classification and the modified Vancouver classification (1). In regards to the AO classification, a femoral shaft fracture can be spiral (A1-3), oblique (B1-3) or complex (C1-3); While in the modified Vancouver classification, the fracture is categorized to assess the location of the fracture.

- A: for the trochanteric region;
- B1: just below the distal end of the fixed nail;
- B2: just below the distal end of the loose nail
- B3: just below the distal end of the loose nail and poor bone quality or severely comminuted bone;
- D: inter prosthetic fracture.

This modified system is also used to assess the loosening of the nail, as a loss of distal fixation due to a secondary femur fracture and non-union of the trochanteric fracture.

The most common typeS of fractureS were AO 32.A1 (53.5%) and AO 32.B1 (23.3%), as well as Vancouver C and B1 (1).

Risk Factor

In the actual literature, to determinate the predisposing factor to the secondary fractures, the authors focused their attention on risk factor and surgical-related risk factor patients. When considering patient-related risk factors, the results were the same as those predisposed to any kind of accidental fall.

In regards to risk factors related to the patient, we have to consider anticoagulation therapy for atrial fibrillation, previous myocardial infarction or previous pulmonary embolism (1). Other risk factors are diabetes mellitus, chronic renal insufficiency and old cerebral infarcts, which can be the cause of weakness or accidental fall (2). Lang et al proved that 45% of the patients which sustained a secondary femur fracture had DEXA (Dual Energy X Ray Absorptiometry), proven osteoporosis or osteopenia (1).

Much more debated and complex is the study of

the surgical related risk factor, including the technical procedure and the choice of the device. Many mechanisms can be responsible of a secondary femur fracture after CMN. First of all, an inappropriate entry point, fracture malreduction, over reaming (>1.5 mm) of the shaft, the use of an awl instead of a center drill, multiple drilling to correct insertion of screws (2, 6, 7).

Thanks to the continuous improvement of surgical procedure and characteristic of device, the rates of secondary fracture has decreased in the last year. Norris et al in fact, described the decreasing of secondary fracture in the third generation of gamma nail when compared to the older one (1.7% vs 2.6%) (4). In the Asian population, for example, as the result of an anthropometric study of Chinese femur, a particular nail has been developed to fit in to the anatomy of the Asian proximal femur with the aim to reduce secondary femur fractures. This is shorter and it has less curvature than the other short nail (2, 4, 8, 9).

For a long time in the clinical practice, the gold standard for the intertrochanteric fracture was long intramedullary nail. This surgical approach has been supported by biomechanical evidence stating that

long nails help to distribute axial loads along the entire length of the diaphysis. A study demonstrated that load transferred at the tip of long intramedullary implant was not significantly different from that of an intact femur (5-10).

In the last years instead, short intramedullary nailing were preferred because short nails required less operative time, lower estimated blood loss, fewer blood transfusion and lower risk to damage anterior cortex of the femur shaft, which, in older patients, is thinner because of the low bone quality (11-12). Many recent studies demonstrate that the incidence of secondary fracture around short nail is about 0-2.7%, with no statistically significant difference from long nails (4, 12- 15).

Daner et al, in a cadaveric study with 10 pairs of cadaveric femur, studied the axially and torsional stress to failure. They implanted CMN in intact femur, a short nail in the left femur and a long one in the right femur. The femur were postoperatively tested in internal rotation and axial stress. The short nail failed typically with a spiral fracture at the tip of the nail, in the mid shaft, around the interlocking screw. The long nail failed with distal spiral fracture involving the distal interlocking screws. The chance of failure for the short nail was significantly higher than for the long one; the tolerance of rotation was also significantly higher for the group of short nails, when compared to the group of long nails (5).

Norris et al compared the incidence of postoperative secondary femoral shaft fracture in biaxial and uniaxial nail. They demonstrate that there is a significant difference. Uniaxial nail is a fixation system with a single screw, pin or blade that goes across the femoral fracture; a biaxial nail has two screws pins or blades, instead. The incidence in biaxial nail was lower than uniaxial nail (4).

Treatment

In the current literature and in our clinical practice, the treatment of choice is based on type of device, on the characteristic of the fracture and on the clinical state and functional require of patient. In the cases of short nails, the fracture is often localized at femur mid shaft. In this cases the best treatment is removing the short nail and replacing it with a longer



Fig 1. X-Ray of secondary femur fracture after CMN.

one and eventually adding circles wires, to sustain the bone continuity because of the line fracture is often spiral (2).

In the cases of a secondary femur fracture around long nail, that are usually localized more distally, the pre-operative planning is based on the presence or not of bone healing. If the fracture is consolidated the treatment of choice is removing the long nail and fixing the new fracture with plate, screws and eventually circles wires. If there isn't bone healing instead, it's not possible to remove the nail and the new distal spiroid fracture can be fixed, after removing the distal interlocking screws, with a condylar plate, monocortical proximal screws or eventually circles wires or a lap plate and bicortical distal screws. The same surgical treatment could be used for fracture that occurs in distal shaft after CMN with short nail (Fig. 2).

Moreover, in the literature the attention is focused on the cases of device failure. It occurs often in non-union of the fracture, where the load brings a mechanical stress that determines the break of



Fig 2. Surgical treatment with plate, screws and wires in Vancouver C



Fig. 3. Device failure in nonunion fracture after 7 months of surgery.

device with multiple micro-motion at the site of the fracture. (Fig. 3) In this case, the surgical technique of choice is removing the nail and replacing it with a longer one, if there is a good bone stock, which make it possible. If there is an important compromising of coxo-femoral articulation and bone reabsorption, it can be necessary to implant a mega-prosthesis.

Outcome

There is no significant influence on mortality complication, duration of surgery or postoperative mobility if the secondary fracture occurs less than or longer than 6 months after initial surgery for trochanteric fracture (1).

Lang et al described that early mortality, within 30 day after surgery, was observed in 7.1% of patient; intermediate mortality, within 90 days, was 14.3% generally for cardiopulmonary failure; late mortality was 9.5% (1). Griffiths et al reported a 10% mortality rate in patients within 30 days (16-18). The bone healing of the secondary femur fracture was observed within 8-27 weeks in 95.3% of patients (1).

REFERENCES

1. Lang NW, Joestl J, Payr S, Platzer P, Sarahrudi K. Secondary femur shaft fracture following treatment with cephalomedullary nail: a retrospective single-center experience. *Arch Orthop Trauma Surg* 2017; 137(9):1271-8.
2. Cheng MT, Chiu FY, Chuang TY, Chen CM, Chen TH. Experience in the use of the long Gamma nail for 16 femoral shaft fracture that have occurred following initial Asian Pacific Gamma nail fixation for pertrochanteric fractures. *Injury* 2006; 37:994-9.
3. Vaughn J, Cohen E, Vopat BG, Kane P, Abbood E, Born C. Complications of short versus long cephalomedullary nail for intertrochanteric femur fractures, minimum 1 year follow-up. *Eur J Orthop Surg Traumatol* 2015; 25:665-70.
4. Norris R, Bhattacharjee D, Parker MJ. Occurrence of secondary fracture around intramedullary nails used for trochanteric hip fractures: a systematic review of 13,568 patients. *Injury* 2012; 43(6):706-11.
5. Daner WE III, Owen JR, Wayne JS, Graves RB, Willis MC Jr. Biomechanical evaluation of the risk of secondary fracture around short versus long cephalomedullary nails. *Eur J Orthop Surg Traumatol* 2017; 27(8):1103-8.
6. Albareda j, Laderiga A, Palanca D, et al. Complication and technical problems with the gamma nail. *Int Orthop* 1996; 20(1):47-50.
7. Lyddon DW Jr. The prevention of complications with the Gamma Locking Nail. *Am J Orthop (Belle Mead NJ)* 1996; 25(5):357-63.
8. Leung KS, So WS, Shen WY, Hui PW. Gamma nails and dynamic hip screws for peritrochanteric fractures. A randomised prospective study in elderly patients. *J Bone Joint Surg Br* 1992; 74(3):345-51.
9. Leung KS, Chen CM, So WS, et al. Multicenter trial of modified Gamma nail in East Asia. *Clin Orthop* 1996; 323:146-54.
10. Bostrom MP, Lyden JP, Ernberg JJ, Missri AA, Berberian WSA biomechanical evaluation of the long stem intramedullary hip screw. *J Orthop Trauma* 1995; 9:45-52.
11. Boone C, Carlberg KN, Koueiter DM, Baker KC, Sadowski J, Wiater PJ, Nowinski GP, Grant KD. Short versus long intramedullary nails for treatment of intertrochanteric femur fractures (OTA 31-A1 and A2). *J Orthop Trauma* 2014; 28:e96-e100.
12. Kleweno C, Morgan J, Redshaw J, Harris M, Rodriguez E, Zurakowski D, Vrahas M, Appleton P. Short versus long cephalomedullary nails for the treatment of intertrochanteric hip fractures in patients older than 65 years. *J Orthop Trauma* 2104; 28(7):391-7.
13. Utrilla AL, Reig JS, Mun˜oz FM, Tufanisco CB. Trochanteric gamma nail and compression hip screw for trochanteric fractures: a randomized, prospective comparative study in 210 elderly patients with a new design of the gamma nail. *J Orthop Trauma* 2015; 19:229-33.
14. Hou Z, Bowen TR, Irgit KS, Matzko ME, Andreychik CM, Horwitz DS, Smith WR. Treatment of pertrochanteric fractures (OTA 31-A1 and A2): long versus short cephalomedullary nailing. *J Orthop Trauma* 2013; 27:318-24.
15. Bhandari M, Schemitsch E, Joˆnsson A, Zlowodzki M, Haidukewych GJ. Gamma nails revisited: gamma nails versus compression hip screws in the management of intertrochanteric fractures of the hip: a meta-analysis. *J Orthop Trauma* 2009; 23:460-4.
16. Griffiths EJ, Cash DJ, Kalra S, Hopgood PJ. Time to surgery and 30-day morbidity and mortality of periprosthetic hip fractures. *Injury* 2013; 44:1949-52.
17. Nijmeijer WS, Folbert EC, Vermeer M, Slaets JP, Hegeman JH. Prediction of early mortality following hip fracture surgery in frail elderly: the Almelo Hip Fracture Score (AHFS). *Injury* 2016; 47(10):2138-43.
18. Shields E, Behrend C, Bair J, Cram P, Kates S. Mortality and financial burden of periprosthetic fractures of the femur. *Geriatr Orthop Surg Rehabil* 2014; 5:147-53.

MUELLER-WEISS DISEASE: REVIEW OF THE LITERATURE

C. PERISANO¹, T. GRECO^{1,2}, R. VITIELLO^{1,2}, G. MACCAURO^{1,2}, F. LIUZZA¹,
F.C. TAMBURELLI^{1,2} and F. FORCONI³

¹Fondazione Policlinico Universitario A. Gemelli IRCCS, Roma, Italia; ²Università Cattolica del Sacro Cuore, Roma, Italia; ³Ospedale San Giovanni Calibita, Fatebenefratelli, Ordine Ospedaliero San Giovanni di Dio, Roma, Italia

Müller-Weiss (MW) disease is a spontaneous osteonecrosis of the tarsal navicular bone in adults. It is a rare cause of chronic medial midfoot pain and deformity characterized by the collapse of the dorso-lateral part of the navicular, progressive navicular fragmentation and talonavicular joint destruction. This study provides a review of the literature about the epidemiology, etio-pathogenesis, clinical, radiological findings and therapeutic alternatives.

Historical background and epidemiology

In 1927, for the first time, Walther Mueller described the condition, attributing it to the compression exerted by the adjacent bones on the navicular (1). A year later, he published another study proposing that this alteration was due to a congenital defect without trauma. In 1929 Konrad Weiss, a radiologist, described a similar condition, but suggested osteonecrosis as a triggering cause (2).

Osteochondrosis of the navicular, Koehler's disease (KD), is a distinct entity from (MW), for both the age group of the population involved and the prevalence and clinical course; in fact, KD has a higher prevalence in male sex, is often less symptomatic, leaves no deformity and is self-limiting with spontaneous resolution.

There are not definite data on the incidence and prevalence of MW disease, but in its most common presentation it occurs in females, in the fourth to sixth decade of life, it is usually bilateral and results in deformity with various degrees of flatfoot typically associated with a varus hindfoot (3).

Anatomy and etio-pathogenesis

The tarsal navicular is a keystone of the medial column of the foot, contributing to the integrity of both the medial longitudinal and transverse arches. The plantar face of the navicular is supplied by the medial plantar artery, while dorsal and lateral face are supplied by the *Dorsalis pedis*. As a result of these circumferential and centripetal blood vascularization, there is a paucity of vascular perfusion in the central zone of the navicular and it is also the area of maximum shear stress. This feature increases with age and is the reason for the greatest incidence of stress fractures and osteonecrosis in this navicular zone (4).

The etiology remains controversial and unclear. Several theories have been proposed including congenital malformation, primary osteonecrosis, traumatic or biomechanical stress, navicular osteoarthritis, abnormal evolution of Kohler's disease. The role of systemic diseases such as diabetes mellitus and lupus, drugs (steroids, antineoplastic) and excessive alcohol consumption has also been discussed (5). It should be noted that patients often have a high BMI and the highest incidence in Europe

Key words: Müller-Weiss disease, tarsal navicular bone, foot, arthrodesis

Corresponding author:

Dr Tommaso Greco,
Department of Geriatrics, Neuroscience and Orthopaedics,
University Hospital "Agostino Gemelli",
Catholic University of the Sacred Heart School of Medicine,
L.go A. Gemelli 1, 00168 Rome, Italy
Tel.: +39-3807582118 - Fax: +39-063055032
e-mail: tommaso.greco91@libero.it

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indicating a possible environmental and nutritional influence.

Maceira et al, in the largest series of patients, proposed a combination of delayed ossification of the tarsal navicular and abnormal plantar pressure distribution as a cause.

The delay in ossification, due to local or generalized disorders (extrinsic - as nutritional deficiencies - or intrinsic - e.g. endocrinopathies), make the bone more vulnerable to deformation and ossification under strong compression causes an abnormal shaped navicular bone.

The delay in ossification is necessary for the disease to occur, along with an abnormal distribution of force that exerts a compressive force on the lateral half of the navicular and leads to deformity and

fragmentation.

Hrestoni et al studied the distribution of plantar pressure in patients diagnosed with MW disease, comparing it with healthy patients, showing an increased plantar pressure in the midfoot and a reduction in finger pressure. This different pattern could be responsible for pain and discomfort of patients (6) and can help in the choice of possible corrections and non-surgical treatments (insoles, arch support, hard platform shoes).

Conditions leading to an uneven distribution of compressive stress on the navicular with an increase on its lateral half [including primary subtalar joint varus, first ray brachymetatarsia (congenital or acquired), and compound conditions such as mild or misdiagnosed forms of clubfoot and pathological



Fig 1. Oblique (A), anteroposterior (B) and lateral (C) radiographs of both feet showing: subchondral sclerosis, irregularity, narrowing of the talonavicular joint, apparent osteophyte formation, positive “cuboid-sign” and comma-like shaped navicular bone (arrow) due to decreased coverage of the navicula. (C) Lateral weight bearing radiograph shows dorsal protrusion of navicular.

metatarsal formula], may cause the failure of the lateral half of the bone. Shearing forces may appear between the talar head and the lateral cuneiforms that lead to increased deformity and eventual fragmentation of the cartilaginous navicular. The lateral displacement of the talar head implies a fixed hindfoot varus, which is a constant feature in MW disease.

Clinical Presentation

Most patients referred chronic and progressive pain in the mid-foot and hind foot, without prior trauma that increases and worsens with weight bearing and walking (7, 8).

Clinical examination can show swelling and tenderness over dorsomedial aspect of the midfoot, a hindfoot varus, with a normal or altered medial longitudinal plantar arch depending on the stage of disease (9). The prominence at the medial side of the midfoot of the navicular tuberosity can give a false clinical impression of valgus hind foot (pseudo hindfoot valgus) (10). A hallmark of advanced disease is a paradoxical pes planus varus, due to a varus hindfoot and a pes planus deformity (11).

Other clinical findings are external rotation of the tibia, secondary to varus hindfoot and a consequent reposition of the external malleolus compared to the internal malleolus. This situation results in a patellar maltracking and often the patients complain of anterior knee pain. Suspecting the diagnosis, a careful anamnesis should be performed to search for possible environmental (socioeconomic) or personal factors (systemic diseases, endocrinopathies) that can compromise normal development and normal growth during infancy.

Diagnosis

Clinical examination and weight-bearing radiographs in the frontal and lateral projections are usually sufficient to make a diagnosis (12).

Radiological features are (Fig. 1):

- Comma (or hourglass) shaped of the navicular bone, instead of its normal “boat-shaped” configuration: this is the result of internal rotation of the medial half and compression with collapse of the lateral half between the talar head and lateral cuneiform.

- The navicular shows decreased size, increased radiodensity, fragmentation, and medial or dorsomedial protrusion (13).
- Medial shift of the cuboid relative to the calcaneus (“cuboid sign”).
- Large open sinus tarsi (due to hindfoot supination).
- Hypertrophic second metatarsal (due to lateral shift of compressive forces from the first to the second metatarsal) (14).
- Degenerative changes on perinavicular joint.
- Absence of index plus metatarsal (a relative short first metatarsal – index minus).

Based on plain weight bearing lateral radiographs, Maceira classified patients into five stages basing on sagittal plane deformity of navicular bone measured through the angle formed by the intersection of the axis of the talus and the axis of the first metatarsal on weight-bearing lateral view (Meary-Tomeno’s angle/axis):

- stage 1. Radiographically, navicular is normal or with minimal changes, MRI can show intraosseous edema and there may be a subtle subtalar varus deformity.
- stage 2. Lateral displacement and dorsal subluxation of the talar head with subtalar varus; initial dorsal angulation of M-T line.
- stage 3. Compression or splitting of navicular, flattening of longitudinal arch, reduction of the space between the talar head and the cuneiforms. M-T line alignment is neutral.
- stage 4. Further navicular compression with rearfoot equinization. Paradoxical pes planus varus; M-T lines intersect on the plantar side and reveal marked longitudinal arch flattening.
- stage 5. Defined by complete extrusion of the navicular with formation of talocuneiform articulation.

This is a radiological classification and often there is no correlation between radiological deformity/fragmentation of navicular bone and severity of symptoms (15). The other imaging procedures play a role for completing the diagnostic procedure and in preoperative planning.

MRI helps in the differential diagnosis with stress fractures, osteonecrosis and infections; it has a

high sensibility in detecting intraosseous edema and early perinavicular arthritic changes and of course for the study of the surrounding soft tissues. CT scan with 3D reconstruction is useful for pre-operative planning and assessing bone destruction.

Histological examination via bone biopsy allows to exclude osteonecrosis, but it is very controversial and the histological characteristics depend a lot on the age of the patient and on the stage of the disease.

Treatment

At present, there is no gold standard for surgery, although many surgical options have been described.

It is important to make a diagnosis in the early stages to allow a conservative treatment, which includes anti-inflammatory, supportive or accommodative orthotics (with a lateral wedge below the heel to correct the varus deformity of the hindfoot), short-leg casts and activity restriction (15). These conservative treatments may effectively decrease pain and improve function.

Pain can be tolerated for years (16) and surgery is reserved only for cases that do not respond to non-specific, conservative and supportive therapy (17). The surgical indication is based more on the severity of the symptoms rather than on the severity of the deformation, though in most of the studies the cases that undergo surgical treatment are the most advanced stages of the classification of Maceira (stage 3 and above) (14, 15, 16). The objectives of all the surgical options proposed are fusion with

arthrodesis of symptomatic degenerative joints to relieve pain and restoration of the plantar vault and medial longitudinal arch (through the correction of the Meary-Tomeno line). Various kinds of operative interventions have been proposed including:

- Percutaneous core decompression of the navicular only in the early stages (in advanced stages patients have already developed advanced deformity and this decompression is not enough) (16);
- internal fixation of the navicular (often it is not possible for bone deficiency in the more advanced stages);
- debridement and complete excision of diseased navicular bone and reconstruction of medial tarsal column with bone graft (18);
- various kinds of arthrodesis of the involved joints;
- isolated talonavicular arthrodesis, may be an effective treatment option in patients who have relatively healthy subtalar and calcaneocuboidal joints but has a high risk of pseudoarthrosis because the altered naviculocuneiform joint is not repaired (19, 20, 21);
- talonavicular-cuneiform arthrodesis (perinavicular arthrodesis) (TNC);
- open or arthroscopic triple arthrodesis (does not address the symptoms due to naviculocuneiform arthrosis) (22);
- triple plus the naviculocuneiform joints (23, 24);



Fig 2. 40-year-old woman with chronic foot pain and advanced Mueller-Weiss syndrome. Preoperative CT scan imaging confirms fragmented navicular (arrow) and perinavicular osteoarthrosis.

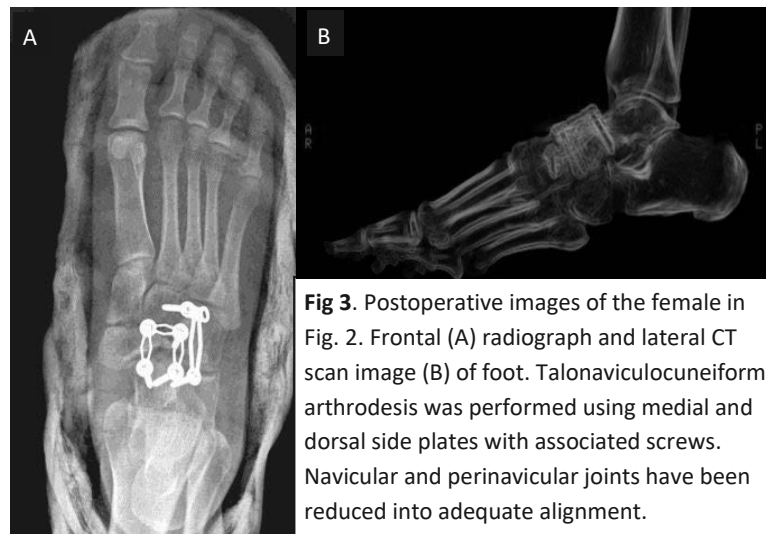


Fig 3. Postoperative images of the female in Fig. 2. Frontal (A) radiograph and lateral CT scan image (B) of foot. Talonaviculocuneiform arthrodesis was performed using medial and dorsal side plates with associated screws. Navicular and perinavicular joints have been reduced into adequate alignment.

The arthrodesis can restore the length of the medial column and relieve the pain caused by removal between the fragments or osteoarthritis. (Fig. 2, 3). Talo-navicular-cuneiform arthrodesis, with bone grafting, satisfies the 2 main objectives of surgery and provided the best results in most of the published papers.

The 2 most important studies on surgical option are from Retana et al (25) and Cao et al (20). The surgical approaches most used are the dorsal approach between the anterior tibial tendon and extensor hallucis longus or medial approach, with use of bone graft, autograft or allograft. For the stabilization of the graft and for arthrodesis, some studies suggest the use of screws or plate instead Retana et al showed as the internal fixation of the graft is not necessary if the graft is correctly applied, as it is usually quite stable and self-locking.

Others possible corrective surgical procedures must be value and can be added, such as calcaneal osteotomy and Achilles tendon lengthening. Postoperative rehabilitation requires at first an initial cast immobilization from 8 to 12 weeks following by a progressive weight bearing.

CONCLUSION

MW disease is a tarsal navicular osteonecrosis related to an abnormal plantar pressure on a bone with suboptimal ossification and must be suspected especially if bilateral and in female. In patients who do not respond to conservative treatments, radiology is crucial to evaluate the joints involved preoperatively. TN or TNC arthrodesis for advanced stage of disease have good clinical outcomes in terms of solid fusion rate and improvement of the quality of life. A large sample size and long-term clinical follow-up studies are required to evaluate the efficacy and safety of the various surgical options.

REFERENCES

1. Mueller W. Ueber eine eigenartige doppelseitige Veraenderung des os naviculare pedis beim Erwachsenen. Dtsch Z Chir 1927; 1-2:84-9.
2. Weiss K. Uber die "malazie" des os naviculare pedis. Fortschritte auf dem Gebiete der Rontgenstrahlen 1927; 45:63.
3. Goedhart LM, Somford MP, Kempink DR, Zeegers

- AV Müller-Weiss disease: idiopathic avascular necrosis of the navicular bone *Ned Tijdschr Geneeskd* 2015; 159:A9036.
4. Bartolotta RJ, McCullion JC, Belfi LM, Hentel KD. Mueller-Weiss syndrome: imaging and implications. *Clin Imaging* 2014; 38(6):895-8.
 5. Aktaş E, Ayanoğlu T, Hatipoğlu Y, Kanatlı U. Spontaneous and bilateral avascular necrosis of the navicula: Müller-Weiss disease. *Eklemler Hastalıkları Cerrahisi* 2016; 27(3):179-82.
 6. Hetsroni I, Nyska M, Ayalon M. Plantar pressure distribution in patients with Müller-Weiss disease. *Foot Ankle Int* 2007; 28(2):237-41.
 7. Goedhart LM, Somford MP, Kempink DR, Zeegers AV. (Müller-Weiss disease: idiopathic avascular necrosis of the navicular bone. *Ned Tijdschr Geneeskd* 2015; 159:A9036.
 8. Kani KK, Mulcahy H, Chew FS. Case 228: Mueller-Weiss Disease. *Radiology*. 2016; 279(1):317-21.
 9. Nguyen AS, Tagoylo GH, Mote GA. Diagnostic imaging of the mueller-weiss Syndrome: findings of a rare condition of the foot. *J Am Podiatr Med Assoc* 2014; 104(1):110-4.
 10. Maceira E, Rochera R. Müller-Weiss disease: clinical and biomechanical features. *Foot Ankle Clin N Am* 2004; 9:105-25.
 11. Maceira E. The paradoxical pes planus varus in Muller-Weiss disease. Presented at the Second Combined Meeting of Foot and Ankle Surgeons (CoMFAS) Congress, Venice, Italy, September 16-18, 1998.
 12. Mohiuddin T, Jennison T, Damany D. Müller-Weiss disease: review of current knowledge. *Foot Ankle Surg* 2014; 20:79-84.
 13. Haller J, Sartoris DJ, Resnick D, Pathria MN, Berthoty D, Howard B, Nordstrom D. Spontaneous osteonecrosis of the tarsal navicular in adults: imaging findings. *AJR Am J Roentgenol* 1988; 151:355-8.
 14. Samim M, Moukaddam HA, Smitaman E. Imaging of Mueller-Weiss Syndrome: A Review of Clinical Presentations and Imaging Spectrum. *AJR Am J Roentgenol* 2016; 207(2):W8-W18.
 15. Levinson H, Miller KJ, Adams SB, Parekh SG. Treatment of spontaneous osteonecrosis of the tarsal navicular with a free medial femoral condyle vascularized bone graft: a new approach to managing a difficult problem. *Foot Ankle Spec* 2013; 7:332-7.
 16. Janositz G, Sisák K, Tóth K. Percutaneous decompression for the treatment of Mueller-Weiss syndrome. *Knee Surg Sports Traumatol Arthrosc* 2011; 19:688-90.
 17. Miltner O, Niedhart C, Piroth W, Weber M, Siebert CH. Transient osteoporosis of the navicular bone in a runner. *Arch Orthop Trauma Surg* 2003; 123:505-8.
 18. Tan A, Smulders YC, Zpoel OT. Use of remodeled femoral head allograft for tarsal reconstruction in the treatment of Müller-Weiss disease. *J Foot Ankle Surg* 2011; 50(4):721-6.
 19. Cao HH, Tang KL, Xu JZ. Peri-navicular arthrodesis for the stage III Müller-Weiss disease. *Foot Ankle Int* 2012; 33(6):475-8.
 20. Hu D, Zhang Y, Liang X, Li Y, Zhao H. Effectiveness of talonavicular arthrodesis for Müller-Weiss disease 2017; *Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi* 2017; 31(12):1434-7.
 21. Harnroongroj T, Chuckpaiwong B. Müller-Weiss Disease: Three- to Eight-Year Follow-Up Outcomes of Isolated Talonavicular Arthrodesis. *J Foot Ankle Surg* 2018; 57(5):1014-9.
 22. Lui TH. New technique of arthroscopic triple arthrodesis. *Arthroscopy* 2006; 22:464.e1-464.e5
 23. Fornaciari P, Gilgen A, Zwicky L, Horn Lang T, Hintermann B. Isolated talonavicular fusion with tension band for Müller-Weiss syndrome. *Foot Ankle Int* 2014; 35(12):1316-22.
 24. Yu G, Zhao Y, Zhou J, Zhang M. Fusion of talonavicular and naviculocuneiform joints for the treatment of Müller-Weiss disease. *J Foot Ankle Surg* 2012; 51(4):415-9.
 25. Fernández de Retana P, Maceira E, Fernández-Valencia JA, Suso S. Arthrodesis of the talonavicular-cuneiform joints in Müller-Weiss disease. *Foot Ankle Clin* 2004; 9(1):65-72.

TRANSILIOSACRAL FIXATION USING THE O-ARM2® AND STEALTHSTATION® NAVIGATION SYSTEM

F. LIUZZA¹, L. CAPASSO¹, M. FLORIO¹, F. MOCINI¹, G. MASCI¹, G. CAZZATO¹,
G. CIOLLI¹, N. SILLUZIO² and G. MACCAURO¹

¹A. Gemelli University Hospital Foundation IRCCS, Catholic University, Roma, Italy; ²Ospedale Maggiore, Bologna, Italy

Pelvic fractures are quite uncommon, they represent only 2-8% of all fractures. Osteosynthesis with percutaneous trans-Iliosacral screw is recognized as one of the standard procedures for the treatment of unstable posterior pelvic ring lesions. Because of the high number of complications associated with the conservative treatment of these kind of lesions such as pain, limb heterometry, difficulty in walking and sexual disability, percutaneous fixation with trans-iliosacral screw has found wide use and has become very popular among orthopedic surgeons. This technique is indicated for the treatment of dislocations of the sacro-iliac joint, some types of sacral and iliac fractures or combinations of these lesions.

Pelvic fractures are quite uncommon, they represent only 2-8% of all fractures (1). Osteosynthesis with percutaneous trans-Iliosacral screw is recognized as one of the standard procedures for the treatment of unstable posterior pelvic ring lesions (2-4).

Because of the high rate of complications associated with the conservative treatment of this kind of lesions such as pain, limb heterometry, difficulty in walking and sexual disability, percutaneous fixation with trans-iliosacral screw has found wide use and has become very common among orthopedic surgeons. This technique is indicated for the treatment of dislocations of the sacro-iliac joint, some types of sacral and iliac fractures or combinations of these lesions. Previously, an open reduction with internal fixation was performed, but it was seen to be associated with complications such as perioperative hemorrhage, postoperative infection and neurological deficits. Although the percutaneous insertion of iliosacral screws has the

advantage of being minimally invasive, resulting in less intra-operative blood loss and a low percentage of postoperative infections, unfortunately it has the risk of an incorrect placement of the screw due to the complex anatomy and the high inter-individual variability of the upper sacral tract. Furthermore, the presence of pathological obesity, intra-abdominal contrast or excessive intestinal meteorism can alter the quality of the fluoroscopic image, especially during the outlet view.

High precision is required to prevent screws misalignment that can cause neurovascular damages. In order to avoid these complications during surgery, surgical times are lengthened as well as radiological exposure time for the medical team and for the patient (5, 6). In literature, a wide range of incorrect positioning rates has been reported during iliosacral fixation (7).

In order to minimize the incidence of an incorrect screw positioning, several imaging techniques have been used as a guide during surgery over

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Corresponding author:

Dr. Luigi Capasso,
Department of Orthopaedics,
A. Gemelli University Hospital Foundation IRCCS, Rome, Italy
Catholic University, Rome, Italy
Tel.: +39 0818806697
e-mail: luigicapasso88@gmail.com

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the years, i.e., traditional fluoroscopy and 3D, guided TC techniques, intraoperative neurological monitoring and fluoro-assisted navigation from computer (8), but despite the more precise imaging of the bone structures provided by the CT scan (9, 10), conventional fluoroscopy remains the most widespread technique because there is a greater availability of the image intensifier in operating theaters. In this technique, pelvic radiographs are used in antero-posterior view (AP), inlet, outlet and lateral in order to determine the screw insertion point and its trajectory (11, 12). Moreover, in order to obtain these radiographs, the surgeon only needs a radiolucent operating table and an intra-operative fluoroscopy machine. Zwingmann et al., in a systematic review, reported a low percentage of incorrect screws placement and, consequently, a low revision rate (1.3% in 445 positioned screws) due to the use of computer assisted navigation (13).

A standardized system is required to compare different studies. The aim of this study is to show the benefits that navigation system can bring in terms of surgical time, learning curve, incorrect screws placement and radiation exposure.

MATERIALS AND METHODS

All fractures of the posterior pelvic ring that arrived at the Orthopedic First Aid Unit of the A. Gemelli University Hospital IRCCS, Rome, Italy from June 2018 to September 2018 were included in the study. We performed a preliminary study on 9 patients (4 males and 5 females) with an average age of 46 years, a maximum age of 58 and a minimum of 21. All cases of fractures or traumatic dislocations of the sacrum and of the sacro-iliac joint treated in percutaneous with trans-ileosacral screws by means of the O-ARM 2 / STEALTH STATION S8 Navigation System were included. All the conservatively treated cases were consequently excluded.

They were sacral fractures or sacroiliac lesions associated with anterior pelvic ring fractures classified as B1 (four cases), C1-2 (four cases) and C1-3 (one case) according to TILE / AO 14.

All patients underwent a graphical RX examination in 3 pelvic views (antero-posterior, inlet and outlet), a preoperative CT study and a postoperative CT study

to evaluate possible incorrect screws positioning. In addition to age and gender, the type of fracture, possible malpositioning of the screws, the time of surgery and the time of exposure to radiation were also recorded. The score described by Smith¹⁵ to highlight any perforation and the angle of the screws was used. The sum of both values corresponds to the “screw score” that goes from a minimum of 0 points (perfect positioning) to a maximum of 6 points (positioning needs revision).

All the CTs, pre- and post-operative were performed by radiologists skilled in musculoskeletal who were not aware of our study. A systematic review of the literature was also carried out using the common dedicated search engines (PUBMED, GOOGLE SCHOLAR, COCHRANE) in order to compare our methodology with the results published in the literature regarding the various techniques. Fisher’s exact probability test was used. A 5% significance level was applied for all tests ($P < 0.05$)

Surgical procedure

The procedure was performed on a radiolucent carbon bed where the patient assumed a supine position. All the patients in the study were operated by the same surgical team; the navigation system O-ARM 2 / STEALTHSTATION S8 (Medtronic Sofamor Danek) was used.

All patients had a monolateral ligament injury or a fracture of the anterior and posterior pelvic ring (Fig. 1). They were operated in supine decubitus on a radiolucent board in carbon, positioned on the lateral edge of the board and lifted from it by about 20 cm by means of sheets positioned below the column to facilitate the introduction of the guide wire and subsequently of the trans-iliosacral screw which, in the supine position, requires a postero-anterior inclination of about 25°.

First of all the reduction and synthesis of the anterior open pelvic ring was performed and then, always in the supine position and with a single operative field, the trans-iliosacral screw was positioned. In particular, the navigator detector was first applied on the iliac crest of the healthy hemipelvis with 4 mm fiches (Fig. 2).

Soon after, a CT scan of the pelvis (Fig. 3) with O-Arm 2 (Medtronic Sofamor Danek) and the acquisition with the Navigator (Stealth Station S8



Fig. 1. O-Arm 2 positioning in operating room with carbon operating table.

Navigation System) were performed. Subsequently the recognition by the navigator of the cannulated trocar that allows the introduction of the 2.8 mm guide wire was performed. Using this navigated trocar it is possible to identify the correct entry point of the guide wire and its direction on the three plans of the space. It was also possible to insert the diameter of the definitive screw (7.3 mm) in the navigator and to measure the length of it.

Once verified that the screw had a correct position on the axial, coronal and sagittal plane, the guide wire was inserted, the reaming was performed and finally the definitive screw was put in. In the first three cases, intermediate CT acquisitions were performed after positioning the guide wire and the cannulated screw to verify the actual accuracy of the positioning. In the following cases, only one CT scan was performed to avoid exposing the patient to further unnecessary radiation. In fact, it is possible to state that it is sufficient to perform a single CT scan to do it properly. In all patients there were no complications due to a malposition of the screws, the bleeding was about 10 cc and the average time of placement of the trans-iliosacral screw was 50 minutes.

RESULTS

In all the cases treated by the O-ARM 2/ STEALTHSTATION S8 Navigation System an execution time superimposable to non-navigational techniques was observed: 51 min on average (± 20.5) of the navigated technique against the 58.8 (± 19.4) of the conventional technique, although the difference did not appear statistically significant (p value 0.65). During the surgery, exposure to radiation was almost

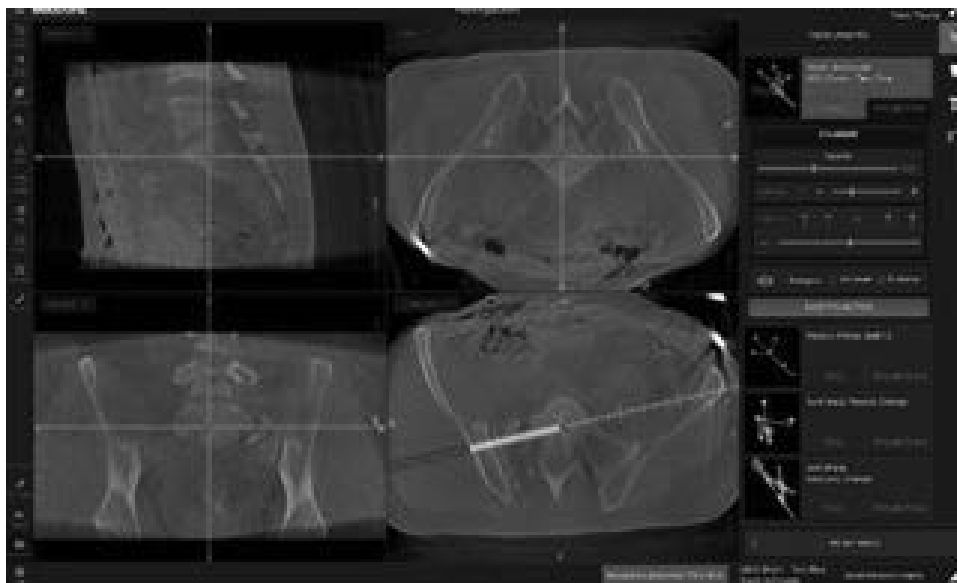


Fig. 2. Real-time intra-operative multiplanar 2D images.

zero for the medical team and it was minimal for the patient: 163.9 seconds on average (± 166.2) for each screw using the conventional technique against 96.6 (± 81.6) for each screw with the navigated technique with a p value of 0.03 which is statistically significant.

Radiation rate for the medical team was zero because during the CT acquisitions the medical staff came out of the operating room. Two CT acquisitions were made: the initial one detected the pelvic volume and the second after positioning the guide wire to check the correct positioning; only in the first case, a third acquisition was performed to verify the final positioning of the screw.

From the post-operative CT study, there were no cases of poor positioning in any of the 18 implanted screws.

DISCUSSION

Preliminary studies reported in the literature were performed on saw bone, on corpses with intact basins or in patients with compound or minimally decomposed sacral wing fractures. In fact, for a precise navigated positioning of ilio-sacral screws, stability

of the sacral fracture is essential because either composed or made stable by a temporary synthesis with wires or with an external fixator. The goal of operative treatment for unstable pelvic ring fractures and sacroiliac dislocations is an anatomic reduction to restore the biomechanical axis and a rigid fixation to facilitate an early mobilization. Early operative treatment decreases immobilization-associated risks like thrombo-embolic events, pneumonia, decubital ulcer and psychological decompensation (16). It is essential, in fact, that there is no movement of the fracture and that the patient's pelvis is fixed on the operating bed in order not to alter the spatial references acquired by the navigator. For this reason, some surgeons connect the patients to the operating bed by means of external fixation fishes positioned in the posterior iliac crest or in the anterior iliac crests connected to a Matta type frame. In this way, the patient is fixed to the operating table, preventing movements of the patient and therefore of the pelvis during the reduction maneuvers. In case of unstable Tile's type B or C lesions, it is therefore necessary to carry out the surgical time of open reduction of the fracture first or, if possible, percutaneously. In the less displaced type B lesions, it is sufficient to



Fig. 3. *Postoperative X-Ray of open book lesion.*

make a reduction and synthesis of the anterior pelvic ring with a plaque on the pubic symphysis. In type C, it is necessary to reduce the posterior fracture in advance or, in some cases, to reduce and synthesize the sacroiliac anteriorly and simultaneously the symphysis and only after obtaining stability, the ileo-sacral screws can be positioned.

Lehmann et al. (1934) (17) was the first report to of an open screw fixation of a sacroiliac dislocation, but the technique of percutaneous fixation with iliosacral screw to treat the sacro-iliac dislocations and sacral fractures was developed above all by Letournel (18). Since then, the technique has undergone several modifications, based on Matta's widely established and standardized fluoroscopic projections (inlet and outlet) to evaluate the pelvic ring lesion and control the screw placement intraoperatively (19). An additional true lateral view further increased the accuracy of transiliacal screw placements, especially for the screw placement in S2 (20). Even the most experienced surgeons reported mean fluoroscopy times of 126 seconds to place iliosacral and 200 seconds for placing retrograde superior pubic ramus screws (21). In some cases, a mean fluoroscopy time of up to 18 min was needed for iliosacral and

transiliacal screw positioning (22). To avoid time-consuming changes of the c-arm position, the simultaneous use of two fluoroscopes was reported. It significantly reduces the fluoroscopy time (320 vs 270 seconds/screw), as well as operating time (23). Disadvantages of this procedure are the need for two fluoroscopic units and limited space around the operating-table.

Using a 2D based navigation system, three fluoroscopic images have to be acquired once and then the movement of the guide wire CAN be visualized and virtually controlled on these images simultaneously. This technique reduces the fluoroscopy time and enables "first pass" guide wire placement reported in experimental settings so far (24).

The use of recently introduced 3D fluoroscopy for navigation seems to further increase the accuracy of iliosacral screw placement compared to 2D fluoroscopy, as reported in two cadaver studies (25). The CT like visualization of osseous structures and additional reformations, displaying orthogonal planes in front of the drill tip and offering a "view ahead", seems to be an advantage, but randomized clinical studies are warranted. Advantages of

	CONVENTIONAL TREATMENT	NAVIGATION TREATMENT	P VALUE
FEMALE	25	5	0,598
MALE	11	4	
AGE, MEAN	63,6 (25-90)	46 (21-58)	0,284
TILE B	9	4	0,498
TILE C	27	5	
SCORE DI SMITH < 3	22	9	0,095
SCORE DI SMITH > 3	14	0	
SURGICAL TIME PER SCREW	58.8	51.0	0,65
EXPOSURE TIME TO RADIATION PER SCREW	163.9	96.6	0,03

computed tomography (CT)-guided sacral fixation are the direct visualization of the course of the screws and the absence of significant wound complications. This technique provides superior visualization of the nerve roots and sacral canal compared to fluoroscopic methods.

Finally, the Robot-assisted Navigation Procedures were introduced (26), a system which collects two fluoroscopic images using C-arm in different directions during the surgery for screw trajectory planning and spatial positioning. The imaging principle of C-arm conforms to the imaging principle of a pinhole camera and acquires the spatial location of the surgical trajectory through a biplane localization algorithm. In contrast to navigation, robots not only allow the surgeon to know the alignment of the guide wire in real time corresponding to intraoperative fluoroscopy, but also guide or assist the surgeon to perform the operation.

Medical robotic systems for orthopedic surgery have been developed, but most studies about robot-assisted iliosacral surgery are based on studies using cadavers (27) or saw bone. That is a different scenario from real life, with no soft-tissue tethers. The safety and accuracy of robot-assisted orthopedic surgery, measured through the number of drilling attempts, screw positioning, and radiation time, have been shown to be better than the conventional techniques. However, there are some disadvantages with the robot-assisted navigation system:

- 1) The surgeon must be constantly aware of the position of the navigation machine and should avoid the path between the trackers and the detector. Blocking the field of view of these cameras will cause loss of tool reference, which might be frustrating, but not critical for the operation;
- 2) Inserting trackers in the iliac spine for registration is an extra step that must be undertaken. It carries minimal risk but definitely discomfort for the patient. It has to be undertaken so as not to be on the placement site of the implant and it has to be placed so that the navigation machine to detect it at all times;
- 3) Using a robot-assisted navigation system adds to the cost of the surgery, including the cost of the machine, the cost of staff responsible for operating

the machine, and the cost of replacing trackers when they have decreased sensitivity. However, technology could be implemented in other hospital service lines, such as neurosurgery or arthroplasty, and a transfer to orthopedic use is likely to be less demanding in that case.

In order to improve the positioning of the screws, several anatomical studies have been published which the aim to clarify the dimensions of the so-called “safe zone” or the “corridor” with the lowest risk of causing a nerve injury (28, 29). This area has been shown to have an oval shape, with a maximum diameter of 22 mm, a minimum diameter of 11 mm and an average angle for the screw of 20.5° in outlet projection (30, 31).

In a very recent study, Berger Groch et al. demonstrated a precision of the screw positioning equal to 79.03% for the conventional method using conventional fluoroscopy and 86.47% for the navigated method (32). The ideal method to determinate the perfect placement of the screw in a standardized manner is a controversial question. Another study with a similar design showed a great advantage of the navigated method compared to the traditional method, with an accuracy of 81% (54 patients) in the first case compared to 42% (87 patients) of the second 33. One of the largest studies comparing the conventional method to computer-based methods was published in the German pelvic trauma record in 2013 supported by a series of 597 patients and showed that no significant difference was found in the intra-operative and post-operative complication rate (34), but without specific reference to the malposition of the screws. Another thorny topic concerns radiation exposure.

It has been shown that computer-assisted screw placement involves less radiation exposure (35, 36). For example, in the previously mentioned study by Berger Groch, it has been shown that there is a significant reduction in the time of radiation exposure in the navigated group ($p=0.023$) and that the same time is reduced inversely proportional to the experience that the surgeon acquires over the years of using this surgical technique. This is an important factor to minimize long-term radiation damage for operating room staff.

To properly specify misplacement and compare different studies, a standardized system is needed. One reasonable suggestion is the one made by Smith et al., which was applied in this study. One limitation of this study was that we did not have our own control group treated with a conventional method. Another important point is that our record of cases is still very small to be able to compare it with a control group as wide as that one reported in the literature.

The percutaneous positioning of the iliosacral screw is an effective, minimally invasive and safe method with a low complication rates. The literature has shown a much better precision of the group with a navigated procedure in the placement of the screws, but it is useful especially in cases where there are anatomical variants or other risk factors for misplacement. 2D-fluoroscopic navigation is a safe tool providing a high accuracy of screw placement, but in the case of a bilateral iliosacral screw fixation an increased risk for screw misplacement was observed. For this fixation, an intraoperative 3D fluoroscopic evaluation should be considered, if available. Additional retrograde ramus pubic screw fixation requires the pass of the iliopubic eminence by inserted screws to prevent an axial screw loosening. At the same time, navigation has shown to significantly reduce radiation exposure rates that are beneficial for both staff and patients.

From this preliminary experience, the CT navigated technique for the positioning of the trans-iliosacral screws presents some advantages, i.e., the absence of radiation for the medical team, the absolute accuracy in positioning of the cannulated screw, the minimal bleeding and the slight reduction of the overall surgical times of the intervention compared to the traditional fluoroscopy technique; thanks to the fact that, in the treated cases, only one position and only one surgical field was performed. In regards to the dose of radiation to which the patient is exposed, it results to be equal to half of a normal CT and overall is slightly higher than the dose of radiation taken with the traditional technique.

REFERENCES

1. Buller LT, Best MJ, Quinnan SM. A nationwide analysis of pelvic ring fractures: incidence and trends in treatment, length of stay, and mortality. *Geriatr Orthop Surg Rehabil.* 2016; 7: 9-17.
2. Richter PH, Gebhard F, Dehner C, Scola A. Accuracy of computer-assisted iliosacral screw placement using a hybrid operating room. *Injury.* 2016; 47:402-7.
3. Lindsay A, Tornetta P, Diwan A, Templeman D. Is closed reduction and percutaneous fixation of unstable posterior ring injuries as accurate as open reduction and internal fixation? *J Orthop Trauma.* 2016; 30:29-33.
4. Liuzza F, Silluzio N, Florio M, El Ezzo O, Cazzato G, Ciolli G, Perisano C, Maccauro G. Comparison between posterior sacral plate stabilization versus minimally invasive transiliac-transsacral lag-screw fixation in fractures of sacrum: a single-centre experience. *Int Orthop* 2018; [Epub ahead of print].
5. Radetzki F, Wohlrab D, Goehre F, et al. Anatomical conditions of the posterior pelvic ring regarding bisegmental transverse sacroiliac screw fixation: a 3D morphometric study of 125 pelvic CT datasets. *Arch Orthop Trauma Surg* 2014; 134:1115-20.
6. Routt ML, Simonian PT, Agnew SG, Mann FA. Radiographic recognition of the sacral alar slope for optimal placement of iliosacral screws: a cadaveric and clinical study. *J Orthop Trauma* 1996; 10:171-7.
7. Theologis AA, Burch S, Pekmezci M. Placement of iliosacral screws using 3D image-guided (O-arm) technology and stealth navigation: comparison with traditional fluoroscopy. *Bone Jt J* 2016; 98B:696-702.
8. Zhang J, Hamilton R, Li M, et al. Evaluation of partial cut-out of sacroiliac screws from the sacral ala slope via pelvic inlet and outlet view. *Spine* 2015; 40: E1264-8.
9. Duwelius PJ, Van Allen M, Bray TJ, Nelson D. Computed tomography-guided fixation of unstable posterior pelvic ring disruptions. *J Orthop Trauma* 1992; 6:420-6.
10. Ebraheim NA, Rusin JJ, Coombs RJ, Jackson WT, Holiday B. Percutaneous computed-tomographystabilization of pelvic fractures: preliminary report. *J Orthop Trauma* 1987; 1:197-204.
11. Jacob AL, Messmer P, Stock KW, et al. Posterior

- pelvic ring fractures: closed reduction and percutaneous CT-guided sacroiliac screw fixation. *Cardiovasc Intervent Radiol* 1997; 20:285-94.
12. Routt ML Jr, Nork SE, Mills WJ. Percutaneous fixation of pelvic ring disruptions. *Clin Orthop Relat Res* 2000; (375):15-29.
 13. Zwingmann J, Hauschild O, Bode G, et al. Malposition and revision rates of different imaging modalities for percutaneous iliosacral screw fixation following pelvic fractures: a systematic review and meta-analysis. *Arch Orthop Trauma Surg* 2013; 133:1257-65.
 14. Tile M. Acute Pelvic Fractures: Causation and Classification. *J Am Acad Orthop Surg* 1996; 4(3):143-151.
 15. Smith HE, Yuan PS, Sasso R, et al. An evaluation of image-guided technologies in the placement of percutaneous iliosacral screws. *Spine* 2006; 31:234-8.
 16. Goldstein A, Phillips T, Sclafani SJ, et al. Early open reduction and internal fixation of the disrupted pelvic ring. *J Trauma*. 1986; 26:325-333.
 17. Lehmann J. Dislocation of a Hemipelvis. *Zentralbl Chir* 1934; 37:2149-52.
 18. Letournel E. Pelvic fractures. *Injury* 1978; 10:145-8.
 19. Shuler TE, Boone DC, Gruen GS, et al. Percutaneous iliosacral screw fixation: early treatment for unstable posterior pelvic disruptions. *J Trauma* 1995; 38:453-57.
 20. Moed BR, Geer BL. S2 iliosacral screw fixation for disruptions of the posterior pelvic ring: a report of 49 cases. *J Orthop Trauma* 2006; 20:378-83.
 21. Routt ML, Jr., Simonian PT, Mills WJ. Iliosacral screw fixation: early complications of the percutaneous technique. *J Orthop Trauma* 1997; 11:584-9.
 22. Starr AJ, Walter JC, Harris RW, Reinert CM, Jones AL. Percutaneous screw fixation of fractures of the iliac wing and fracture-dislocations of the sacro-iliac joint (OTA Types 61-B2.2 and 61-B2.3, or Young-Burgess lateral compression type II pelvic fractures). *J Orthop Trauma* 2002; 16:116-23.
 23. Peng KT, Huang KC, Chen MC, Li YY, Hsu RW. Percutaneous placement of iliosacral screws for unstable pelvic ring injuries: comparison between one and two C-arm fluoroscopic techniques. *J Trauma* 2006; 60(3):602-8.
 24. Smith HE, Yuan PS, Sasso R, Papadopolous S, Vaccaro AR. An evaluation of image-guided technologies in the placement of percutaneous iliosacral screws. *Spine* 2006; 31(2):234-8.
 25. Briem D, Rueger JM, Begemann PG, Halata Z, Bock T, Linhart W, Windolf J. Computer-assisted screw placement into the posterior pelvic ring: assessment of different navigated procedures in a cadaver trial. *Unfallchirurg* 2006; 109:640-6.
 26. Wang JQ, Wang Y, Feng Y, et al. Percutaneous sacroiliac screw placement: a prospective randomized comparison of robot-assisted navigation procedures with a conventional technique. *Chin Med J* 2017; 130:2,527-34.
 27. Oszwald M, Westphal R, Bredow J, et al. Robot-assisted fracture reduction using three-dimensional intraoperative fracture visualization: an experimental study on human cadaver femora. *J Orthop Res* 2010; 28:1140-4.
 28. Carlson DA, Scheid DK, Maar DC, et al. Safe placement of S1 and S2 iliosacral screws: the "vestibule" concept. *J Orthop Trauma* 2000; 14:264-9.
 29. Mendel T, Appelt K, Kuhn P, Suhm N. Bony sacroiliac corridor. A virtual volume model for the accurate insertion of transarticular screws. *Unfallchirurg* 2008; 111:19-26.
 30. Gardner MJ, Morshed S, Nork SE, et al. Quantification of the upper and second sacral segment safe zones in normal and dysmorphic sacra. *J Orthop Trauma* 2010; 24:622-9.
 31. Tonetti J, Cloppet O, Clerc M, et al. Implantation of iliosacral screws. Simulation of optimal placement by 3-dimensional X-ray computed tomography. *Rev Chir Orthop Reparatrice Appar Mot* 2000; 86:360-9.
 32. Josephine Berger-Groch et al. Accuracy of navigated and conventional iliosacral screw placement in B- and C-type pelvic ring fractures. *Eur J Trauma Emerg Surg* 2018; [Epub ahead of print].
 33. Zwingmann J, Konrad G, Mehlhorn AT, et al. Percutaneous iliosacral screw insertion: malpositioning and revision rate of screws with regards to application technique (navigated vs. conventional). *J Trauma* 2010; 69:1501-6.
 34. Zwingmann J, Südkamp NP, König B, et al. Intra- and postoperative complications of navigated and conventional techniques in percutaneous iliosacral screw fixation after pelvic fractures: results from

- the German Pelvic Trauma Registry. *Injury* 2013; 44:1765-72.
35. Abdullah KG, Bishop FS, Lubelski D, et al. Radiation exposure to the spine surgeon in lumbar and thoracolumbar fusions with the use of an intraoperative computed tomographic 3-dimensional imaging system. *Spine* 2012; 37: E1074-8.
36. Gebhard FT, Kraus MD, Schneider E, et al Does computer-assisted spine surgery reduce intraoperative radiation doses? *Spine* 2006; 31:2024-7.

THE BIOMOLECULAR INTERACTIONS BETWEEN ENDOTHELIUM AND BONE CELLS: AN OVERVIEW

C. CARULLI¹, M. INNOCENTI¹, F. LAZZARINI¹, M. VILLANO¹, G. PASTA²,
M.L. BRANDI³ and M. INNOCENTI¹

¹Orthopaedic Clinic, University of Florence; ²Operative Unit of Orthopaedics and Traumatology, Fond. IRCCS Policlinico S.an Matteo, Pavia; ³Bone Metabolic Disease Unit, University of Florence

Bone turnover is a complex set of different molecules pathways and it is strictly connected with bone vasculature. It includes every bone process concerning bone modelling and remodelling such as skeletal growth and healing process. A fundamental component of this bone architecture is played by the endothelium, that acts in a paracrine fashion on other bone stromal cells via humoral factors, growth factors and chemokines/cytokines. The alteration of those biochemical interactions between endothelium, vasculature and bone tissue may cause various pathological manifestations. Understanding the bases of the interaction between those different pathways could provide novel therapeutic strategies for bone disease. The Authors present an updated overview of the most common communication biomolecules between bone cells and endothelium and their interactions both in healthy and pathological conditions. Furthermore, focusing on gene and related therapies, possible future therapeutic strategies for bone vasculature/metabolic diseases are presented.

The microscopic structure of the bone is characterized by a high vascularization and an intense bone turnover. This bone turnover is strictly connected with bone vasculature and includes every bone process concerning bone modelling and remodelling such as skeletal growth and healing process. These processes are made possible by a unique interaction between the bone matrix, the growth factors, the chemokines and the cells as described according to the “diamond concept” (1). Endothelium, a fundamental component of this bone architecture, plays an important role in the local bone metabolism both anabolic or catabolic, acting in a paracrine fashion on other bone stromal cells via humoral factors, growth factors and chemokines/cytokines (2, 3).

Several hypotheses of this “cross-talk” have been suggested during the past decades but the exact

molecular mechanisms are not completely clear yet (4, 5). We know that, under normal conditions, this complex bone homeostasis is realized by the balance between bone formation and bone resorption, processes regulated by osteoblasts and osteoclasts, respectively (6) (Fig. 1). Conditions like trauma, metabolic or genetic disorders that causes alteration of biochemical paracrine interactions between endothelium, vasculature and bone tissue could cause bone pathologies such as osteonecrosis (idiopathic or secondary), Gorham-Stout disease, Klippel-Trénaunay syndrome. In those conditions, the metabolism is often inhibited causing bone resorption or reduced bone deposition.

We wondered if a vascular alteration or tissue damage occurred in a bone segment could modify the paracrine function resulting in metabolic dysfunctions at bone level. We overviewed the

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Corresponding author:

Christian Carulli, MD

Orthopaedic Clinic, University of Florence

Largo P. Palagi 1, 50139 Florence

Tel.: 0039 055 7948200

Fax: 0039 055 486201

e-mail: christian.carulli@unifi.it

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literature about the most common biomolecules for communication between bone cells and endothelium and their interaction both in healthy and pathological conditions, we also analysed the possible future therapeutic.

The biomolecular base of the interactions between endothelial and bone cells

The communication between endothelium and bone cells is complexly based on the release and interaction of humoral factors, growth factors and chemokines/cytokines. For instance, in case of fracture healing, to ensure the correct bone healing process the osteoinduction is build upon the neovascularization that is allowed by the expression of a signature profile of VEGF on the mesenchimal stromal cells (MSCs). Indeed it has been demonstrated that in patients affected by Osteonecrosis (ON) of the hip, the pattern of VEGF expression is different than the one in healthy population. For instance, some studies assessed that in human and animal models of late stage ON the VEGF expression in MSCs is elevated, probably to induce a reparative blood supply in the osteonecrotic bone (7, 8). Moreover a study in Chinese population affected by ON of the femoral head demonstrated the presence of polymorphism in the VEGF genotype (634G/C) (9). However VEGF alone is not able to promote effective osteogenesis and angiogenesis or bone regeneration, indeed it may have synergistic actions with other biomolecules like prostaglandins (PGE₂) or BMPs (BMP-2, BMP-4) (10, 11). In fact, prostaglandins plays a role in bone metabolism as it is demonstrated by their elevated expression during mechanical load, injury or inflammation. Furthermore the expression of VEGF mRNA in osteoblasts rat's model demonstrated its over expression concomitant with an increased production of PGE₂ (in a dose-dependent fashion) (12). Moreover, once the osteogenesis and angiogenesis process has begun, a high level of Hypoxia-Inducible Factor (HIF-1) and RANK-L expressed by chondrocytes (13, 14) both express VEGF. The induction of bone formation is also linked to other factors, such endothelin-1 and angiotensin-II (15, 16), after which the action of the endothelium can coordinate all these cross-

talks during bone growth, bone development, bone remodelling, and bone repair (17). To complicate this delicate system the activity of growth factors is also modulated by the endocrine system. Indeed ACTH, TSH and FSH modulate angiogenesis and osteogenesis through the control of the synthesis of VEGF and RANK/RANK-L, the release of the VEGF and the bone resorption through the TNF- α pathway (FSH induced). Moreover, parathyroid hormone (PTH) has shown a vasodilating effect on bone vasculature (18). As a demonstration of those endocrine-modulated actions, some pathologic bone condition or the chronic corticosteroids use (as in ON of the hip) are strictly associated with an uncontrolled VEGF release ACTH mediated. It seems to create hyperpermeability of the endothelial and so insufficient bone repair due to vasculature insufficiency (19, 20). A central role in osteoclastogenesis/bone homeostasis is played by cytokines both for osteoclasts up-regulation (IL-1, IL-6, IL-7, IL-8, IL-11, IL-15, IL-17, IL-23, IL-34, TNF- α) and osteoclast down-regulation (IFN- α , IFN- β , IFN- γ , IL-3, IL-4, IL-10, IL-12, IL-27, IL-33) (21). Disregulation of interleukins levels plays a role in the development of bone diseases (22). Other cytokines act to promote the differentiation of osteoclasts from the monocyte/macrophage lineage, the macrophage colony-stimulating factor (M-CSF) and receptor activator of nuclear factor-kappa B (NF- κ B) ligand (RANKL) that activate NF- κ B signalling by recruiting TRAF6 (TFN receptor-associated factor 6) to its receptor. From this interaction numerous signalling pathways start to induce bone formation and activation as well as autophagosomal degradation of TFN receptor-associated factor 3 (TRAF3) that finally have a positive regulatory differentiation role of MSCs into osteoblastic precursors. Instead the role of IFN- γ in osteoclastogenesis is more conflicted resulting in a balance of direct or indirect effect. Indeed, IFN- γ could both target RANK-RANKL and inhibits osteoclasts differentiation as well as it activates the immune response determining an increase of osteoclastic factors. Nevertheless, there is no possibility to maintain the normal bone mass in case of loss of IFN- γ signal. Moreover many transcription

regulators [microphthalmia transcription factor (MITF), c-Fos, NF- κ B, and nuclear factor-activated T cells c1 (NFATc1)] control osteoclast differentiation.

Understanding the base of the interaction between those different pathways with future studies could provide novel therapeutic strategies for bone disease as it could be for the control of NFATc1 (23) or for the inhibition of TRAF3 degradation that interrupts bone resorption through osteoclast inhibition and stimulates bone formation through MSCs differentiation into osteoblasts (24). More debating is the direct administration of IFN- γ that could cause bone loss or resorption at the same time depending on the dose, usage and frequency (25).

The altered bone/vasculature complex and related clinical conditions

The altered relationship between endothelium and bone cells causes many different bone pathologies such as idiopathic or secondary osteonecrosis, bisphosphonate-related osteonecrosis of the jaws, Gorham–Stout disease, Klippel–Trénaunay syndrome and vertebral hemangioma (26).

The idiopathic osteonecrosis is the most common disease related to a defect of bone vasculature. It is probably linked to an ischemic injury that causes an over-expression of TRAP (tartrate-resistant acid phosphatase) protein on osteoclasts. In this pathological condition, there's a collapse of bone architecture, mostly localized on long bone epiphysis. The main symptom is a localized pain on the interested bone and it is extremely helpful for diagnosis using x-ray or MRI. Nitric oxide has possible protective role in the pathogenesis of the idiopathic osteonecrosis. Indeed, in Korean patient with idiopathic avascular necrosis of femoral head a polymorphism in intron 4 of Endothelial nitric oxide synthase was significantly found.

On the other hand, osteonecrosis could be linked to several congenital disorders (secondary osteonecrosis). This condition is common in patients affected by sickle cell anemia, primary thrombophilia and hyperfibrinolysis, mainly in thrombophilic Leiden mutation of the factor V gene and prothrombin 20210A gene mutation. Probably the main reason of this relationship concerns the

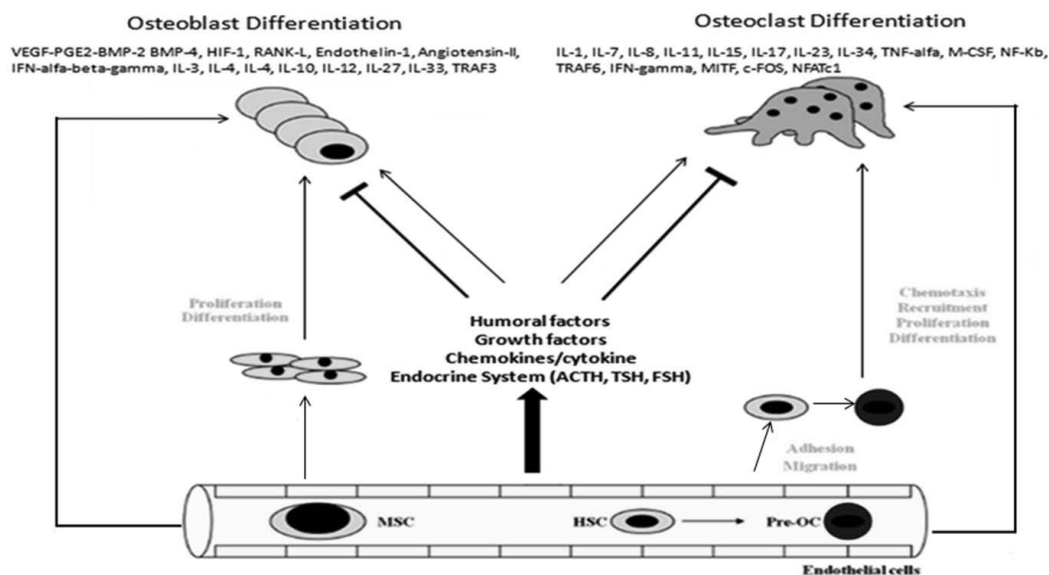


Fig. 1. A summary of the biomolecular pathways between the endothelium and the bone cells. **Ang II:** angiotensin-II; **BMPs:** bone morphogenetic proteins; **c-FOS:** Fos proto-oncogene AP-1 transcription factor subunit; **EC:** endothelial cell; **ET-1:** endothelin-1; **HIF-1:** Hypoxia-Inducible Factor; **HSC:** hematopoietic cell; **IFN α - β - γ :** Interferon alpha-beta-gamma; **IL-:** interleukin; **MITF:** microphthalmia transcription factor; **MSC:** mesenchymal stem cell; **NFATc1:** nuclear factor-activated T cells c1; **Nf- κ B:** nuclear factor-kappa B; **OB:** osteoblast; **OC:** osteoclast; **PGE2:** prostaglandin E2; **RANK-L:** Receptor activator of nuclear factor kappa-B ligand; **TRAF3-6:** TNF receptor-associated factor 3-6; **VEGF:** vascular endothelial growth factor.

increase in intravascular coagulation.

The bisphosphonate-related osteonecrosis of the jaws is a condition largely documented in literature caused by a relationship between the using of high doses of oral or endovenous bisphosphonate therapy and the incidence of ON, mainly concerning mandible or maxillar bone. The knowledge of the action mechanism, demonstrated in vitro or animal experiment, probably concerns the intracortical bone remodeling suppression induced by bisphosphonates, the reduction of the angiogenic processes and the inhibition of T-lymphocyte activation and proliferation that increases the infection risk.

Known as “phantom bone”, Gorham–Stout disease (GSD) is a pathological condition characterized by disseminated multifocal vascular lesions of the skeleton with possible visceral aspect. It is characterized by a hyperemia with subsequent excess of bone destruction and osteoclastic activity respect to bone formation. The pathogenetic

mechanism is linked to the over-expression of OPG (osteoprotegerin), able to capture RANK-L and OPN (osteopontin) that modify the apoptotic and regenerative balance of bone methabolism. It is important to underline that the osteolytic process usually is painless and the patients discover to be affected by this disease after a pathological fracture.

Klippel–Trénaunay syndrome (KTS) is a congenital malformation probably related to mutation of genes encoding for angiogenic factor, such as VG5Q and RASA1 located in chromosome 5. It is characterized by mixed vascular malformations associated with abnormal growth in the extremities, muscle hypoplasia and intramuscular lymphatic lesions. There is a simple form, with a segmental port-wine stain and good prognosis, and a complex form that includes deep venous system aplasia or hypoplasia with geographic PWS and higher risk of lymphatic involvement.

Vertebral hemangioma is a hamartomatous mass

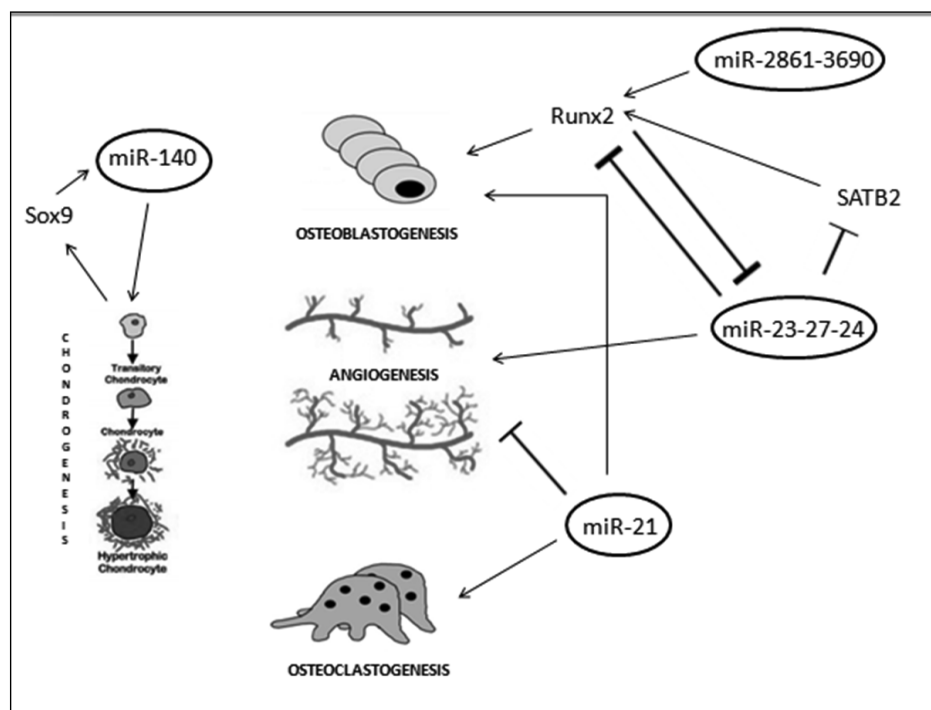


Fig. 2. The complex mRNA network. Various mRNAs play a selective role in bone homeostasis but a single mRNA has multiple ascertained effects in more than one type of cells at the same time: such numerous simultaneous effects make hard a specific therapy.

composed of thin-walled vessels lined by flat, usually unknown and discovered as “incidentaloma” at MRI. It’s a disease age-related that mostly concerns thoracic and lumbar spine. Usually confined to the vertebral body, VH may extend towards the posterior elements and compress the spinal cord and nerve roots.

The presence and future prospectives of “bone-metabolism drugs”

The study of the different pathways involved in the regulation of bone metabolism and bone vascularization both in normal and pathological conditions helps in the production of specific drugs acting in the altered typical processes of some bone diseases. Nevertheless antiresorptive drugs like biphosphonates still remain the elective treatment to any type of vasculature/metabolic bone disease (27-30). The biphosphonates’s mechanism of action is wide and it spreads from a strong antiangiogenetic activity to an inhibition of osteoclast activity resulting in the reduction of bone loss, bone resorption and bone hypervascularization. These molecules have been one of the pivot treatments in the early stage of ON of the femoral head along with other treatment as the limitation of weight bearing, the daily life activity modification and the instrumental physical activity (31). Because of their mechanism against the bone vascular proliferation, biphosphonates are also the main treatment in those pathologies related to a redundant bone vasculature as it is in GSD (32). Indeed the intravenous Pamidronate induce a tangible reduction of serum OPN and IL-6 levels. Another possible pharmacologic treatment in this pathological condition is the use of the monoclonal antibody called Denosumab that has already been registered as a treatment for fragility fractures in GSD (33, 34).

More recently, the scientific research of therapeutic strategies to act against bone metabolic/vasculature diseases have been focused in therapies related to genes and their expression (35). One strategy is to create and deliver a so called “suicide gene” to selectively eliminates the expression of a gene targeted as the cause of the disease. This could act in the altered endothelium cells to specifically inhibit the angiogenesis without systemic toxicity

(36). Another future perspective for the treatment of bone pathologies is the use of miRNAs, molecules involved in the process of RNA-silencing and post-transcriptional regulation of gene expression. Focusing in bone metabolism miRNAs can determine the commitment of progenitor cells to the osteoclast lineage as well as controlling their differentiation and bone resorbing function.

One of the main problems about the use of miRNAs is their immunogenicity even if in the last few years some excellent advances have been made to increase their stability and efficacy while decreasing immunogenicity. Moreover, another problem is that a single miRNA can target many different mRNAs and a single mRNA can have multiple miRNA binding sites Furthermore miRNAs have documented effects on more than one cell type (37). In bone repair, this concept is further complicated because the repair process progresses through a series of overlapping stages, involving at least four distinct cell types (Fig. 2). Therefore, the most important challenge is to try to reduce the side effects due to exposure of non-target cell types to therapeutic miRNA. It means that there is much work to be done in this field.

Moreover, because bone repair is a dynamic process, it is possible that modulation of one miRNA may facilitate repair if given early in the process, but impede it if given later. Therefore, it is important to study both miRNAs and validated miRNA targets in different tissues, in order to predict the safety and efficacy of miRNA-based therapeutics and their mechanisms of action. So the future of bone disease therapy will be probably based on miRNAs and it certainly include to overcome the problems related with miRNA-based therapeutics in bone repair that include to target the correct cells, to understand the correct timing of delivery, and to deepen the knowledge of their potential side effects and mechanisms of action (38).

What has emerged from this overview of possible treatments for bone vasculature/metabolism diseases is that gene and miRNA therapies represent a promising intervention but the scientific word needs much more studies to create a molecule that can selectively act in one target sparing the other physiological normal cellular pathways.

REFERENCES

1. Giannoudis P V, Einhorn TA, Marsh D. Fracture healing: The diamond concept. *Injury* 2007; 38(Suppl4):S3-6.
2. Brandi ML, Collin-Osdoby P. Vascular biology and the skeleton. *J Bone Miner Res* 2006; 21(2):183-92.
3. Cleaver O, Melton DA. Endothelial signaling during development. *Nat Med* 2003; 9(6):661-8.
4. Kern PA, Knedler A, Eckel RH. Isolation and culture of microvascular endothelium from human adipose tissue. *J Clin Invest* 1983; 71(6):1822-9.
5. Pasqualini R, Arap W, McDonald DM. Probing the structural and molecular diversity of tumor vasculature. *Trends Mol Med* 2002; 8(12):563-71.
6. Walsh MC, Kim N, Kadono Y, Rho J, Lee SY, Lorenzo J, et al. Osteoimmunology: interplay between the immune system and bone metabolism. *Annu Rev Immunol* 2006; 24:33-63.
7. Varoga D, Drescher W, Pufe M, Groth G, Pufe T. Differential expression of vascular endothelial growth factor in glucocorticoid-related osteonecrosis of the femoral head. *Clin Orthop Relat Res* 2009; 467(12):3273-82.
8. Mwale F, Wang H, Johnson AJ, Mont MA, Antoniou J. Abnormal vascular endothelial growth factor expression in mesenchymal stem cells from both osteonecrotic and osteoarthritic hips. *Bull NYU Hosp Jt Dis* 2011; 69(Suppl1):S56-61.
9. Liu B, Cao Y, Wang D, Yao G, Bi Z. Vascular Endothelial Growth Factor -634G/C Polymorphism Associated with Osteonecrosis of the Femoral Head in a Chinese Population. *Genet Test Mol Biomarkers* 2012; 16(7):739-43.
10. Keramaris NC, Calori GM, Nikolaou VS, Schemitsch EH, Giannoudis P V. Fracture vascularity and bone healing: A systematic review of the role of VEGF. *Injury* 2008; 39(Suppl2):S45-57.
11. Bouletreau PJ, Warren SM, Spector JA, Peled ZM, Gerrets RP, Greenwald JA, et al. Hypoxia and VEGF up-regulate BMP-2 mRNA and protein expression in microvascular endothelial cells: Implications for fracture healing. *Plast Reconstr Surg* 2002; 109(7):2384-97.
12. Harada SI, Nagy JA, Sullivan KA, Thomas KA, Endo N, Rodan GA, et al. Induction of vascular endothelial growth factor expression by prostaglandin E2 and E1 in osteoblasts. *J Clin Invest* 1994; 93(6):2490-6.
13. Kartsogiannis V, Zhou H, Horwood NJ, Thomas RJ, Hards DK, Quinn JMW, et al. Localization of RANKL (receptor activator of NFκB ligand) mRNA and protein in skeletal and extraskeletal tissues. *Bone* 1999; 25(5):525-34.
14. Gerber HP, Vu TH, Ryan AM, Kowalski J, Werb Z, Ferrara N. VEGF couples hypertrophic cartilage remodeling, ossification and angiogenesis during endochondral bone formation. *Nat Med* 1999; 5(6):623-8.
15. Escobar E, Rodríguez-Reyna TS, Arrieta O, Sotelo J. Angiotensin II, cell proliferation and angiogenesis regulator: biologic and therapeutic implications in cancer. *Curr Vasc Pharmacol* 2004; 2(4):385-99.
16. Veillette CJH, Von Schroeder HP. Endothelin-1 down-regulates the expression of vascular endothelial growth factor-A associated with osteoprogenitor proliferation and differentiation. *Bone* 2004; 34(2):288-96.
17. Yang YQ, Tan YY, Wong R, Wenden A, Zhang LK, Rabie ABM. The role of vascular endothelial growth factor in ossification. *Int J Oral Sci* 2012; 4(2):64-8.
18. Streeten EA, Brandi ML. Biology of bone endothelial cells. *Bone Miner* 1990; 10(2):85-94.
19. Zhang G, Sheng H, He YX, Xie XH, Wang YX, Lee KM, et al. Continuous occurrence of both insufficient neovascularization and elevated vascular permeability in rabbit proximal femur during inadequate repair of steroid-associated osteonecrotic lesions. *Arthritis Rheum* 2009; 60(10):2966-77.
20. Blair HC, Robinson LJ, Sun L, Isaacs C, Davies TF, Zaidi M. Skeletal receptors for steroid-family regulating glycoprotein hormones: A multilevel, integrated physiological control system Skeletal receptors for pituitary hormones. *Ann NY Acad Sci* 2011; 1240:26-31.
21. Amarasekara DS, Yun H, Kim S, Lee N, Kim H, Rho J. Regulation of osteoclast differentiation by cytokine networks. *Immune Netw* 2018; 18(1):e8.
22. Amarasekara DS, Yu J, Rho J. Bone loss triggered by the cytokine network in inflammatory autoimmune diseases. *J Immunol Res* 2015; 2015:832127.
23. Kim JH, Kim N. Regulation of NFATc1 in osteoclast differentiation. *J Bone Metab* 2014; 21(4):233-41.
24. Boyce BF, Li J, Xing L, Yao Z. Bone remodeling and

- the role of TRAF3 in osteoclastic bone resorption. *Front Immunol* 2018; 9:2263.
25. Tang M, Tian L, Luo G, Yu X. Interferon-gamma-mediated osteoimmunology. *Front Immunol* 2018; 9:1508.
 26. Carulli C, Innocenti M, Brandi ML. Bone vascularization in normal and disease conditions. *Front Endocrinol (Lausanne)* 2013; 4:106.
 27. Coxon FP, Thompson K, Rogers MJ. Recent advances in understanding the mechanism of action of bisphosphonates. *Curr Opin Pharmacol* 2006; 6(3):307-12.
 28. Wood J. Novel Antiangiogenic Effects of the Bisphosphonate Compound Zoledronic Acid. *J Pharmacol Exp Ther* 2002; 302(3):1055-61.
 29. Allen MR, Kubek DJ, Burr DB, Ruggiero SL, Chu TM. Compromised osseous healing of dental extraction sites in zoledronic acid-treated dogs. *Osteoporos Int* 2011; 22(2):693-702.
 30. Mont MA, Jones LC, Seyler TM, Marulanda GA, Saleh KJ, Delanois RE. New treatment approaches for osteonecrosis of the femoral head: an overview. *Instr Course Lect* 2007; 56:197-212.
 31. Aaron RK, Ciombor DM. Therapeutic effects of electromagnetic fields in the stimulation of connective tissue repair. *J Cell Biochem* 1993; 52(1):42-6.
 32. Moschetta M, Di Pietro G, Ria R, et al. Bortezomib and zoledronic acid on angiogenic and vasculogenic activities of bone marrow macrophages in patients with multiple myeloma. *Eur J Cancer* 2010; 46(2):420-9.
 33. Marcucci G, Masi L, Carossino AM, Franchi A, Capanna R, Sinigaglia L, et al. Cystic bone angiomas: A case report treated with aminobisphosphonates and review of the literature. *Calcif Tissue Int* 2013; 93(5):462-71.
 34. McClung MR, Lewiecki EM, Cohen SB, et al. Denosumab in postmenopausal women with low bone mineral density: Commentary. *Obstet Gynecol Surv* 2006; 354(8):821-31.
 35. Rafii S, Lyden D. Therapeutic stem and progenitor cell transplantation for organ vascularization and regeneration. *Nat Med* 2003; 9(6):702-12.
 36. De Palma M, Venneri MA, Roca C, Naldini L. Targeting exogenous genes to tumor angiogenesis by transplantation of genetically modified hematopoietic stem cells. *Nat Med* 2003; 9(6):789-95.
 37. Carulli C, Luzi E, Macera A, Innocenti M, Brandi ML. RNA-Based Therapies for Bone Diseases. *MicroRNA Regen Med* 2014; doi.org/10.1016/B978-0-12-405544-5.00040-X.
 38. Franceschetti T, Delany AM. miRNAs in Bone Repair. *MicroRNA Regen Med* 2014; doi.org/10.1016/B978-0-12-405544-5.00025-3.

HOW TO OBTAIN BETTER RESULTS, CHOICE OF IMPLANT OR SURGICAL TECHNIQUE? A NEW SURGICAL TECHNIQUE WITH AN EXTRAMEDULLARY REFERENCED CUTTING GUIDE AND A BLENDED ALIGNMENT METHOD

S.M.P ROSSI¹, M. GHIARA¹, A. CASTELLI¹, A. IVONE^{1,2}, E. JANNELLI^{1,2} and F. BENAZZO^{1,2}

¹*Clinica Ortopedica, Fondazione IRCCS Policlinico San Matteo;* ²*Università degli Studi di Pavia, Pavia Italy*

Measured resection and ligament balancing techniques are the most used in TKA; both require, in the majority of knee arthroplasty systems, a femoral intramedullary canal referencing for the distal femoral cut. Fat embolism, activation of coagulation and bleeding may occur from the reamed canal; this can be avoided with different solutions such as navigation, patient specific instruments or the use of an extramedullary device. The FuZion™ tensor was created as a blended method with the purpose to overcome the downsides of both techniques by fusing them. The aim of our study is to present the technique and the preliminary results of a cohort of patients where this instrument was customized and used as an extramedullary cutting guide for the distal femur, matching the distal femoral cut with the proximal tibial cut in order to obtain a guided, kinematically adjusted, alignment and a reduction of post-op blood loss.

In most surgical techniques, the distal femur resection is commonly made using a fixed angle (pre-operatively planned) relative to an intramedullary rod (1-12, 13-16, 17-20, 21-23).

Intramedullary instruments are the most widely accepted way to ensure proper component positioning, but the rates of outliers (more than 3° off the neutral mechanical axis), both in the sagittal and coronal planes, reportedly range from 10% to 20% (23).

In addition, the distal femoral cut is performed independently from the tibial cut, and the rotational alignment determined upon fixed landmarks (measured resection) or ligament driven (gap balancing) (5, 19, 21). The alignment obtained is standardized and imposed to the patients and not guided by their functional anatomy.

Two important topics of discussion are on the table at the present time. The first deals with neutral

alignment as the best option for having satisfied patients or if a more "kinematically aligned" implant would be suitable for better outcomes (2-4). The concept of an individual alignment of the implants is of growing interest in recent years (7, 11, 18, 26, 27). The second is whether a ligament balancing or a measured resection technique can give better outcomes or if a hybrid technique is preferable in order to achieve a personalization of the surgery (5, 19, 21).

Our hypothesis is that matching the distal femoral cut with the proximal tibial cut, adopting an extramedullary driven device (with customized instruments added to "Fuzion" tensor-blending the measured resection and the ligament balancing techniques), we can achieve a customized alignment for each patient with clinical outcomes at least comparable or better than a standard technique and a reduced, postoperative blood loss.

Key words: total knee arthroplasty, blood loss, extramedullary

Corresponding Author:
Dr Stefano Marco Paolo Rossi,
Clinica Ortopedica e Traumatologica, Università degli Studi di Pavia,
Fondazione IRCCS Policlinico San Matteo,
P.le Golgi 19, 27100 Pavia, Italy
Tel.: +393331058877
e-mail: rossi.smp@gmail.com

MATERIALS AND METHODS

We prospectively evaluated 45 patients (28 females, 17 males), who underwent total knee arthroplasty surgery from September 2016 to July 2017. Mean age was 70 years (range 47-85).

All patients were operated with the Persona PS (Posterior Stabilized) prosthesis (Zimmer, Warsaw, IN) through a medial parapatellar approach without everting the patella, using the customized version of FuZion[®] tensor, with an extramedullary referenced distal femoral cutting guide.

All the patients signed for informed consent before the surgery. All the procedures were performed by 2 experienced surgeons in the field of total knee arthroplasty (senior authors). No tourniquet was used, nor was a drain inserted after surgery. The rehabilitation protocol consisted in assisted full weight bearing from the first postoperative day and early free mobilization. After 7 to 15 days, we allowed free walking without crutches but the patients were authorized to leave the aids at their compliance.

The mean follow-up was 12 months (range 8-18 months). Weight bearing long leg films were administered to all patients (standard AP view and lateral view), preoperatively and at 3 months post-op. We evaluated the preoperative and postoperative mechanical axis using HKA (hip-knee-ankle).

Patients were evaluated clinically, using the international Oxford Knee Score preoperatively, at 6 months and one year after the surgery.

We evaluated the blood haemoglobin levels preoperatively and in the first, third and fifth postoperative days. The values were compared to those obtained by a matched group of 45 patients in which we used intramedullary referenced distal femoral cutting guide.

Baseline characteristics were expressed as the mean (standard deviation). We examined the distribution of continuous variables through the Kolmogorov-Smirnov test. The *t*-test was used for normally distributed continuous variables, and the Mann-Whitney test was used for continuous variables not normally distributed. A value of $P < 0.05$ was considered statistically significant. Data were statistically analyzed using IBM SPSS Statistics for Windows (Version 22.0; IBM, Armonk, NY, USA).

We used GraphPad Prism 5.0 for the statistical analysis. We studied the variations between the preoperative and

postoperative scores and the variations of the preoperative and postoperative hemoglobin between the two groups (extramedullary alignment and control group). This study was approved by the Ethical Committee (No. 2015001968).

Surgical technique

The tensor device named FuZion[®] has been conceived to enable the surgeons to blend the measured resection and the ligament balancing techniques, optimizing the femoral rotation at 90° of flexion without the need of defining less then precise bony landmarks (the epicondyles), and the flexion/extension spaces (8). Originally, the use of this sort of “mechanical” navigation could be started after the distal femoral cut (with the traditional endomedullary technique) while the proximal tibial cut was made independently. Assuming that the proximal tibial cut should be done at 90°, with the appropriate slope, we thought that the distal femoral cut could be bound to the tibial cut with a minimal modification of the tensor device, achieving a rectangular extension space driven by the purposely tensioned ligaments and removing the need of an intramedullary violation of the femur. In order to do that we customized the tensor and the surgical technique as follows:

- The proximal tibial cut must be at 90° precisely, with the proper slope according to the design of the implant
- Ligaments tension in extension must be equal medial and lateral, and the required adjustments can be performed to achieve it (mandatory removal of the osteophytes, needle crusting if necessary)
- The alignment rods will prove that the leg is properly aligned in extension: the distal rod has to be parallel to the tibia
- Distal femoral cutting jig is placed according to the balanced and properly tensioned ligaments (it can be also displaced in steps of two mm in order to achieve the perfect amount of bone resection required)
- Sizing and rotational alignment of the femoral component can be done at this point according to the original FuZion technique, and the following steps done subsequently (Fig. 1-3).

RESULTS

No patients were lost at follow up and no major

complications were found in the follow up of this series of patients. At preoperative long leg standing X-rays of all patients, we had 7 valgus knees (15%) with a mean HKA alignment of -7.6° ($SD \pm 3.2$) and 30 varus knees (67%) with a mean HKA alignment of 7.8° ($SD \pm 3.7$); 8 (18%) knees could be classified as with a neutral pre-op alignment ($\pm 2^\circ$).

At 3 months postoperatively all patients had a long leg standing X-ray control and we measured 6 valgus knees (mean HKA angle $-3.7^\circ \pm 1.5^\circ$) within the pre-op valgus group and 25 varus knees (mean HKA angle $3.9^\circ \pm 1.8^\circ$) within the pre-op varus group. Fourteen knees were classified neutral in the postop x-rays with a HKA angle of $\pm 2^\circ$.



Fig 1. Two magnetic paddles tapered at 5° , necessary to compensate the tibial slope and to reach the full extension of the knee.



Fig. 3. The distal cutting jig is positioned, the amount of bone to reset can be checked, and the jig displaced accordingly.

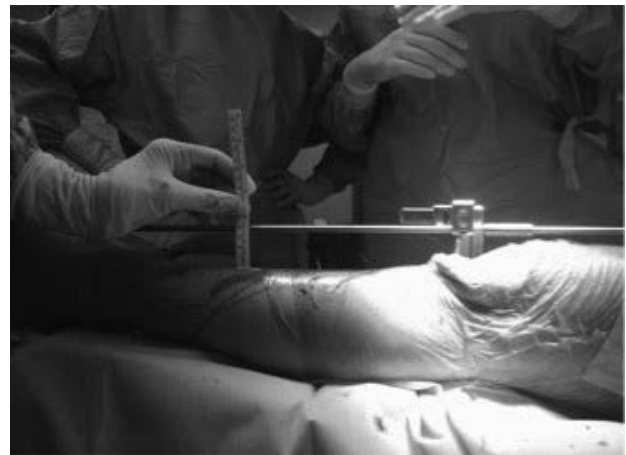


Fig 2. The alignment rods will prove that the leg is properly aligned: rod is placed parallel to the tibia, in order to guarantee the proper flexion of the distal femoral cutting jig, with the position of the two pins on the distal femur.

Preoperatively the mean OKS was $21.1 (\pm 8.1)$ while at 6 months and at 1 year after surgery it was respectively $41.5 (\pm 6.5)$ and $44.8 (\pm 3.5)$, statistically significant if compared with preoperative values ($p < 0.001$) and less if compared to each other ($p < 0.05$).

We analyzed blood loss comparing hemoglobin values in study group and in a control group of the same number and age and found that in the preoperative phase there was no difference of hgb levels in the two groups ($p = 0.07$); while on day 5 postoperatively, the mean hgb level in the control group was 9.75 g/dL and in the study group it was 10.35 g/dL ($p < 0.05$). There was a different number of blood transfusions in the two groups studied although without significant difference.

DISCUSSION

With the customization of the tensor device FuZion[®], the distal femoral cut with extramedullary alignment is possible and feasible. Compared to previous studies that present results on EM alignment technique (3), our aim was not to achieve a neutral alignment on the coronal plane, but to give the knee a “ligament driven” alignment based on a 90° tibial cut.

Our hypothesis was sustained by previous papers presenting the so called “kinematic alignment”, demonstrating that a residual deformity (especially

in varus) is not only well tolerated but can lead to better clinical outcomes as patients experience the feeling of a more normal knee (9, 10, 14, 17). Compared to a Kinematic alignment technique (2, 4, 11, 25-27), our technique for the distal femoral cut is driven by the ligament tension adjusted on a 90° tibial resection and it is not carried out for the purpose of replacing the worn cartilage and bone with the thickness of the implant. The results of our series in terms of coronal alignment show the tendency in the majority of the patients to leave a residual deformity, confined within 5° of a neutral alignment. This deviation from a neutral alignment did not correlate with worse clinical outcomes. Our hypothesis was that, as demonstrated in previous studies presenting results on kinematically aligned implants (2, 4, 11, 24-27), patients would benefit from a more physiological knee and therefore gain better results on PROMS. Our study showed a significant reduction of Haemoglobin loss at 5 days post-op, compared to a previous group of comparable patients treated with an IM alignment technique, but no significant difference was found in terms of blood transfusion.

Blood loss after TKA is multifactorial, and this study was not exactly powered for that purpose. We believe that, to expect a consistent and evident reduction in blood loss by changing only one intraoperative variable, a study with a larger cohort and strict patient selection criteria would be required. Our study have several limitations. The surgical technique is applicable only with this customized tensor system, but in order to perform the distal femoral cut with a balanced extension space, the same instrumentation set could be used independently with any kind of implant. With the due modifications, the technique can be applied to all prosthetic designs. At the moment we only have short term follow up results and further follow up and investigations are necessary to confirm the results and to evaluate survival at mid-term and long-term follow-up.

REFERENCES

1. Abdel MP, Oussedik S, Parratte S, Lustig S, Haddad FS. Coronal alignment in total knee replacement: historical review, contemporary analysis, and future direction *Bone Joint J* 2014; 96-B(7):857-62.
2. Almaawi AM, Hutt JRB, Masse V, Lavigne M, Vendittoli PA. The Impact of Mechanical and Restricted Kinematic Alignment on Knee Anatomy in Total Knee Arthroplasty. *J Arthroplasty* 2017; 32(7):2133-40.
3. Baldini A, Adravanti P. Less Invasive TKA Extramedullary Femoral Reference Without Navigation *Clin Orthop Relat Res* 2008; 466:2694-700.
4. Courtney PM, Lee GC. Early Outcomes of Kinematic Alignment in Primary Total Knee Arthroplasty: A Meta-Analysis of the Literature. *J Arthroplasty* 2017; 32(6):2028-32.
5. Daines B, Dennis D. Gap Balancing vs. Measured Resection Technique in Total Knee Arthroplasty *Clin Orthop Surg* 2014; 6(1):1-8.
6. Ewald FC. The Knee Society total knee arthroplasty roentgenographic evaluation and scoring system. *Clin Orthop Relat Res* 198; (248):9-12.
7. Hommel H, Perka C, Pfizner T. Preliminary results of a new surgical technique in total knee arthroplasty (TKA) using the native ligament tension for femoral implant positioning in varus osteoarthritis. *Arch Orthop Trauma Surg* 2016; 136(7):991-7.
8. Jones C, Nawaz Z, Hassan A, White S, Khaleel A. The variability in the external rotation axis of the distal femur: an MRI-based anatomical study. *Eur J Orthop Surg Traumatol* 2016; 26(2):199-203.
9. Kim JM, Hong SH, Kim JM, Lee BS, Kim DE, Kim KA, Bin SI. Femoral shaft bowing in the coronal plane has more significant effect on the coronal alignment of TKA than proximal or distal variations of femoral shape. *Knee Surg Sports Traumatol Arthrosc* 2015; 23(7):1936-42.
10. Lording T, Lustig S, Neyret P. Coronal alignment after total knee arthroplasty. *EFORT Open Rev* 2017; 1(1):12-17.
11. Lee YS, Howell SM, Won YY, Lee OS, Lee SH, Vahedi H, Teo SH. Kinematic alignment is a possible alternative to mechanical alignment in total knee arthroplasty *Knee Surg Sports Traumatol Arthrosc* 2017; 25(11):3467-79.
12. Luyckx T, Zambianchi F, Catani F, Bellemans J,

- Victor J. Coronal alignment is a predictor of the rotational geometry of the distal femur in the osteoarthritic knee. *Knee Surg Sports Traumatol Arthrosc* 2013; 21(10):2331-7.
13. Maderbacher G, Schaumburger J, Baier C, Zeman F, Springorum HR, Birkenbach AM, Grifka J, Keshmiri A. Appropriate sagittal femoral component alignment cannot be ensured by intramedullary alignment rods. *Knee Surg Sports Traumatol Arthrosc* 2016; 24(8):2453-60.
 14. Nam D, Vajapey S, Haynes JA, Barrack RL, Nunley RM. Does use of a variable distal femur resection angle improve radiographic alignment in primary total knee arthroplasty? *J Arthroplasty* 2016; 31(9S):91-6.
 15. Park A, Stambough JB, Nunley RM, Barrack RL, Nam D. The inadequacy of short knee radiographs in evaluating coronal alignment after total knee arthroplasty. *J Arthroplasty*. 2016; 31(4):878-82.
 16. Pfitzner T, Von Roth P, Perka C, Matziolis G. Intramedullary control of distal femoral resection results in precise coronal alignment in TKA. *Arch Orthop Trauma Surg* 2014; 134(4):459-65.
 17. Rivière C, Iranpour F, Auvinet E, Howell S, Vendittoli PA, Cobb J, Parratte S. Alignment options for total knee arthroplasty: A systematic review. *Orthop Traumatol Surg Res* 2017; 103(7):1047-56.
 18. Rivière C, Iranpour F, Auvinet E, Aframian A, Asare K, Harris S, Cobb J, Parratte S. Mechanical alignment technique for TKA: Are there intrinsic technical limitations? *Orthop Traumatol Surg Res* 2017; 103(7):1057-67.
 19. Rahm S, Camenzind RS, Hingsammer A, Lenz C, Bauer DE, Farshad M, Fucentese SF. Postoperative alignment of TKA in patients with severe preoperative varus or valgus deformity: is there a difference between surgical techniques? *BMC Musculoskelet Disord* 2017; 18(1):272.
 20. Shah NA, Patil HG, Dhawale AS, Khedkar BM. Limited femoral navigation versus conventional intramedullary femoral jig based instrumentation for achieving optimal restoration of mechanical axis post total knee arthroplasty: a prospective comparative study of 200 knees. *J Arthroplasty* 2015; 30(4):559-63.
 21. Sheth NP, Husain A, Nelson CL. Surgical techniques for total knee arthroplasty: measured resection, gap balancing, and hybrid. *J Am Acad Orthop Surg* 2017; 25(7):499-508.
 22. Stucinskas J, Robertsson O, Lebedev A, Wingstrand H, Smailys A, Tarasevicius S. Measuring long radiographs affects the positioning of femoral components in total knee arthroplasty: a randomized controlled trial. *Arch Orthop Trauma Surg* 2016; 136(5):693-700.
 23. Tang Q, Shang P, Zheng G, Xu HZ, Liu HX. Extramedullary versus intramedullary femoral alignment technique in total knee arthroplasty: a meta-analysis of randomized controlled trials. *J Orthop Surg Res* 2017; 12(1):82.
 24. Vandekerckhove PJ, Lanting B, Bellemans J, Victor J, MacDonald S. The current role of coronal plane alignment in Total Knee Arthroplasty in a preoperative varus aligned population: an evidence based review. *Acta Orthop Belg* 2016; 82(1):129-42.
 25. Victor J. Optimising position and stability in total knee arthroplasty. *EFORT Open Rev* 2017; 2(5):215-20.
 26. Waterson HB, Clement ND, Eyres KS, Mandalia VI, Toms AD. The early outcome of kinematic versus mechanical alignment in total knee arthroplasty: a prospective randomised control trial. *Bone Joint J* 2016; 98-B(10):1360-8.
 27. Young SW, Walker ML, Bayan A, Briant-Evans T, Pavlou P, Farrington B. The Chitranjan S. Ranawat Award: No difference in 2-year functional outcomes using kinematic versus mechanical alignment in TKA: a randomized controlled clinical trial. *Clin Orthop Relat Res* 2017; 475(1):9-20.

HYDATID CYST IN THE SPINE. THE ALLIANCE BETWEEN SURGEON AND INFECTIOLOGIST IS WINNING: A CASE REPORT.

F. CUZZOCREA¹, E. JANNELLI^{1,3}, A. IVONE^{1,3}, R. VANELLI¹, M. GHIARA¹, A. FIORUZZI¹, E. BRUNETTI² and F. BENAZZO^{1,3}

¹*Clinica di Ortopedia e Traumatologia, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy;*

²*Clinica di Malattie Infettive, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy;* ³*Università degli Studi di Pavia, Pavia, Italy*

Hydatid Cyst is a protozoal disease, whose spinal localization may be cause of pain and severe inability due to neurological symptoms and instability. Treatment combines the difficulties to eradicate the cyst with the spine regional constraints. The case of a 45-year-old woman is reported, affected by hydatid cyst in the spine, submitted to combination of medical treatment and surgery (double approach gross total excision and reconstruction) and followed up for 6 years. The treatment strategy allowed an excellent quality of life without pain and neurological symptoms at the latest follow-up. This case supports the validity to combine anthelmintic medical treatment and excisional surgery in the treatment of hydatid cyst of the spine. The medical treatment makes surgery more effective maintaining the result over long term. Subtotal or total excision of the cysts can be performed, including decompression of neural structures and adequate spine reconstruction.

Cystic echinococcosis has a cosmopolitan distribution and represents a major public health problem in some regions. It is considered endemic in areas such as Peru, Chile, Argentina, Uruguay, southern Brazil, the Mediterranean region, central Asia, western China, and East Africa. Cystic echinococcosis is not found in Antarctica and has been eliminated through comprehensive control programs in Iceland, New Zealand, Tasmania, Falkland Islands, and Cyprus.

The G1 genotype of *Echinococcus granulosus sensu stricto* is responsible for the vast majority (88%) of human cases worldwide. It has a cosmopolitan distribution and is associated with transmission from sheep as an intermediate host. *E. canadensis* G6 and G7 are responsible for 7.3% and 3.7% of infections

worldwide, respectively. There have been no human cases of *E. equinus* described in the literature.

The human incidence can exceed 50 per 100,000 person-years in areas of endemicity, and prevalence rates as high as 5% to 10% can be found in parts of Peru, Argentina, east Africa, and China. Every year, echinococcosis is responsible for the loss of at least 1 million Disability-Adjusted Life Years (DALYs) and of 3 billion dollars in expenses, including treatment and livestock losses.

Cystic echinococcosis typically occurs in poor pastoral regions in which sheep or other livestock are raised and in which dogs are kept, for herding or property guarding, in close proximity to households. Dogs in such regions are frequently fed offal, and for religious and other reasons, their populations

Key words: echinococcus, anterior approach, corpectomy, expandable cage

Corresponding author:

Dr Alberto Fioruzzi,
Clinica di Ortopedia e Traumatologia,
Fondazione IRCCS Policlinico San Matteo,
Viale Camillo Golgi 19, 27100 Pavia, PV, Italy
Tel.: +393289222682
e-mail: alberto.fioruzzi@gmail.com
ORCID-ID: 0000-0002-4412-7375

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might not be curtailed. The prevalence of cystic echinococcosis increases with age and women are affected more frequently than men; this might be related to domestic activities that bring them in closer contact with dogs through feeding, herding, or milking livestock (1).

Case Report

A 45-year-old woman, complaining back pain for many years, was submitted to a CT scan of the thoracic spine in December 2011.

A huge psoas abscess and an invasion of the spinal canal with compression and dislocation of the spinal cord was found, originating from an erosive radiolucent image in the vertebral bodies of T12 and L1. A CT scan of the brain and the abdomen were negative. Imaging was consistent with hydatid cyst disease, confirmed also by the echographic findings.

She was hospitalized for haematological tests, including ELISA, IHA and immunoblotting. Serology for hydatid disease reported positivity with IHA antibodies 1/65536 ($\text{pos} \geq 1/64$), ELISA 4.980 ($\text{pos} \geq 1.1$) and the detection of antibodies with immunoblotting showed specific bands.

The MRI study showed a large paravertebral mass infiltrating and enveloping the left iliopsoas, lapping the diaphragm, embracing the left renal artery, displacing the aorta and touching the right iliopsoas (Fig. 1). The study of the spinal cord vascularization did not show a feeding artery to anterior spinal artery (the so-called Adamkiewicz

artery). The patient complained of persistent back pain, reduced autonomy gait and the occurrence of paresthesia in the lower limbs. The neurological examination showed reduction of walking autonomy, claudication, bilateral hyperreflexia and right lower limb weakness, although electrophysiological examinations were negative.

The patient underwent medical therapy with albendazole (Zentel 400mg day) for about 8 months. Blood tests suddenly showed thrombocytopenia (45000/ul). The patient was not in treatment with heparin and no apparent cause, that may have triggered the reduction of platelets, was found and the values normalized after 25 days without any treatment.

Surgical Treatment

In October 2012, a surgical procedure was planned after evaluation of several surgical options. Considering the kyphosis deformity, the destruction of the anterior column, the symptomatic spinal cord compression, a first posterior procedure for decompression, fixation and postero-lateral grafting for fusion and a second anterior procedure for mass removal and reconstruction of the anterior column were scheduled.

First procedure: Fixation T10-L3. With the patient in prone position with monitoring of MEP and SSEP, the decompression, the removal of the posterior elements of T12 and L1 (bilateral T12 and L1 laminartrectomy) and removal of the affected

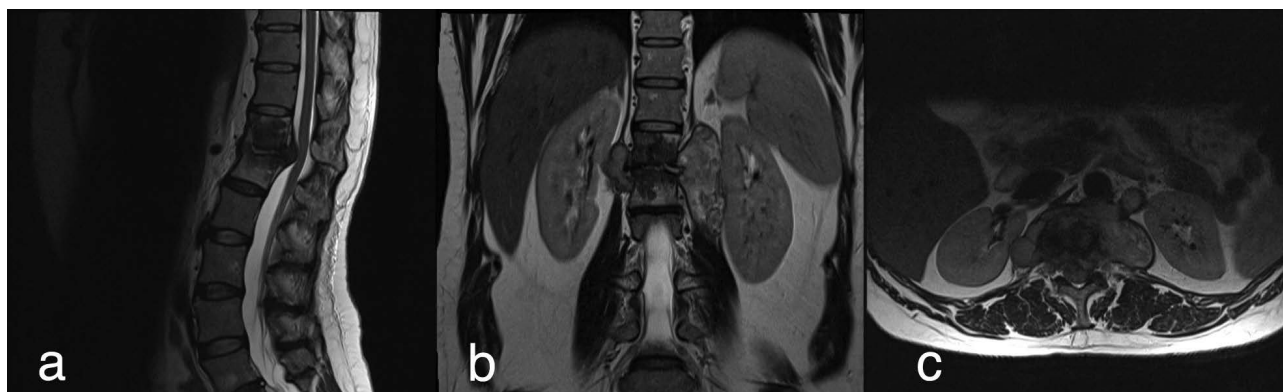


Fig. 1. Preoperative MRI T1. **a)**: Sagittal plane; **b)**: Coronal plane; **c)**: Transverse plane.

pedicles were performed. The posterior longitudinal ligament was intact without solution of continuity with the lesion, nor presence of cysts inside the canal. It was decided not to remove the anterior compression, caused by the pressure of the cyst on the ligament and the kyphosis, to avoid the spreading of the disease inside the spinal canal. (Fig. 2a).

Second procedure: Three weeks after complete healing of the surgical scar the anterior procedure was performed (Fig. 2b). The patient was in right lateral decubitus with monitoring of SSEP and MEP. The Zielke access was performed (trans-diaphragmatic-retroperitoneal-lombotomic). Because of the involvement of the renal artery and the aorta, the vascular surgeon performed the release of the vessels: the mass had completely incorporated the left renal artery and a cleavage plane with the vessel was not present because of the impressive inflammatory reaction between the cysts and the vessel walls. Therefore, the cysts' walls were not isolated from the artery's wall, but had acceptable margins. Within the left psoas, there was fluid that had collected. The vertebral bodies of T12 and L1 were eroded by the cyst and the left foramina of T12 was partially infiltrated. A substantial part of the mass was removed, by removing the T11-T12 disc, the vertebral body of T12, the D12-L1 disc, the body of L1 and the L1-L2 disc and then an expandable cage was implanted and filled with bone chips from bone

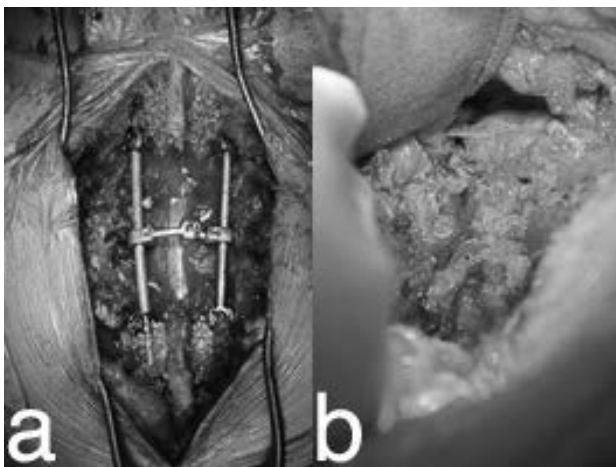


Fig. 2. Intraoperative pictures. *a*): Posterior instrumentation; *b*): Anterior expandable cage.

bank. In the end, repeated washings with hypertonic NaCl solution were performed (5).

RESULTS

The postoperative CT scan and Rx controls showed the correct placement of the implants and the removal of 80% of the mass (Fig. 3). Histological examination and bacteriological tests performed on intraoperative samples confirmed the diagnosis of hydatid disease. The neurological symptoms resolved completely after the first procedure with complete recovery of walking autonomy. Patient was evaluated at six months after surgery and every year using standardized clinical questionnaires (Oswestry Disability Index - ODI, Visual-Analogue-Scale for low back pain - VAS LBP) (7).

At six months, follow up ODI was 32%, with moderate disability and the VAS LBP was 5. At one year, follow-up ODI was 18% and VAS LBP was 2; finally, at four year follow-up, no recurrence of disease was found with consolidation of the arthrodesis and stabilization of the clinical status, with ODI 10% and VAS LBP 1.

DISCUSSION

The location of spinal hydatid disease, although considered rare, is frequently encountered in clinical practice (2, 3). The palliative treatment and the wide laminectomy without removal of the mass have a very high percentage of implant failure and as a result the progression of the disease. The surgical revision interventions are extremely complicated for the numerous adhesions and the presence of pathological tissue. Early diagnosis and treatment can dramatically prevent the mass growth, which can complicate its removal.

The preoperative therapy is intended to inactivate the cysts turning them into inactive tissue and reduce the risk of relapse. A decompression of the neurological structures (in the case of spinal cord compression), the reconstruction of anterior column with bone (possibly from bank) to ensure a biological support are mandatory. Intraoperative washing with hypertonic saline is favoring sclerosis of the cyst (5).

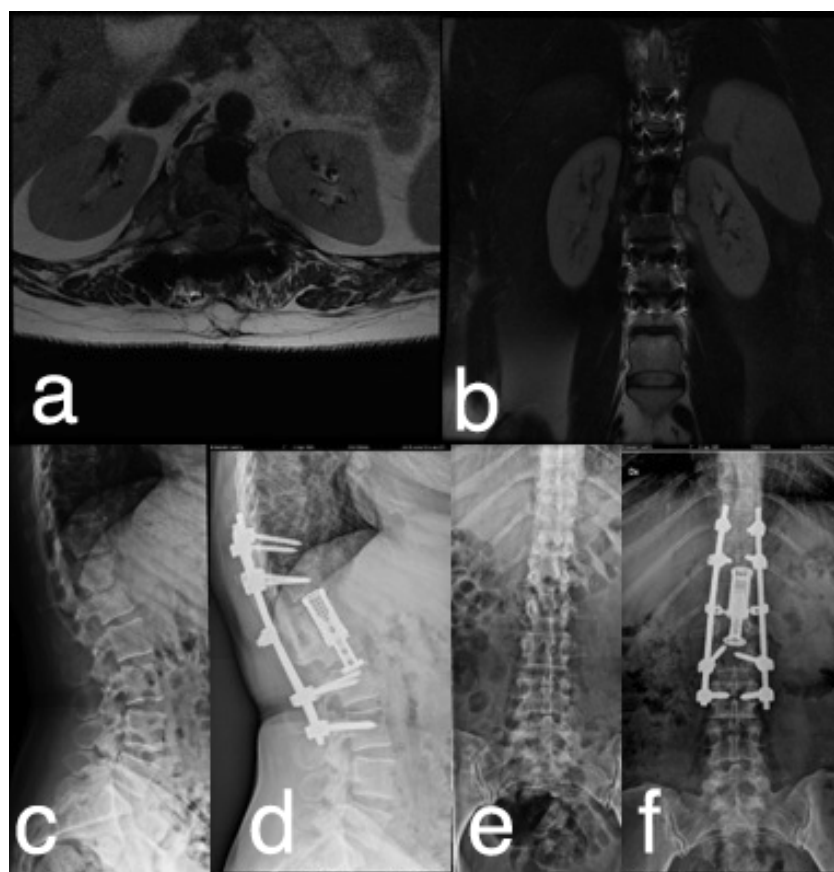


Fig. 3. Postoperative control. **a**): Trasverse T2 MRI; **b**): Coronal T2 MRI; **c**): Preop lateral view X-ray; **d**): 70 months follow up lateral view X-ray; **e**): Preop AP view X-ray; **f**): 70 months follow up AP view X-ray.

Treatment should be as aggressive as large as the volume of the mass, providing two or three accesses. The risk of anaphylactic shock, widely described in the literature is actually very low, comparable to a drug-related anaphylactic side effect (6).

Almost two centuries after Reydellet performed the first surgical intervention on a patient with spinal CE (1819), surgery remains the treatment of choice (8). Despite significant advances in diagnostic imaging techniques and surgical treatment, spinal echinococcosis remains associated with a high degree of morbidity, disability and mortality.

Surgery is the treatment of choice with decompression of a compromised spinal cord and stabilization of a compromised spinal column being the two primary tasks. The long-term outcome depends on complete resection of all parasitic lesions,

which is frequently hampered by the infiltrative nature of the disease. Recurrence rates of spinal CE are high, especially in cases with vertebral bone involvement. The currently existing data on follow-up of spinal CE cases is largely insufficient to judge the long-term outcome of surgery.

Besides surgery, the intraoperative use of scolicalids and the pre-, peri-, and post-operative use of albendazole is currently considered the treatment standard of spinal CE. Therapy with benzimidazole derivatives has shown to be effective in visceral CE (9) although a recent systematic review concluded that treatment response depends on cyst stage and size, and that efficacy of medical treatment may have been overstated in the past (10, 11). The role of combination drug therapy, percutaneous treatment and radiotherapy in spinal CE remains to be defined.

REFERENCES

1. Agudelo Higuera NI, Brunetti E, McCloskey C. Cystic echinococcosis. *J Clin Microbiol* 2016; 54(3):518-23.
2. Sami A. et al. Hydatidose vertebro-medullaire. *Neurochirurgie* 1996; 42:281-7.
3. Ferrandez HD, Gomez-Castresana F, Lopez-Duran L, Mata P, Brandau D, Sanchez-Barba A. Osseous hydatidiosis. *J bone Joint Surgery Am* 1978; 60:685-90.
4. Fitzpatrick S.C. Hydatid disease of the lumbar vertebrae. *J Bone Joint Surg Br* 1965; 47:286-91.
5. Besim H, Karayalçın K, Hamamci O, Güngör C, Korkmaz A. Scolicidal agents in hydatid cyst surgery. *HPB Surg* 1998; 10(6):347-51.
6. Neumayr A, Troia G, de Bernardis C, Tamarozzi F, Goblirsch S, Piccoli L, Hatz C, Filice C, Brunetti E. Justified concern or exaggerated fear: the risk of anaphylaxis in percutaneous treatment of cystic echinococcosis-a systematic literature review. *PLoS Negl Trop Dis* 2011; 5(6):e1154.
7. Fairbank JCT, Pynsent, PB. The Oswestry Disability Index. *Spine* 2000; 25(22):2940-53.
8. Davidson M, Keating JL. A comparison of five low back disability questionnaires: reliability and responsiveness. *Phys Ther* 2002; 82(1):8-24.
9. Neumayr A, Tamarozzi F, Goblirsch S, Blum J, Brunetti E. Spinal cystic echinococcosis – a systematic analysis and review of the literature: part 2. Treatment, follow-up and outcome. *PLoS Negl Trop Dis* 2013; 19:7(9):e2458.
10. Gil-Grande LA, Rodriguez-Caabeiro F, Prieto JG, et al. Randomised controlled trial of efficacy of albendazole in intra-abdominal hydatid disease. *Lancet* 1993; 342 (8882):1269-72.
11. Stojkovic M, Zwahlen M, Teggi A, et al. Treatment response of cystic echinococcosis to benzimidazoles: a systematic review. *PLoS Negl Trop Dis* 2009; 3(9):e524.

EVALUATION OF THE USE OF AUTOLOGOUS MICRO-FRAGMENTED ADIPOSE TISSUE IN THE TREATMENT OF KNEE OSTEOARTHRITIS: PRELIMINARY RESULTS OF A RANDOMIZED CONTROLLED TRIAL

G.M. PERETTI^{*1,2}, M. ULIVI^{*1}, L. DE GIROLAMO¹, V. MERONI¹, M.D. LOMBARDO³,
F. VERDONI¹ and L. MANGIAVINI¹

¹*IRCCS Istituto Ortopedico Galeazzi, Milan, Italy;* ²*Department of Biomedical Sciences for Health, University of Milan, Milan, Italy;* ³*Residency Program in Orthopaedics and Traumatology, University of Milan, Milan, Italy*

*These authors equally contributed to the work.

Articular cartilage injuries are still unsolved due to the limited intrinsic healing potential of this tissue. Unlike other tissues, inflammation in the synovial joint causes perpetual damage and progressively leads to the development of osteoarthritis. Previous *in vitro* and *in vivo* studies have demonstrated the efficacy of mesenchymal stem cells isolated from adipose tissue in modulating inflammation. In this study, we analyzed the role of these cells in modifying the pathological microenvironment present in knee osteoarthritis. This is an interventional, prospective, randomized, controlled study. Starting from June 2017, 39 patients with grade III and IV knee osteoarthritis of Kellgren-Lawrence were enrolled, aged between 45 and 75 years, with pain greater than or equal to 6 according to the VAS scale, without ligament instability, with an axial deviation not greater than 10° and with a BMI between 18 and 30. The control group underwent an arthroscopic debridement, while the experimental group underwent an arthroscopic debridement and a subsequent intra-articular injection of autologous micro-fragmented adipose tissue. Patients were evaluated before surgery and at 6 months after the procedure, by radiological analysis (MRI) and functional outcome measures. The main purpose of the study is to evaluate the symptomatic improvement by comparing the functional outcome scores between the two groups. At 6 months after treatment, preliminary results on 39 patients showed pain reduction and functional improvements in the experimental group without a significant difference due to the low number of patients. The radiological and biochemical analyses are still ongoing. To date, the study has not revealed any side effects. These preliminary results demonstrate an encouraging positive trend in the experimental group. Patient recruitment is still ongoing to finalize the statistical analyses and to confirm our hypothesis.

Knee osteoarthritis is the most common cause of knee pain and its social impact is steadily increasing due to the continuing increase in the average age and active population (1). Joint cartilage injuries, due to the limited intrinsic healing potential, are difficult to treat and they remain a difficult problem

for orthopaedic surgeons and physiatrists. Cartilage regeneration capacity is limited due to its isolation from systemic homeostasis and the absence of vessels and nerve endings (2). Unlike other tissues, none of the inflammatory processes is useful for healing the cartilage and the chondrocytes cannot migrate

Key words: knee osteoarthritis, inflammation, adipose tissue, stem cell

Corresponding author:

Dr. Laura Mangiavini,
IRCCS Istituto Ortopedico Galeazzi,
Via R. Galeazzi 4,
20161 Milan, Italy
Tel: +39 0266214930
e-mail: laura.mangiavini@grupposandonato.it

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from an intact site to the injury (3). Biomechanical, metabolic and biological changes, as well as traumas and focal cartilaginous defects, can lead to the loss of homeostasis in the tissue, resulting in accelerated degeneration of the articular surface, leading to severe forms of osteoarthritis. Knee osteoarthritis has a major impact on the quality of life and it is ranked among the top 10 causes of disability in the world. Therefore, one of the objectives of modern medicine is to improve the quality of life and increase the years of athletic activity in the population affected by cartilage and osteoarthritis injuries.

A variety of non-invasive solutions have been proposed, with the aim to decrease pain, to improve joint function and to modify the course of joint degeneration. Drug treatment usually involves analgesia and the use of anti-inflammatory agents (3); the large variability of patient drug response, the absence of clear clinical data regarding the therapeutic potential and the possible presence of side effects limit the use of these drugs. The use of topical agents has shown only short-term usefulness for the management of minor pain. Intra-articular injection of corticosteroids leads to only short-term benefits on pain and joint function (4, 5). Moreover, various evidences show that corticosteroids do not alter the degenerative course in the slightest, but rather worsen it.

Among the different therapeutic choices, injections of hyaluronic acid is extremely widespread in clinical practice, with good results reported in several studies in spite of apparent contradictions and controversies regarding the effectiveness of its use (6). Current clinical solutions, however, suffer from some significant limitations and do not allow for a complete recovery of patient mobility and for a good quality of life.

Given the current limitations of minimally invasive procedures, surgical procedures such as cleaning arthroscopy, cartilage repair, osteotomies (HTO) and major joint replacement interventions are used.

With recent interest in regenerative medicine, many studies have focused on regenerating articular cartilage with stem cells (7). The first methods involved the use of cell cultures, with

proliferation and consequent differentiation of mesenchymal cells (MSC), but in reality, there are numerous disadvantages related to the costs and complexity in the management of these procedures, today considered advanced cellular therapies. Consequently, the possibility of using MSCs in an autologous way, through minimal manipulation, is extremely interesting. Several studies have revealed that injections of MSC, from bone marrow or adipose tissue, leads to improvement of symptoms and joint function. Among the various tissue sources, adipose tissue is of great interest because it is richer in frequency in MSC than in bone marrow and it is easier to harvest (8).

MSC from adipose tissue (ADSC) can be used at least through three routes. The first is the culture and possible *in vitro* expansion. By selecting cells that adhere to the plastic, cells with mesenchymal cell markers are obtained (CD105+, CD73+, CD90+, CD45-, CD34-, CD11b-, CD14-, CD79-, HLA-DR-) that are able to differentiate into the three mesodermal lines (bone, cartilage and fat). This option, however, is scarcely applicable to clinical practice except through Good Manufacturing Practice procedures. Moreover, the same concentrated bone marrow MSC (BM-MSC) compared to those grown in the laboratory are more effective as well as more practical in terms of risks, costs and time (9).

The second alternative is the enzymatic digestion of adipose tissue that delivers a heterogeneous population of cells that contain endothelial cells, leukocytes and pre-adipocytes in addition to MSC. This sub-product of enzymatic digestion is called vascular-stromal fraction (SVF). Finally, the autologous use of ADSC can be performed by micro-fragmented adipose tissue (μ FAT, microfat). While SVF is a heterogeneous population of cells separated from each other and falling under the concept of dose efficacy, μ FAT is a true transplantation of minimally manipulated tissue, which relies more on cellular quality rather than cellular quantity. The Lipogems® system, working through minimal mechanical forces, allows to obtain μ FAT by reducing the adipose tissue in clusters up to a size of 200 to 500 μ m in diameter. In these clusters, there are the cells that also make up the SVF wedged in an adipocyte

scaffold that protects the cells from death due to lack of adhesion or other possible stresses. The MSCs contained in the clusters have great vitality and can differentiate in the three mesodermal lines to prove their multipotency (10).

Because cellular quality is more important than cellular quantity, it has been demonstrated in an animal model of chronic peripheral ischemia that an injection of 500µl of Lipogems® (containing about 2x10⁴ ADSC) recovers the perfusion of the paw better than the sole 1x10⁶ injection of ADSC (11). Lipogems® has demonstrated *in vitro* its potential benefit in orthopedic practice for the regeneration of musculoskeletal system tissues. In particular, as far as tendon regeneration is concerned, it has been demonstrated that Lipogems® is able to stimulate the tendon stem cells *in vitro*, increasing their proliferation and inducing the transcription of VEGF (12). As far as cartilage is concerned, Lipogems® is able to catch selectively on the damaged cartilage *in vitro* and subsequently differentiate into chondrocytes through the cells that compose it (13).

Moreover, Lipogems® in soil enriched with chondrogenic factors forms a compact structure with increased elastic properties compared to the original state (75% increase in compression force necessary to cause tissue breakage and with 70% increase in Young's modulus) (13).

The intra-articular use of micro-fragmented adipose tissue has proved to be safe in animal models and it is effective from a regenerative point of view for cartilage (14). In clinical practice, the same procedure showed a significant reduction in pain at rest and in movement, a decrease in stiffness and an increase in mobility, without experiencing any side effects in patients with severe osteoarthritis (15). Other studies confirm that therapy with MSC from adipose tissue (both infiltrative and intra-articular deposition) is effective in reducing arthritic pain and in cartilage regeneration (16). In the world, over 7000 patients have already been treated with Lipogems, in cosmetic, orthopaedic and general surgery, with promising results and without obvious contraindications.

Knee arthroscopy of "debridement" with the regularization of degenerative meniscal lesions is

one of the first steps in the treatment of the knee affected by degenerative osteoarthritis. Recently, it has been shown that the cells contained in the stromal fraction of the adipose tissue exert a trophic and immunomodulatory role on the cartilage, thus making the use of this biological product very useful in the treatment of patients suffering from isolated or diffuse lesions affecting the articular cartilage. Lipogems®, a CE marked medical device class IIa, allows to obtain easily and quickly a micro-fragmented adipose tissue, starting from a minimal amount of lipoaspirate isolated from the patient. Our hypothesis is therefore that Lipogems® can lead to a significant improvement in the treatment of knee osteoarthritis.

The aim of the study is to evaluate the improvement in clinical and radiological terms of patients undergoing arthroscopic surgery and injection of micro-fragmented adipose tissue (Lipogems®, case group) compared to the group of patients undergoing only arthroscopy (control group).

MATERIALS AND METHODS

The study is a monocentric, interventional, prospective, randomized and controlled. Patients, homogeneous by gender, age and physical activity, were randomly divided into two treatment groups (Arthroscopy or Arthroscopy + Lipogems®) by a computer generated 1:1 table. The total number of participants is 78:

- Group 1: Composed of 39 patients with knee osteoarthritis (OA) undergoing arthroscopic debridement (Control Group);
- Group 2: composed of patients affected by knee osteoarthritis undergoing arthroscopic debridement and injection of autologous micro-fragmented adipose tissue (Lipogems®) (Case Group).

The inclusion criteria are:

- Patients affected by knee OA (Kellgren-Lawrence 3-4)
- Age between 45 and 75 years included
- Joint pain ≥ 6 with VAS (Visual Analog Scale) at the time of study enrolment
- Ligament instability $\leq$ Grade II
- Mechanical axis with axial deviation in load no higher than 10°
- Availability to active participation in the rehabilitation

protocol and to the follow-up foreseen by the study

- Coagulation parameters in the standard: PT (INR) <1.5
- BMI between 18 and 30 kg / m² included

The exclusion criteria are:

- Patients previously treated for traumatic lesions (tibial osteotomies, tibial plateau fractures) of the knee
- Previous visco-supplementation or steroid infiltrations within 3 months from the procedure
- Vascular necrosis / osteonecrosis
- Meniscal surgery within 3 months prior to enrolment
- Systemic or local autoimmune or inflammatory pathology such as rheumatoid arthritis
- Previous infection in the 6 weeks before
- Surgical intervention, radiation therapy, chemotherapy, immunosuppression or steroid therapy in the previous 8 weeks
- Severe concomitant diseases that could influence experimentation
- Diabetes
- Pregnant or lactating women
- Patients suffering from malignant neoplasms

- Patients suffering from metabolic diseases
- Patients with cardiac, pulmonary, neurological or other conditions that may increase the operative risk
- Drug addicts, alcoholics, large smokers or psychiatric disorders
- Patients unable to sign an informed consent
- Patients enrolled in another clinical study within 4 weeks prior
- Patients with coagulation disorders

Patients were evaluated before and after 6 months by radiological clinical and biochemical evaluations. Clinically, several scores were administered: VAS (17), KOOS (18), KSS (19), WOMAC (20) and SF12.

The radiological evaluations took into consideration the femorotibial angle, the WBL (Weight-bearing line) and Kellgren-Lawrence classification (21). Biochemical analyzes were aimed at identifying the serum levels of the following parameters: collagen type II C-telopeptide (CTX-II) (22) and collagen type III N-propeptide (PIIINP) (22). A magnetic resonance of the knee was performed before enrolment and after 6 months from the surgical procedure.

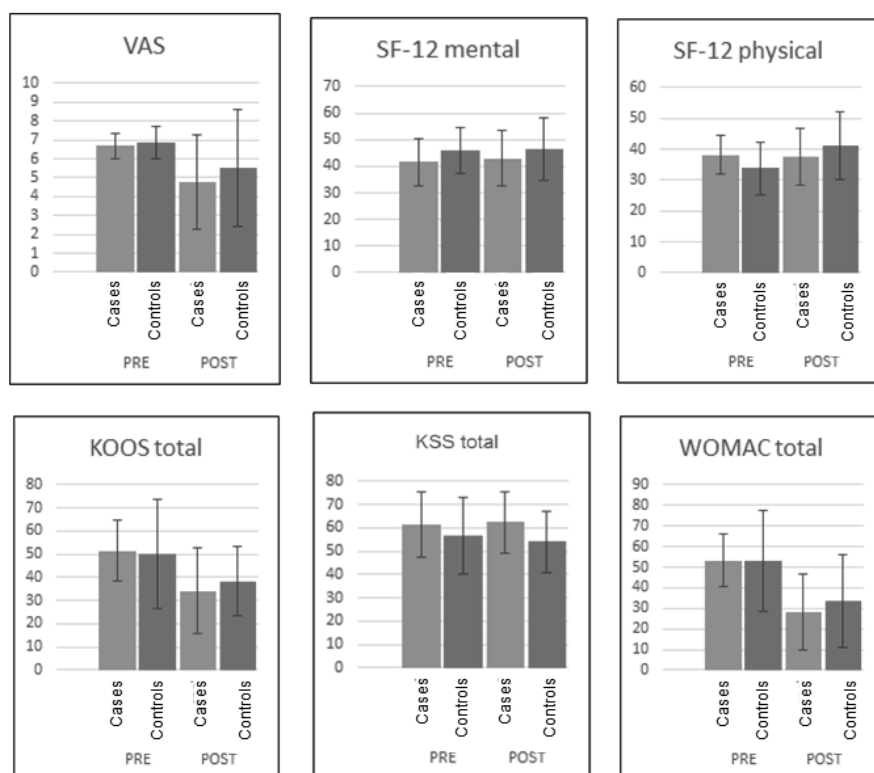


Fig. 1. Functional outcome scores in control and case groups before surgery (PRE) and after 6 months (POST) from the treatment.

RESULTS

On 01/11/2018, 39 surgical procedures were performed, 20 patients (11 cases and 9 controls) also completed the follow-up period. Unfortunately, there were 4 drop-outs (1 in the control group and 3 in the case group) due to non-adherence to the inclusion criteria (1 patient), detection of major disease not related to the study (1 patient) and failure to follow-up within the deadline (2 patients).

The control group was composed of 75% males and 25% females, the average age was 60.375 with standard deviation of 8.365. The case Group included 70% males and 30% females and the average age was 56.25 with standard deviation of 8.396.

Regarding the surgical procedure, the average surgical duration was 20.625 minutes in the control group with a standard deviation of 6.781. The average in the case group was 41.25 minutes with standard deviation of 11.572. Therefore, between the two groups we record an average difference of 10.47 minutes with a standard deviation of 5.73. The increased surgical time in the cases was due to the additional procedure of adipose tissue harvesting.

For the case group, we estimated that for Lipogems® procedure, the average volume of

processed adipose tissue was 77.5 ml with a standard deviation of 19.086 and the volume of injected tissue was 8.125 with a standard deviation of 0.64.

Our preliminary data showed a decrease in the VAS scores in both groups, especially in the case group. However, no significant difference was found. Moreover, we also observed an improvement in the functional score KSS and an amelioration of the quality of life (SF12 score) in both samples (Fig. 1). We noted the main difference in the KOOS and WOMAC scores, where there is a positive trend in the case group, though no significant difference was measured, probably due to the low number of patients analysed. At the radiological evaluation, we often noted a decrease in synovial fluid and a decrease in bone oedema (Fig. 2). The complete clinical, radiological and biochemical analyses are still ongoing.

DISCUSSION

Osteoarthritis is a degenerative disease of the joint that leads to disability. Due to its high frequency it is crucial to find a therapy able to decelerate and stop the process. This disease is based on a cartilage injury, which advances due to its scarce healing

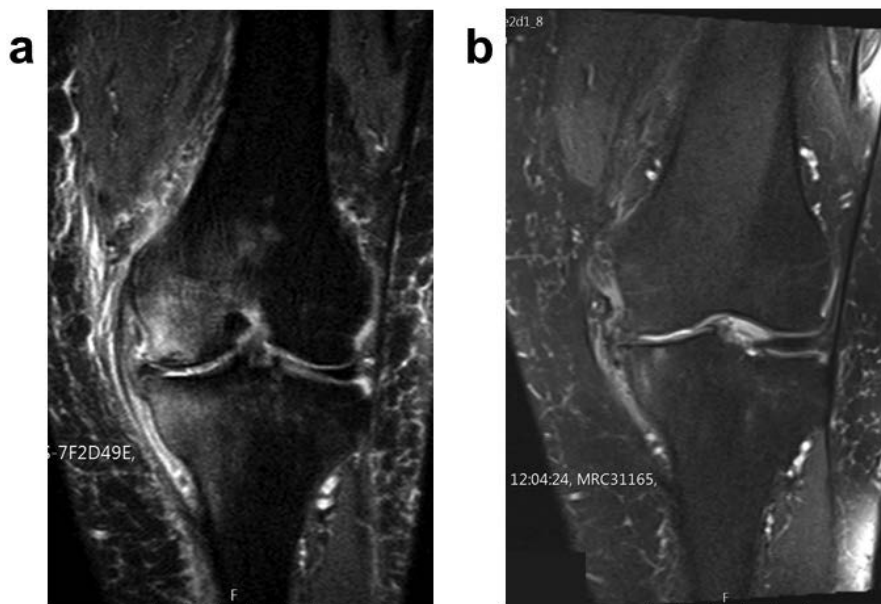


Fig. 2. Representative MRI images of a CASE patient before (a) and after 6 months (b) from surgery.

potential.

Nowadays, several therapies are available for knee OA: physiotherapy, anti-inflammatory drugs or several surgical procedures. Moreover, different regenerative procedures involving multipotent cells have been introduced in the clinical practice. Currently, several clinical trials are studying the use of autologous expanded mesenchymal cells from bone marrow (BM-MSCs) or adipose tissue (ADSCs) followed by an intra-articular injection without the use of scaffolds or major surgery.

Several studies show that the intra-articular injection of ADSCs in association with their Stromal Vascular Fraction is a safe and potentially effective therapy for knee osteoarthritis and the results are comparable with the intra-articular injection of hyaluronic acid or Platelet Rich Plasma (PRP). The results of the studies with a single injection showed that there was a temporary improvement in the first 6-8 months from the treatment. Multiple or regular MSC intra-articular injections may be necessary although, the definitive solution may be represented by a single injection of MSC in combination with growth factors, which could regenerate the damage of the articular cartilage and lead to a lasting tissue that eliminates the pain.

Our study aims to evaluate the effectiveness of a single intra-articular injection of autologous micro-fragmented adipose tissue following an arthroscopic joint debridement.

Currently, data are available only for the first 20 patients. We observed improvements at 6 months follow-up in all the functional scores (VAS, WOMAC, KSS, KOOS, SF-12) in both groups, but especially in the case study. These preliminary results are not statistically significant, although the trends are positive, probably due to the small number of patients analyzed and to the fact that the selected patients had a very advanced degree of osteoarthritis. However, our preliminary results are promising as they show that the intra-articular injection of autologous micro-fragmented adipose tissue may decrease the pain and therefore may increase the functional outcomes by acting on inflammation patterns (23).

A future study to evaluate the benefits of micro-

fragmented adipose tissue should involve patients with less severe osteoarthritis and should compare the results with the outcomes of hyaluronic acid injections.

REFERENCES

1. Curl WW, Krome J, Gordon ES, Rushing J, Smith BP, Poehling GG. Cartilage injuries: A review of 31,516 knee arthroscopies. *Arthroscopy* 1997; 13(4):456-60.
2. Buckwalter JA, Brown TD. Joint injury, repair, and remodeling: Roles in post-traumatic osteoarthritis. *Clin Orthop Relat Res* 2004; 423:7-16.
3. Ochi M, Uchio Y, Kawasaki K, Wakitani S, Iwasa J. Transplantation of cartilage-like tissue made by tissue engineering in the treatment of cartilage defects of the knee. *J Bone Joint Surg Br* 2002; 84:571-8.
4. Hochberg MC, Altman RD, Brandt KD, Clark BM, Dieppe PA, Griffin MR, Moskowitz RW, Schnitzer TJ. Guidelines for the medical management of osteoarthritis. Part II. Osteoarthritis of the knee. American College of Rheumatology. *Arthritis Rheum.* 1995; 38(11):1541-6.
5. Niethard FU, Gold MS, Solomon G. Efficacy of topical diclofenac diethylamine gel in osteoarthritis of the knee. *J Rheumatol* 2005; 32:2384-92.
6. Bellamy N, Campbell J, Robinson V, Gee T, Bourne R, Wells G. Viscosupplementation for the treatment of osteoarthritis of the knee. *Cochrane Database Syst Rev* 2005; 19:CD005321.
7. Centeno CJ, Busse D, Kisiday J, Keohan C, Freeman M, Karli D. Regeneration of meniscus cartilage in a knee treated with percutaneously implanted autologous mesenchymal stem cells. *Med Hypotheses* 2008; 71(6):900-8.
8. Baer PC, Geiger H. Adipose-derived mesenchymal stromal/stem cells: tissue localization, characterization, and heterogeneity. *Stem Cells Int* 2012; 2012:812693.
9. Murphy MB, Moncivais K, Caplan AI. Mesenchymal stem cells: environmentally responsive therapeutics for regenerative medicine. *Exp Mol Med* 2013; 45:e54.
10. Bianchi F, Maioli M, Leonardi E, et al. A new non-enzymatic method and device to obtain a fat tissue

- derivative highly enriched in pericyte-like elements by mild mechanical forces from human lipoaspirates. *Cell Transplant* 2013; 22(11):2063-77.
11. Bianchi F, Olivi E, Baldassarre M, et al. Lipogems, a new modality of fat tissue handling to enhance tissue repair in chronic hind limb ischemia. *CellR* 2014; 4:2-6.
 12. Randelli P, Menon A, Ragone V, et al. (2016). Lipogems product treatment increases the proliferation rate of human tendon stem cells without affecting their stemness and differentiation capability. *Stem Cells Int* 2016; 2016:4373410.
 13. Bosetti M, Borrone A, Follenzi A, Messaggio F, Tremolada C, Cannas M. Human lipoaspirate as autologous injectable active scaffold for one-step repair of cartilage defects. *Cell Transplant* 2016; 25(6):1043-56.
 14. Moshref S, Jamal Y, Hummdi L, Kaki A, Al-Hibshi A. Intra-articular injection of autologous fat micro graft in sheep hind knee joints. *Life Sci J* 2013; 10(4):2115-20.
 15. Moshref S, Kaki AM, Al-Hibshi AM, Jamal S. Intra-articular injection of autologous fat micro-graft for the treatment of knee osteoarthritis: preliminary experience. *Life Sci J* 2014; 11(2):55-60.
 16. Michalek J, Moster R, Lukac L, et al. Osteoarthritis therapy using adipose tissue-derived stromal vascular fraction cells. *Cell Transplant* 2014; [Epub ahead of print].
 17. Joyce CR, Zutshi DW, Hrubes V, Mason RM. Comparison of fixed interval and visual analogue scales for rating chronic pain. *Eur J Clin Pharmacol* 1975; 8(6):415-20.
 18. Roos EM, Roos HP, Lohmander LS, Ekdahl C, Beynnon BD. Knee Injury and Osteoarthritis Outcome Score (KOOS)-development of a self-administered outcome measure. *J Orthop Sports Phys Ther* 1998; 28(2):88-96.
 19. Insall JN1, Dorr LD, Scott RD, Scott WN. Rationale of the Knee Society clinical rating system. *Clin Orthop Relat Res* 1989; 248:13-4.
 20. Bellamy N, Buchanan WW, Goldsmith CH, Campbell J, Stitt LW. Validation study of WOMAC: a health status instrument for measuring clinically important patient relevant outcomes to antirheumatic drug therapy in patients with osteoarthritis of the hip or knee. *J Rheumatol* 1988; 15(12):1833-40.
 21. Scott WW Jr, Lethbridge-Cejku M, Reichle R, Wigley FM, Tobin JD, Hochberg MC. (1993). Reliability of grading scales for individual radiographic features of osteoarthritis of the knee. The Baltimore longitudinal study of aging atlas of knee osteoarthritis. *Invest Radiol* 1993; 28(6):497-501.
 22. Jiao Q, Wei L1, Chen C, Li P, Wang X, Li Y, Guo L, Zhang C, Wei X. Cartilage oligomeric matrix protein and hyaluronic acid are sensitive serum biomarkers for early cartilage lesions in the knee joint. *Biomarkers* 2016; 21(2):146-51.
 23. Caplan AI. Why are MSCc therapeutic? New data: new insight. *J Pathol* 2009; 217(2):318-24.

TOTAL HIP ARTHROPLASTY IN RAPIDLY PROGRESSIVE OSTEOARTHRITIS OF THE HIP: CLINICAL AND RADIOLOGICAL OUTCOME OF A CONSECUTIVE SERIES OF PATIENTS

T. CHOMPOOSANG, R. D'APOLITO, C. LACAGNINA, G. PERETTI AND L. ZAGRA

Hip Department, IRCCS Istituto Ortopedico Galeazzi, Milan, Italy

Rapidly progressive osteoarthritis (RPOA) is an uncommon subset of osteoarthritis (OA) characterized by rapid joint degeneration and destruction. The treatment of choice for these patients has traditionally been total hip arthroplasty (THA) because nonoperative treatments modalities are not effective. RPOA can lead to acetabular bone loss and consequently intraoperative technical difficulties making joint reconstruction in such patients a challenge for the surgeon. The purpose of this study was to determine the clinical and radiological results of patients suffering from RPOA and treated with hybrid or cementless THA at a single institution. We retrospectively review of all patients who underwent THA for RPOA from January 2011 to December 2016. Twenty-three hips in twenty-one patients were included in this study, with a mean age at surgery of 74 years (range, 41-82) and a mean follow-up of 30 months (range, 24-60). In all patients an uncemented cup, either plasma-spray or trabecular titanium, was implanted. In eighteen cases a cemented femoral stem was used. In eight cases autologous bone graft was used to replace the acetabular defect. Radiographic and clinical follow-up was performed at six months postoperatively, twelve months and yearly thereafter. At radiographic follow-up, no signs of prosthetic loosening or migration were seen. Harris Hip Score improved from 65.3 preoperatively to 89.2 at latest follow-up. The use of both hybrid and uncemented THA in the treatment of RPOA was found to be successful in our patients with a mean 30 months follow-up. Hemispherical cementless cups with both plasma spray and high porosity outer surface (used in the worse cases) were effective in getting primary stability and bone ingrowth.

Rapidly progressive osteoarthritis (RPOA) is an uncommon subset of osteoarthritis (OA) characterized by rapid joint degeneration and destruction. It was described for the first time in the late fifties by Forestier (7), who reported a rapidly progressive hip disease resulting in destruction of both the femoral and the acetabular aspects of the hip joint, with almost complete disappearing of the femoral head within a few months. During the seventies Lequesne (17) and Postel and Kerboul (20) firstly coin the term "rapid destructive hip osteoarthritis". The original definition described RPOA as a radiographic joint space narrowing (>2 mm in 1 year or 50% joint-space narrowing in 1 year) in the setting of pain and

disability (16, 17). The incidence of RPOA has not been clearly determined, but in some studies the prevalence is reported to range from 4.25% to 14% (12) amongst patients referred to specialist centers. The etiology is still unclear, despite drug toxicity, cytokine-mediated immunological mechanisms, autoimmune reactions, or subchondral insufficiency fractures have been hypothesized in the pathological mechanisms. Total hip arthroplasty (THA) has traditionally been the treatment of choice for these patients as non-operative treatments modalities are not effective. For such patients a long waiting list is a matter of concern because RPOA can lead to acetabular bone loss and consequently intraoperative

Corresponding author:

Prof. Giuseppe M. Peretti,
IRCCS Istituto Ortopedico Galeazzi,
Via R. Galeazzi 4,
20161 Milan, Italy
Tel.: +39 0266214930
e-mail: giuseppe.peretti@unimi.it

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technical difficulties along with increased operating time and increased intraoperative blood loss. As a consequence of bone damage, joint effusion, poor soft tissues and synovitis, a higher complications rate could be expected in these patients including post-operative infection, dislocation, implant loosening. On the other hand, septic or neurologic arthritis should be excluded as causes of such articular damages. Nevertheless, few papers reported about THA outcomes in these patients, with results similar to those of patients treated for common OA (3,19,20). The purpose of this study is to determine the clinical and radiological results of patients suffering from RPOA and treated with hybrid or cementless THA at a single institution.

MATERIALS AND METHODS

A retrospective study was carried out on all patients who underwent a primary total hip replacement in our department from January 2011 to December 2016 (five-year period). Patients were identified from our institutional database, and all revisions and acute trauma cases were excluded. Among 1388 primary THAs, 23 were performed for RPOA. The diagnosis of RPOA was based on a recent history of worsening pain (from 1 to 6 months), radiographic appearance of rapidly progressive atrophic bone destruction of the hip, absence of clinical or laboratory evidence of infectious, neurologic, metabolic, endocrine or inflammatory disorders. Post-traumatic hip arthritis, as well as septic hip arthritis were excluded from this study. Superior bony destruction occurred in thirteen hips (eleven patients). These patients underwent preoperative computed tomography (CT) to better define the defect before reconstruction. The diagnosis was confirmed intraoperatively by the operating surgeon (LZ). Two patients had minor previous bilateral developmental hip dysplasia, a condition that is not associated with RPOA in its natural history.

All surgeries were performed via a posterolateral approach, the piriformis and conjoint tendons and the posterior capsule were repaired in separate layers using a trans-osseous technique. The choice of the fixation for the femoral component was based on patient's age and Dorr's classification (5). Patients aged over 70 years or with a Dorr type C femur or poor femoral bone were treated using

cemented stems. In all cases, a BioloX Delta ceramic head was used, coupled with a BioloX Delta ceramic, a highly cross-linked polyethylene insert, or as part of a dual mobility construct in those patients presenting a higher risk of dislocation (eight cases). Eighteen hips in sixteen patients were treated with total hip arthroplasty with a hybrid configuration. The acetabular component consisted of either a non-cemented titanium (Ti) plasma-sprayed hemispherical cup or a high porosity hemispherical cup (trabecular Ti) outer surface, which was used in eleven cases with poor acetabular bone quality. A cemented Charnley-type femoral component was used. Second generation cementing technique and antibiotic loaded cement were used. Five hips in five patients were treated with uncemented total hip arthroplasty. Type 3C (13) grit-blasted stems were used in these patients. In the group of all cementless implant, plasma-sprayed Ti cups were used in two patients, whereas high porosity Ti cups in three patients.

All patients were assessed by means of Harris hip score (HHS) and hip range of motion pre-operatively, at six months, twelve months and yearly thereafter. Clinical data were collected by an independent investigator. With regard to the radiological evaluation, anteroposterior and lateral views of the hips were appraised for position of the implants, implant migration, bone ingrowth, osteolysis and periprosthetic radiolucencies lines using De Lee and Charnley's classification (8) on the acetabular aspect and Gruen's classification (9) on the femoral side. All X-rays were reviewed by an arthroplasty fellow.

RESULTS

Twenty-one patients (23 hips), six males and fifteen females, were included in this study with a mean follow up of 30 months (range, 24 to 60 months). The mean age at surgery was 74 years (range, 41 to 84), whereas the mean BMI was 28.3 (range, 27.6 to 36.1). The average operative time was 1.5 hours. No intra-operative complications were reported.

Clinical outcomes as assessed by the HHS were rated from good to excellent. Seventeen hips in fifteen patients had excellent results. Five hips in five patients had good result. Only one patient had fair results due the onset of spine disease. Mean

preoperative HHS improved from 65.3 preoperatively to 89.2 postoperatively. Mean functional score increased from a mean value of 27.5 preoperatively to 36.6 at the latest follow-up. The range of motion (ROM) raised from a mean of 42° of flexion, 4° of extension, 16° of abduction, 5° of adduction, 12° of internal rotation, 10° of external rotation to a mean of 89° of flexion, 12° of extension, 41° of abduction, 15° of adduction, 22° of internal rotation, 27° of external rotation at 12 months follow up.

Radiological assessment on the acetabular side at six months follow up showed bone ingrowth in De Lee and Charnley zones 1, 2, 3 in nine cases, whereas thirteen cases revealed ingrowth in zones 1, 2; in one case ingrowth was detectable just in zone 1. At 12 months follow up, all but one cases showed bone ingrowth in zones 1, 2, 3. The only case of limited bone ingrowth did not display signs of loosening, despite non-progressive radiolucencies were seen in zone 2 and 3. In eight cases in which autograft was used to replace the defect, bone healing was noticed on the x-rays at 6 months for seven patients and 12 months in one patient.

With regard to the femoral side, all hips had complete filling of the medullary canal, without radiolucent lines between the cement and the bone at initial post-operative x-ray. One uncemented stem

showed radiolucency lines at zone 1 and 7 since the first 6 months follow-up, unchanged at final follow up. No signs of subsidence or malposition of components were reported in the uncemented stem group. Bone ingrowth was seen in zone 2-6 at 6 months in four cases, in one case at 12 months.

Despite a slower and more difficult post-operative recovery due to the very poor preoperative capability of some of these patients compared to cases of common OA, no major complications occurred in this cohort of patients: no dislocations, no surgical site infections, no loosening of the prosthesis.

DISCUSSION

RPOA is a radiological and clinical condition of the hip joint that can pose a challenge for the orthopaedic surgeon. Diagnosis can be misleading as a septic arthritis must be always excluded. The rapid worsening of the clinical condition can seriously affect the walking capability of the patient. Symptoms can include shortening of the lower limb and a very painful hip. Bone loss, especially on the acetabular side and poor bone quality, joint effusion and synovitis can be problematic.

The purpose of this paper was to determine the outcome of THA in the setting of RPOA. Both

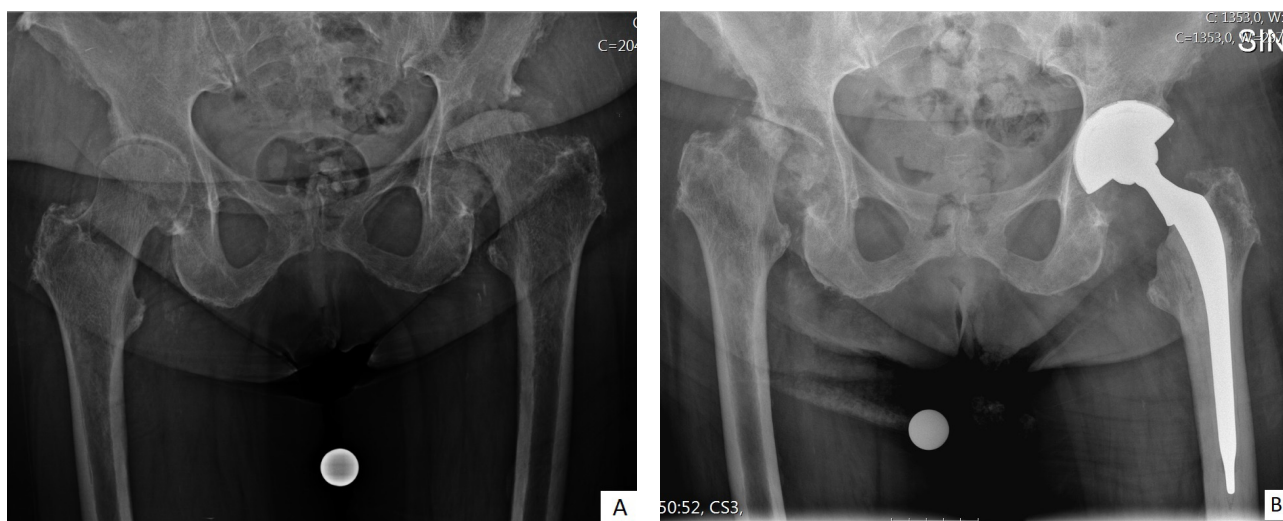


Fig. 1. This (A) is the preoperative X-rays of a 78-year-old patient suffering from RPOA on the left hip and scheduled for THA. The same patient presented a rapid worsening of symptoms on the contralateral side and four months later (B) showed a full-blown RPOA on the right hip.

hybrid and full uncemented reconstructions showed good clinical and radiological results at a short-term follow-up. Our results are in line with those of the other published series. The first paper reporting on patients suffering of RPOA and treated with THA (McKee–Farrar or Charnley prostheses) by Postel and Kerboul, with a maximum 3-year follow-up, concluded that the clinical results were similar to THA performed for ordinary OA. However, the Authors mentioned the technical difficulties encountered due to bone loss, along with four cases of cup loosening at the X-rays. Kuo et al., reported on ten patients with an average follow-up of 6 years confirming that, despite the rapid progression of disease and preoperative bony destruction in this set of patients, hybrid and non-cemented total hip arthroplasty were effective and safe treatments for RPOA (15). Similarly, Thompson and colleagues (23) reported the outcomes of 35 patients founding no significant difference in the Oxford Hip Score between the normally progressive and rapidly progressive groups. Charrois and colleagues (3) showed good functional results in a series of 100 patients treated with cemented Charnley-Kerboul prostheses at 7 years of follow-up, with loosening of the cup in six cases and of femoral component in

three cases.

In this scenario, acetabular bone loss can make these procedures technically challenging and determine the need for bone graft or special implants for adequate reconstruction. These patients may lose up to 15 mm of bone stock annually. In our series, eight cases required autologous bone grafting on the acetabular aspect. At twelve months of follow-up, bone healing was detectable on the X-rays for all cases. High porosity Ti cups were used in 14 cases with major bone loss and worse bone quality. This type of surface finishing is not for routine use in our practice which considers Ti plasma sprayed cups for standard cases. However, in such cases a surface with high grip and with high bone ingrowth capability can lead to a significant improvement and can be able to overcome technical difficulties of the past and possible failures of cemented cups as previously reported (3). Several authors (1,12,23) highlighted the need for priority treatment for these patients due to the potentially rapid and extensive loss of bone stock that can occur.

Despite RPOA has been known for more than fifty years, the pathogenesis is still unclear. It could result from the interaction of three factors: mechanical stress, cartilage degeneration, and bone

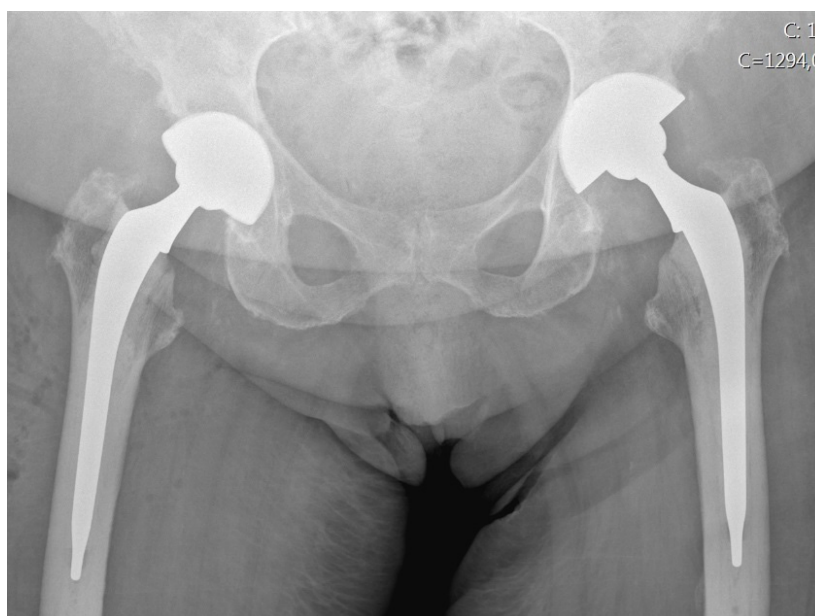


Fig. 2. The same patient at one-year of follow-up after the second THA.

response. If cartilage degeneration is rapid and bone response is poor, then atrophic RPOA can occur; otherwise, if the degenerative process is slow and bone response is good, reparative sclerosis and osteophyte formation are the result (10). Subchondral insufficiency fracture, subchondral bone ischemia and cell necrosis have been considered major factors in the development of RPOA (2,4,18). Some authors postulated a possible role of non-steroidal inflammatory drugs in the etiology of RPOA, but it is difficult to establish a causal relationship (21). More recently, Hess et al. reported a 21% incidence of RPOA of the hip in patients receiving intra-articular steroid injection for OA, despite a true relationship is not well defined (11). Compared with hips with conventional osteoarthritis, a marked increase in the osteoclast count was reported at the histology (18). Several biological factors can play a role in the genesis of RPOA: elevated intra-articular levels of interleukin 6, interleukin 1 β , and matrix metalloproteinases have been reported (14,22).

The differential diagnosis is of paramount importance in this instance because multiple conditions can mimic rapid destructive hip diseases, such as infection, malignancy, neuropathic arthropathy, inflammatory arthritis, osteonecrosis, hemophilia, and ochronosis. When the joint destruction is so rapid, other possible causes must be ruled out, bearing in mind that septic arthritis is usually associated with leukocytosis and local and systemic signs of infections, patients with neuropathic arthritis do not present with pain and have a history of syphilis, diabetes mellitus, syringomyelia, or spinal cord injury, inflammatory arthritis will present with multiple joint pain and morning stiffness and the characteristic serologic abnormalities (24). Recently, Hart and Fehring described three patients with preoperative or intraoperative findings consistent with infection, and one of these underwent an unnecessary two-stage procedure due to the appearance of the joint fluid (10). They advocate to perform an infection workup preoperatively when patients present with rapid degenerative changes of their hip joint to diminish the uncertainty of proceeding with arthroplasty.

Radiographic manifestations can help distinguish

RPOA from other conditions. In the early (chondrolysis) stage a joint space narrowing, usually superolateral, and variable subchondral cysts can be seen on the x-ray. Osteophytes, if present, are small and subchondral sclerosis is scant. An edema-like pattern is detectable on the femoral side with similar findings on the acetabular side on the MRI. Focal subchondral fracture may be seen in up to 33% of patients. This band of low signal parallels the overlying subchondral bone and is usually less than 1 cm deep to it. These two characteristics help distinguish a subchondral fracture from avascular necrosis in which the linear band of low signal is concave to the articular surface and serpentine (6). In the late (subchondral destruction) stage, the radiology is less informative, with joint space narrowing (or widening on the non-weightbearing X-rays in some cases) and various degrees of femoral head/acetabulum remodeling(6).

This study has several limitations. First, the cohort was relatively small and lacked a control group of patients suffering from conventional OA. Second, there may be some selection bias in favor of cemented stem due to the relatively high mean age of the patients of this cohort, because we usually choose cemented stem for patients older than 70 years. Third, conventional radiography was used to detect and evaluate periprosthetic osteolysis and may underestimate the true incidence of osteolysis as computed tomographic scan is more sensitive than conventional x-rays in detecting the presence and extent of osteolytic lesions. Fourth, the length of follow-up is relatively short, anyway able to exclude early complications and implant failures related to the poor bone quality. However, RPOA accounted for only 1.66% of all patients undergoing THA in the study period, representing an uncommon subset or OA in our practice. This prevalence is lower than those previously reported in literature, probably due to our major diagnostic criterion for RPOA, namely patients with a history of symptoms lasting less than 6 months. Indeed, subjects suffering from long-standing conventional OA with worsening of symptoms are excluded from this cohort.

The use of both hybrid and uncemented THA in the treatment of RPOA was found to be successful

in our patients with a mean 2-year and half follow-up. Hemispherical cementless Ti cups with both plasma sprayed and high porosity outer surface (used in the worse cases) were effective in getting primary stability and bone ingrowth. Further studies with longer follow-ups are needed to confirm the durability of these reconstructions in this subset of patients and strengthen the findings of the existing literature.

REFERENCES

1. Batra S, Batra M, McMurtrie A, Sinha AK. Rapidly destructive osteoarthritis of the hip joint: A case series. *J Orthop Surg Res* 2008;3(1).
2. Bekki S. [The pathogenesis of rapidly destructive coxarthrosis]. *Nihon Seikeigeka Gakkai Zasshi* 1991;65(9):720–730.
3. Charrois O, Kahwaji A, Rhami M, Inoue K, Courpied JP. [Outcome after total hip arthroplasty performed for rapidly progressive hip destruction]. *Rev Chir Orthop Reparatrice Appar Mot* 2002;88(3):236–244.
4. Davies M, Cassar-Pullicino VN, Darby AJ. Subchondral insufficiency fractures of the femoral head. *Eur Radiol* 2004;14(2):201–207.
5. Dorr LD, Faugere M-C, Mackel AM, Gruen TA, Bogner B, Malluche HH. Structural and cellular assessment of bone quality of proximal femur. *Bone* 1993;14(3):231–242.
6. Flemming DJ, Gustas-French CN. Rapidly Progressive Osteoarthritis: a Review of the Clinical and Radiologic Presentation. *Curr. Rheumatol. Rep.* 2017;19(7).
7. FORESTIER, F. Coxite rhumatismales subaigues et chroniques. Thesis 1957;
8. Goudarz Mehdikhani K, Morales Moreno B, Reid JJ, de Paz Nieves A, Lee Y-Y, González Della Valle A. An Algorithmic, Pie-Crusting Medial Soft Tissue Release Reduces the Need for Constrained Inserts Patients With Severe Varus Deformity Undergoing Total Knee Arthroplasty [Internet]. *J. Arthroplasty*. 2015;
9. Gruen T, McNeice G, Amstutz H. “Modes of failure” of cemented stem-type femoral components: a radiographic analysis of loosening. *Clin Orthop Relat Res* 1979;17–27.
10. Hart G, Fehring T. Rapidly destructive osteoarthritis can mimic infection. *Arthroplast Today* 2016;2(1):15–18.
11. Hess SR, O’Connell RS, Bednarz CP, Waligora AC, Golladay GJ, Jiranek WA. Association of rapidly destructive osteoarthritis of the hip with intra-articular steroid injections. *Arthroplast Today* 2018;4(2):205–209.
12. Irwin LR, Roberts JA. Rapidly progressive osteoarthritis of the hip. *J Arthroplasty* 1998;13(6):642–646.
13. Khanuja HS, Vakil JJ, Goddard MS, Mont MA. Current Concepts Review: Cementless Femoral Fixation in Total Hip Arthroplasty. *J Bone Joint Surg Am* 2011;93:500–509.
14. Komiya S, Inoue A, Sasaguri Y, Minamitani K, Morimatsu M. Rapidly destructive arthropathy of the hip: Studies on bone resorptive factors in joint fluid with a theory of pathogenesis. *Clin Orthop Relat Res* 1992;273–282.
15. Kuo A, Ezzet KA, Patil S, Colwell CW. Total hip arthroplasty in rapidly destructive osteoarthritis of the hip: A case series. *HSS J* 2009;5(2):117–119.
16. Lequesne M, Ray G. LA COXOPATHIE DESTRUCTRICE RAPIDE IDIOPATHIQUE. ETUDE ETIOLOGIQUE PROSPECTIVE DE 27 CAS. *Rev Rhum Mal Osteoartic* 1989;56(2):115–119.
17. Lequesne M, de Seze S, Amouroux J. [Rapidly destructive coxarthrosis]. *Rev Rhum Mal Osteoartic* 1970;37(11):721–733.
18. Mitrovic DR, Riera H. Synovial, articular cartilage and bone changes in rapidly destructive arthropathy (osteoarthritis) of the hip. *Rheumatol Int* 1992;12(1):17–22.
19. Motomura G, Yamamoto T, Nakashima Y, Shuto T, Jinguishi S, Iwamoto Y. Outcome of the Contralateral Hip in Rapidly Destructive Arthrosis After Total Hip Arthroplasty. A Preliminary Report. *J Arthroplasty* 2006;21(7):1026–1031.
20. Postel M, Kerboull M. Total prosthetic replacement in rapidly destructive arthrosis of the hip joint. *Clin Orthop Relat Res* 1970;72:138–144.
21. Ronningen H, Langeland N, Hauge MF. Indomethacin in osteoarthritis of the hip. *Tidsskr den Nor Laegeforening* 1979;99(8).
22. Tamai M, Sagawa K, Kawabata R, Inoue A, Itoh K. Production of IL-6 by T cells from the femoral head of patients with rapidly destructive coxopathy

- (RDC). Clin Exp Immunol 1996;103(3):506–513.
23. Thompson NW, Corr AM, Geddis CJ, O'Brien S, Beverland DE. Rapidly progressive osteoarthritis of the hip. HIP Int. 2004;14(4):217–222.
24. Tiwari A, Karkhur Y, Keeney JA, Aggarwal A. Rapid destructive osteoarthritis of the hip after intra-articular steroid injection. Arthroplast today 2018;4(2):184–186.

TREATMENT OF VANCOUVER B1, C PERIPROSTHETIC HIP FRACTURES WITH PERIPROSTHETIC POLYAXIAL LOCKING PLATE SYSTEM: A 3-YEAR FOLLOW-UP

A. CASTELLI¹, S.M.P. ROSSI¹, L. ROCCA², E. JANNELLI¹ and F. BENAZZO¹

¹*Clinica Ortopedica e Traumatologica, IRCCS Policlinico San Matteo, Università degli studi di Pavia, Italy;* ²*ASST ovest milanese, Ospedale di Magenta, UO Ortopedia e Traumatologia, Magenta, Milano, Italy*

The incidence of periprosthetic fractures after primary knee and hip arthroplasty is around 2.5% and is increasing after revision surgery up to 4%. Management of these fractures is often particularly demanding and expensive. The primary aim of the presented study is to describe our experience in using a precontoured periarticular polyaxial standard plating system in a cohort of patients with homogeneous fracture type (Vancouver B1, C), comparing our experience with other surgical solutions. In stable implants, the primary strategies aim for fracture stabilization, leaving the original prosthesis in place. The results of conventional non-locking implants have been mostly poor with complication rates up to 53%. Therefore, today, monoaxial locking plates are strongly recommended. From May 2013 to December 2014, 30 “non-contact bridging plate” (NCB-PP®) were implanted. All fractures were periprosthetic Vancouver B1 or C fractures. In 24 patients, NCB-PP® plating was performed after periprosthetic femoral fracture as primary treatment, in 6 patients, it was performed as secondary fracture treatment after primary plating failure. All surgeons performed lateral femoral approach with ORIF. Average follow up was 36 months. Bony consolidation was confirmed in all patients, bar one, in an average time of 4 months; none of the patients bar one developed mechanical failure or implant breakage. The GOS at 52 weeks was back to the preoperative level in 18 patients and it did not improve at 24 months. The Harris Hip Score at 52 weeks showed a mean score of 80.14 points. Full weight bearing was allowed at mean time of 100 days. None of the patients developed complications that needed subsequent surgery. The use of NCBPP plates has given excellent results in our clinical practice, allowing early postoperative mobilization and recovery.

The incidence of periprosthetic fractures after primary knee and hip arthroplasty is around 2.5% and increasing after revision surgery up to 4% (1-3). Management of these fractures is often particularly demanding, complex and expensive. In many cases, the surgeon has simultaneously to deal with the problems of implant loosening, bone loss and fracture.

The Vancouver classification (4, 5) has become

the universally accepted classification and is used as a tool that guides the surgeon pre- and intra-operatively. According to most of the established treatment algorithms, the different Vancouver types of PFF (periprosthetic femoral fracture) require certain methods of intra- or extra-medullary fixation, grafting, implant revision, or combinations of the above (6, 7, 8, 9).

Key words: periprosthetic hip fracture, Vancouver classification, polyaxial locking fixation, locked bicortical screw, implant failure

Corresponding author:

Dr A. Castelli,
Clinica ortopedica e traumatologica,
IRCCS Policlinico San Matteo,
Via Golgi 19, 27100 Pavia, Italia,
Tel.: +393334754427
e-mail: albecastell@libero.it

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Non-operative treatment has been advocated in the past, (10, 11, 12) but because of its high morbidity, more aggressive rationales have been established (13). On the other hand, even in recent literature, important complication rates up to 41% and revision rates up to 29% have been reported for surgical treatment of PPFs (14-19).

The stability of the original implant and the configuration of the fracture itself are the basic factors that influence the decision-making process. In a loose prosthesis, exchange for a revision prosthesis is commonly the treatment of choice (20, 21). In these situations, the fixation of the revision implant has to be secured by long stems that are well fixed in the diaphysis of the femur. In stable implants, the primary strategies aim for fracture stabilization, leaving the original prosthesis in place. To date, a variety of strategies for fracture fixation has been described. The results of conventional non-locking implants have been mostly poor with complication rates up to 53% (22).

Therefore, today monoaxial locking plates are strongly recommended in complicated osteoporotic fractures. Although this offers a more stable fixation, the complication rates remain high with up to 29% failure (23-28).

As monoaxial plating systems are sometimes difficult to fix in regions with an intramedullary implant, additional devices such as cerclages are recommended to provide stable fixation.

The primary aim of the presented study is to describe our experience in using a precontoured periarticular polyaxial plating system in patient with PPF B1, C Vancouver comparing our experience with other surgical solutions.

As there is a high inhomogeneity in patients treated for this fracture type with respect to the local (e.g., type of prosthesis) and to the general (e.g., mobility of the patient) conditions, we tried to structure the paper based on the following sub items:

- S1: impact of a new PPF plate with "open approach";
- S2: application of polyaxial locking screws in PPF fixation with cement and cementless implants compared with other surgical solutions;
- S3: radiological healing, outcome and mobility after 12, 24, 36 months.

MATERIALS AND METHODS

From May 2013 to December 2014, 30 NCB-PP® plates were implanted. All fractures were periprosthetic fractures that did not disturb the stability of the arthroplasty. In all cases, the stability of the prosthesis was evaluated by preoperative X-ray, clinical evaluation (e.g., history of pain before the fracture) and finally, surgical evaluation. The classification of the PPFs was based on the Vancouver classification (28).

Informed consent was obtained from all the patients or their relatives. Patients were excluded from the study in cases of:

- Loose hip prosthesis (Vancouver type B2 and B3);
- Pathological fracture caused by local malignant destruction of the bone.

Follow-up time of the study was 36 months in 21 patients: mean follow up 35 months. Twenty-one fractures were left-sided and 9 was right-sided. Mechanism of fracture was more likely a low-energy trauma. Average patients' age was 78 years (66-86 years), 27 females, 3 males. ASA-Score was 1.7 (1-3). Twelve patients had three and more relevant comorbidities, 12 patients had two or less relevant comorbidities and 6 patients had none. Twenty-two patients were admitted to hospital directly from their homes, 7 after traffic accidents and 1 patient was referred from another hospital. None of the patients were bed-ridden prior to the trauma. Out of 30 periprosthetic fractures, 6 patients (20%) had a cemented stem, 24 (80%) cementless, 10 patients (33%) had an endoprosthesis, 20 patients (67%) had total hip prosthesis, 18 patients (60%) had a primary implant and 12 patients (40%) had a revisioned implant. According to Vancouver (28), they were classified as 3×type C and 27×type B1.

In 24 patients (80%), NCB-PP® plating was performed as primary fracture treatment, in 6 patients (20%) NCB-PP® plating was performed as secondary fracture treatment after primary plating failure. Eighteen patients out of 24 (75%) primarily suffered a long spiral diaphyseal fracture with interposition of soft tissue, the other 6 patients (25%) a transverse 3 fragments fracture. All surgeons performed lateral

femoral approach extended distally to the diaphysis with open reduction and internal fixation (Fig. 1).

Preoperative mobility as well as intraoperative parameters were recorded. Follow-up included evaluation of wound healing, functional assessment, bony consolidation (both cortices bridged, fracture line no longer evident, no pain at loading), evaluation of the GOS, HHS (Harris hip score) and general complications. The Glasgow Outcome Scale (GOS) was used to estimate participation in daily life (29) (Table 1). It was used for its easy inter-observer comparability pre- and postoperatively. For easier

comprehension, GOS was inverted (30) (1=good recovery and 5=death) (Table I).

All fractures were reduced and fixed with the periprosthetic polyaxial locking *Non-Contact-Bridging-plate* (NCB-PP® Zimmer Inc., Winterthur, Switzerland) (31). This implant offers the possibility of locking screw fixation where the screws can be directed in an angle of up to 30° in any direction to the plate level to avoid an intramedullary implant. Oversized drill bits and drill guides are useful to reduce the risk of cracks in the cement mantle when placing screws around a cemented prosthesis.

Table I. *Modified Glasgow Outcome Scale.*

GOS 1	Good recovery
GOS 2	Moderate disability (disabled but independent), no assistance in daily life needed
GOS 3	Severe disability (conscious but disabled), needing assistance in daily life
GOS 4	Persistent vegetative state
GOS 5	Death



Fig.1. *a): Vancouver Type C fracture on cemented stem; b): ORIF with NCB-PP.*

RESULTS

The duration from admission to operation was in median 48 h (14 ± 90 h). Surgery lasted 150 m (80 ± 220 m) in median. The intraoperative blood loss measured by intraoperative transfusion was 2 units of erythrocyte concentrate (0 ± 4) on average pro patient. The average stay in hospital was 9 days (5 ± 22). The rehabilitation program was standardized with no weight bearing of the affected extremity for

4 weeks. Twenty-seven patients were transferred to rehabilitation or geriatric centers and 3 patients were discharged home. No patient died in hospital. Six patients (20%) died at a mean age of 80.5 (75 ± 86) years during further follow-up that averaged 2.5 months after surgery. After 36 months, 21 patients (70%) attended the clinical follow-up. Bony consolidation was confirmed in all patients bar one (95.2%), in an average time of 4 months. Range of motion of the knee was sufficient ($0\geq90^\circ$) in all

Table II. GOS-course.

GOS OPn=21	Pre trauma n=30	6weeks post OPn=10	24weeks post OPn=21	52weeks post
1 (good)	27	3	15	18
2	3	27	6	3
3	0	0	0	0
4	0	0	0	0
5 (death)	0	0	6	6



Fig. 2. Vancouver type C fracture on uncemented stem. ORIF with NCB-PP.

cases. The GOS at 52 weeks showed a decrease of one point in 3 (14%) patients and was back to the preoperative level in 18 patients (86%). The Harris Hip Score at 52 weeks showed a mean score of 80.14 points (71±89 points). Full weight bearing was allowed at mean time of 100 days (50±160 days). None of the patients developed complications that needed subsequent surgery. None developed deep infection. Complications without need for surgical revision were seen in 6 patients (28%). A prolonged wound healing in two patients healed conservatively with antibiotics and repeated dressings. Four cases of urinary tract infection were registered; no patients developed a deep vein thrombosis (Table II).

DISCUSSION

Despite the shortcomings outlined above, the study offers an array of relevant information for the treatment of these complicated fractures. The conception of the NCB-PP® polyaxial locking system is a relatively new strategy (31-33).

Because of small cohort numbers and general lack of controlled studies, an optimal treatment for PPFs has not been described. The following discussion is based on the 3 main questions of this study.

- S1: impact of a new PPF plate;
- S2: application and technical advantages of polyaxial locking screws in PPF fixation with cement and cementless implants;
- S3: radiological healing, outcomes and mobility after 12 and 36 months.

S1: impact of a new PPF plate

The recent literature on the treatment of PPFs with locking plates reports 7-41% local complications and 0-29% revisions (23-28, 31). In our analysis of open approach polyaxial locking fixation, a relatively low rate of 20% minor complications were found. These results are the same or better than the outcomes obtained with mini-open or mini-invasive approach.

Although some authors recommend 'minimally invasive' strategies, in order to preserve the blood supply of the bone and to diminish local soft-tissue complications (25, 27, 28), other authors still recommend an open approach (23, 24, 26, 31, 34, 35). The more likely reason of revision is

the implant failure due to a mistake in the fracture classification process. The heart of the problem is distinguish between B1 and B2 fracture with stem loosening. In our opinion, an open-approach technique allows a better intraoperative evaluation of the implant stability, in order to obtain a better reduction, especially for 3 fragments fractures. It can also provide a better fixation with relative stability, with a bridging technique and a fracture periosteal healing *per secundam*. Otherwise, in some cases you can also obtain a complete stability, with bicortical screws and free lag screws with a *per primam* fracture healing, avoiding problems with axis and rotation of the healed fracture. Although the revision rates in the papers describing minimally invasive techniques (0-12.5%) seem to be lower than in studies on open approaches (7-29% revisions), a direct comparison cannot be performed because of the varying fracture types, strategies and implants.

While the mini-invasive technique showed favourable results when applied in A1 fractures, we found important complications such as plate fractures due to failed biological healing and malrotation when this technique was used for B1 and C fractures. Although a mini invasive technique is a viable option in long spiral 2-fragment fractures, this emphasizes the surgical difficulty and gives fair results in transverse and 3-fragment fractures, due to difficulty in close indirect reduction and achieving a perfect rotation.

It is unclear whether healing fracture complication was related to the procedure. However, in these cases we always found a periosteal flap or muscular flap interposed between the fracture fragments. In other studies with MI and MO technique, complications such as malrotation, malreduction, nonunion and plate breakage were documented.

Comparing the different techniques (open approach, MI and MO) with respect to OR time, packed red blood cell (PRBC) administration and need for ICU (intensive care unit) treatment, we did not find statistically significant differences comparing our group with other studies.

Nevertheless, we appreciate the open approach technique, because this approach offers higher perioperative safety than the other approaches and

great and unique possibility of testing the stability of the femoral implant thereby avoiding an important surgical indication mistake. In fact, only for 76% of PPFs do we can have a correct diagnosis of the implant stability before surgery. In an average of 24% of cases, the instability of the implant is accessed during surgery (36, 25, 27, 28).

S2: application and technical advantages of polyaxial locking screws in PPF fixation with cement and cementless implants

For fixation of PPFs, different techniques are described. According to the literature, PPFs around a THA are better fixed by locking screws than by conventional screws (22). Nevertheless, when a stable intramedullary stem is in place, a locking plate is the actual treatment of choice in a PPF. However, in monoaxial locking systems, the bicortical fixation of a plate is difficult to achieve in areas with an intramedullary implant and cemented stem. Monocortical screws can be placed as an

alternative, whereas if only (or mainly) monoaxial unicortical locking screws are set, a high risk of pullout and implant failure exists in osteopenic bone or uncemented implants (23, 26, 27); therefore, some authors recommend additional cable or cerclage fixation in these circumstances (23, 25, 28).

The NCB-PP® system, with polyaxial locking mechanism, allows the positioning of a locked bicortical screw around an intramedullary cemented stem. The wide plate design in periprosthetic region allows for bicortical screw fixation around the prosthesis. Oversized drill bits and drill guides are useful to reduce the risk of cracks in the cement mantle when placing screws around a cemented prosthesis. In our study, we were able to set a minimum of 4 and a mean of 5-8 bicortical screws around a femoral stem. In 12 cases (40%), an 8 bicortical screws fixation, 4 proximal (2 anterior and 2 posterior to the stem) and 4 distal were performed; in 12 cases (40%), an additional cerclage wire was necessary with 8-12 bicortical and monocortical

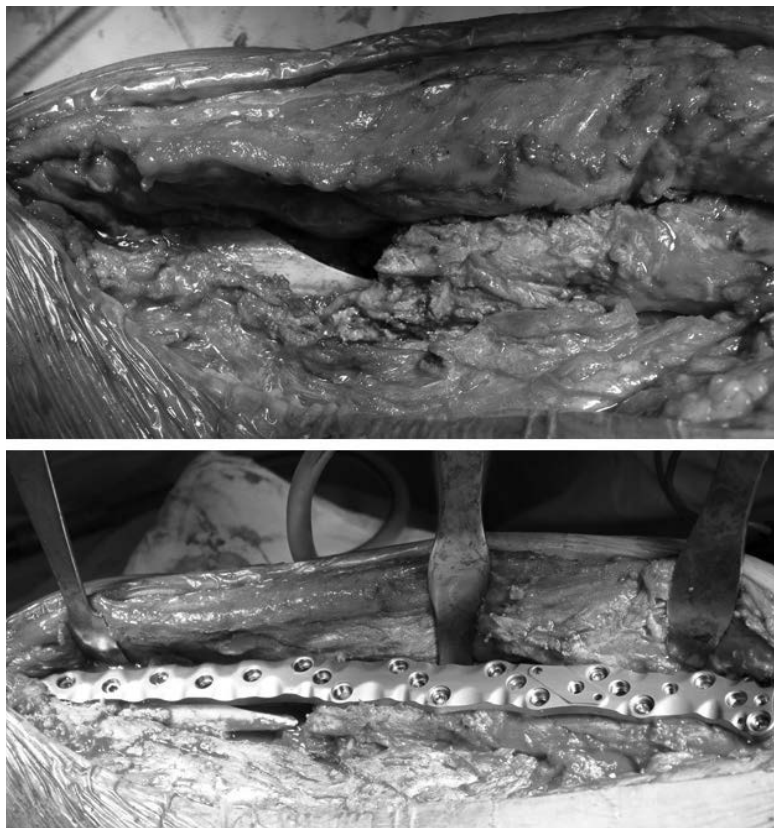


Fig. 3. Intraoperative Reduction and Fixation technique.

screws and in 6 cases, (20%) of precedent plating failure, an autologous-homologous bone graft was performed (3 homologous cortical tibial shaft grafts and 3 autologous iliac bone grafts).

S3: radiological healing, outcome and mobility after 12 months

Comparing the different approaches, we have observed very similar bone healing results and functional outcomes between open approach, mini-invasive and mini-open technique. Bony consolidation was confirmed in all patients in an average time of 4 months. Postoperative mortality on average 2.5 months after surgery, of average 20% of patients, is very close to the values of other studies. None of the patients developed complications that needed subsequent surgery despite general postoperative complications rate after PPF was high. Complications without need for surgical revision were seen in 2 patients (9%). No patients developed deep infection or deep vein thrombosis. The Harris Hip Score at 52 weeks showed a mean score of 80.14 points (71-89 points). This is an encouraging score and is very close to the mean HHS obtained after primary hip arthroplasty surgery.

The use of NCB-PP® plates has given excellent results in our clinical practice, giving good implant stability and allowing early postoperative mobilisation and recovery for our patients. For a successful implant and good functional recovery, the following are essential:

1. A correct surgical indication (ostosynthesis in cases of stable prosthesis only);
2. The choice of plate with the use of a diagonal three hole pattern in the area of greatest prosthesis fullness;
3. Trauma surgery experience.

Despite the small number of cases studied, the results after 4 years from the first implant are certainly encouraging. There are two major limitations of this study:

1. Different types of PPFs of the femur were included but we have a great homogeneity in the femoral implants (only prosthetic stems, no intramedullary nails);
2. Small cohort numbers.

REFERENCES

1. Su ET, DeWal H, Di Cesare PE. Periprosthetic femoral fractures above total knee replacements. J Am Acad Orthop Surg 2004; 12:12-20.
2. Berry DJ. Epidemiology: hip and knee. Orthop Clin North Am 1999; 30:183-90.
3. Lindahl H. Epidemiology of periprosthetic femur fracture around a total hip arthroplasty. Injury 2007; 38:651-4.
4. Duncan CP, Masri BA. Fractures of the femur after hip replacement. Instr Course Lect 1995; 44:293-304.
5. Masri BA, Meek RM, Duncan CP. Periprosthetic fractures evaluation and treatment. Clin Orthop Relat Res 2004; 80-95.
6. Berry DJ. Management of periprosthetic fractures: the hip. J Arthroplasty 2002; 17:11-3.
7. Garbuz DS, Masri BA, Duncan CP. Periprosthetic fractures of the femur: principles of prevention and management. Instr Course Lect 1998; 47:237-42.
8. Lewallen DG, Berry DJ. Periprosthetic fracture of the femur after total hip arthroplasty: treatment and results to date. Instr Course Lect 1998; 47:243-9.
9. Lindahl H, Malchau H, Oden A, Garellick G. Risk factors for failure after treatment of a periprosthetic fracture of the femur. J Bone Joint Surg Br 2006; 88:26-30.
10. Masri BA, Meek RM, Duncan CP. Periprosthetic fractures evaluation and treatment. Clin Orthop Relat Res 2004; 80-95.
11. McElfresh EC, Coventry MB. Femoral and pelvic fractures after total hip arthroplasty. J Bone Joint Surg Am 1974; 56:483-92.
12. Missakian ML, Rand JA. Fractures of the femoral shaft adjacent to long stem femoral components of total hip arthroplasty: report of seven cases. Orthopedics 1993; 16:149-52.
13. Mont MA, Maar DC. Fractures of the ipsilateral femur after hip arthroplasty. A statistical analysis of outcome based on 487 patients. J Arthroplasty 1994; 9:511-9.
14. Wood GCA, Naudie DR, McAuley J, McCalden RW. Locking compression plates for the treatment of periprosthetic femoral fractures around well-fixed total hip and knee Implants. J Arthroplasty 2011; 26:886-982.
15. Buttaro MA, Farfalli G, Paredes Nunez M, Comba

- F, Piccalug F. Locking compression plate fixation of Vancouver type B1 periprosthetic femoral fractures. *J Bone Joint Surg Am* 2007; 89:1964-9.
16. Xue H, Tu Y, Cai M, Yang A. Locking compression plate and cerclage bond for type B1 periprosthetic femoral fractures. *J Arthroplasty* 2011; 26:467-71.
 17. Charkravathy J, Bansal R, Cooper J. Locking plate osteosynthesis for Vancouver type B1 and type C periprosthetic fractures of the femur: a report on 12 patients. *Injury* 2007; 38:725-33.
 18. Fulkerson E, Tejwani N, Stuchin S, Egol K. Management of periprosthetic femur fractures with a first generation locking plate. *Injury* 2007; 38:965-72.
 19. Ehlinger M, Adam P, Abane L, Rahme M, Moor BK, Arlettaz Y, Bonnomet F. Treatment of periprosthetic femoral fractures of the knee. *Knee Surg Sports Traumatol Arthrosc* 2011; 19:1473-8.
 20. Rorabeck CH, Taylor JW. Classification of periprosthetic fractures complicating total knee arthroplasty. *Orthop Clin North Am* 1999; 30:209-14.
 21. Duncan CP, Masri BA. Fractures of the femur after hip replacement. *Instr Course Lect* 1995; 44:293-304.
 22. Herrera DA, Kregor PJ, Cole PA, Levy BA, Jo`nsson A, Zlowodzki M. Treatment of acute distal femur fractures above a total knee arthroplasty – systematic review of 415 cases (1981–2006). *Acta Orthopaedica* 2008; 79:22-7.
 23. Wood GCA, Naudie DR, McAuley J, McCalden RW. Locking compression plates for the treatment of periprosthetic femoral fractures around well-fixed total hip and knee Implants. *J Arthroplasty* 2011; 26:886-982.
 24. Buttaro MA, Farfalli G, Paredes Nunez M, Comba F, Piccalug F. Locking compression plate fixation of Vancouver type B1 periprosthetic femoral fractures. *J Bone Joint Surg Am* 2007; 89:1964-9.
 25. Xue H, Tu Y, Cai M, Yang A. Locking compression plate and cerclage bond for type B1 periprosthetic femoral fractures. *J Arthroplasty* 2011; 26:467-71.
 26. Charkravathy J, Bansal R, Cooper J. Locking plate osteosynthesis for Vancouver type B1 and type C periprosthetic fractures of the femur: a report on 12 patients. *Injury* 2007; 38:725-33.
 27. Fulkerson E, Tejwani N, Stuchin S, Egol K. Management of periprosthetic femur fractures with a first generation locking plate. *Injury* 2007; 38:965-72.
 28. Ehlinger M, Adam P, Abane L, Rahme M, Moor BK, Arlettaz Y, Bonnomet F. Treatment of periprosthetic femoral fractures of the knee. *Knee Surg Sports Traumatol Arthrosc* 2011; 19:1473-8.
 29. Jennett B, Bond M. Assessment of outcome after severe brain damage. *Lancet* 1975; 1:480-4.
 30. Franz KLR. Stand der Beurteilungsmöglichkeit der Prognose nach SHT-Vorbedingungen für eine Aussage über die Prognose. *Neuro Trauma News* 1998; 4-5.
 31. Erhardt JB, Grob K, Roderer G, Hoffmann A, Forster TN, Kuster MS. Treatment of periprosthetic femur fractures with the noncontact bridging plate: a new angular stable implant. *Arch Orthop Trauma Surg* 2008; 128:409-16.
 32. El-Zayat BF, Zettl R, Efe T, Kru`ger A, Ruchholtz S. Minimally invasive treatment of geriatric and osteoporotic femur fractures with polyaxial locking implants (NCB-DF1). *Unfallchirurg* 2012; 115:134-44.
 33. Pressmar J, Macholz F, Merkert W, Gebhard F, Liener UC. Results and complications in the treatment of periprosthetic femur fractures with a locked plate system. *Unfallchirurg* 2010; 113:195-202.
 34. Ebraheim NA, Liu J, Hashimi SZ, Sochacki KR, Moral M, Hirschfeld AG. High complication rate in locking plate fixation of lower periprosthetic distal femur fractures in patients with total knee arthroplasties. *J Arthroplasty* 2011.
 35. Ricci WR, Loftus T, Cox C, Borrelli J. Locked plates combined with minimally invasive insertion technique for the treatment of periprosthetic supracondylar femur fractures above a total knee arthroplasty. *J Orthop Trauma* 2006; 2:190-6.
 36. Lindahl H, Garellick G, Regner H, Herberts P, Malchau H. Three hundred and twenty-one periprosthetic femoral fractures. *JbJs* 2006; 88(6):1215-22.

TARGETING VEGF-A IN CARTILAGE REPAIR AND REGENERATION: STATE OF THE ART AND PERSPECTIVES

G. VADALÀ, F RUSSO, M. MUSUMECI, A GIACALONE, R. PAPALIA and V. DENARO

Department of Orthopaedic and Trauma Surgery, University Campus Bio-Medico of Rome, Rome, Italy

Osteoarthritis (OA) is the most common joint disorders in western populations, and is characterized by a progressive degradation of articular cartilage (AC) leading to loss of joint function. Methods to cure, delay or prevent the onset of OA and/or improve AC repair strategies have high clinical and socioeconomic impact. Possible innovative strategies envisioned for early OA treatments or cartilage repair include the implantation/injection of mesenchymal progenitors (MPs)-based constructs or cell-free bioactive scaffolds/hydrogel coupled with the controlled recruitment and instruction of resident MPs or AC. However, these tissue engineering-based strategies still suffer from unreliable outcomes with poor fibro-cartilaginous repair and blood vessel invasion. In such conditions Vascular Endothelial Growth Factor (VEGF) family has been shown to play a key role in controlling AC catabolism on one hand and angiogenesis on the other as a crucial step for endochondral ossification of MPs, ultimately leading to progressive breakdown of the neo-formed matrix. This review aims to provide a summary of relevant relationships between impaired angiogenesis, OA and cartilage regeneration highlighting how VEGF might play a paramount role in the pathophysiology of cartilage aging or degeneration as well as in cartilage repair.

Articular cartilage (AC) is a highly organized network of extracellular matrix proteins, proteoglycans, collagen, and non-collagenous proteins that maintain a high water content. Chondrocytes are embedded within lacunae in this structural and molecular labyrinth. AC, due to its avascular nature, receives nutrients through synovial fluid during normal compression load bearing cycles. AC plays a key role in loadbearing, force distribution and dissipation while chondrocytes critically maintain its biomechanical properties (1).

Chronic AC pathologies such as osteoarthritis (OA), are a major cause of pain and disability in the elderly population causing a rising demand for better treatment, considering that the surgical treatment in the late stage of the disease still is joint replacement. OA is driven by different degenerative mechanisms associated with chondropathy,

synovitis, osteochondral junction (OJ) remodeling, and osteophyte formation.

Histological analysis and imaging studies have shown marked synovitis in approximately one-third of patients with symptomatic OA, or rather, increased synovial angiogenesis and macrophage infiltration. This increase probably results from an imbalance between pro- and anti-angiogenic factors, such as the enhanced production of VEGF family members and the reduced expression of chondromodulin-1 (ChM-1) and thrombospondin-1 (TSP-1) by the inflamed synovium. In regard hypoxia inducible factor (HIF)-1 α and HIF-2 α are important transcription factors in hypoxia, inducing VEGFA gene responses (2).

OA is characterized by the loss of normal avascular status and an increased tendency to angiogenesis, as it is observed in the cartilage epiphyses of growing bone. Indeed, both matrix

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Corresponding author:

Gianluca Vadalà, MD, PhD,
Department of Orthopaedic and Trauma Surgery,
University Campus Bio-Medico of Rome,
Via Alvaro del Portillo 200, 00128 Rome, Italy
Tel.: +39 06 22 54 19142 - Fax: +39 06 22 54 11638
e-mail: g.vadala@gmail.com

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and signals change toward angiogenesis resulting in decreased essential extracellular matrix components such as proteoglycans (which may reduce the normal barrier to angiogenesis), high-molecular-weight molecules such as hyaluronic acid, type II collagen, N-propeptide suggesting their anti-angiogenic properties (2). Moreover, it has been shown the down-regulation of anti-angiogenic factors such as ChM-1, endostatin, TSP-1, tenomodulin, troponin I, angiostatin and inhibitors of matrix degrading enzymes, including tissue inhibitors of metalloproteinases (TIMPs), secretory leukocyte proteinase inhibitor and plasminogen activator inhibitor-1 (3).

VEGF family is a family of secreted polypeptides and VEGF-A (VEGF), is the founding member of the family. VEGF is also the most studied member of this family and has important roles in mammalian vascular development and in diseases involving abnormal growth of blood vessels. It is one of the most powerful angiogenic factor and it plays an essential role in the endochondral ossification process at the level of the growth plate, modulating cartilage vascularization and cells signaling (4). A recent study revealed that chondrocyte-derived VEGF switches the normal chondrocyte phenotype toward OA phenotype inducing catabolic pathways (5). In addition, VEGF acts as a powerful chemo-attractant for monocytes, leading to synovial hyperplasia in OA (6).

In AC degeneration, increased osteoclast activity causes extension of channels from the subchondral bone into the AC, ultimately passing across the tidemark into the normally avascular noncalcified cartilage. Numerous studies have shown that blood vessels in OA derive from the subchondral bone through the channels and into the cartilage. This leads to ossification of the cartilage and osteophyte formation around the channel (3). Blood vessels are accompanied by extensions of sympathetic and sensory nerves. Therefore, angiogenesis also results in innervation of the AC (7), which may cause pain in OA patients.

These aspects demonstrate the importance in controlling angiogenesis and in particular VEGF in AC lesions and OA management. In this review,

we investigate the physiological and pathological role of VEGF family and its potential modulation to improve both cartilage repair strategies and OA prevention and treatment.

VEGF family

VEGF is an important mediator of angiogenesis. The VEGF family is composed of seven secreted glycoproteins: VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, placental growth factor (PlGF) and VEGF-F (8). They all perform their biologic effect through interaction with different transmembrane tyrosine kinases receptors such as VEGFR-1, VEGFR-2, VEGFR-3 and the neuropilins (NP-1 and NP-2). Within the VEGF family, VEGF-A is the most specific and prominent angiogenic factor. For this reason, VEGF-A is usually called VEGF. VEGF-A has been shown to play an effective role both in physiological and pathological angiogenesis. Indeed, angiogenesis is an important biological process in many pathologies such as cancer. VEGF-VEGF receptor (VEGFR) system has been discovered to induce angiogenesis, mitogenesis and cell survival. Based on these findings, a variety of anti-angiogenic drugs, including anti-VEGF antibodies and VEGFR/multi-receptor kinase inhibitors have been developed and approved for clinical use. For these reasons, VEGF-A is considered a key angiogenic growth factor and its level of expression is a critical marker for detection of diseases. Indeed, under physiological conditions, a healthy adult organism does not demonstrate any active angiogenic process, except for few cases, such as in the female reproductive system and in lung, kidney, liver and brain where VEGF expression may also be detectable (8).

Physiological role of VEGF in endochondral bone formation

VEGF-induced signaling and consequent angiogenesis are fundamental processes during endochondral bone development and tissue repair, such as in fracture. Indeed, treatment with exogenous VEGF promotes fracture repair as well as angiogenesis while the use of a soluble neutralizing VEGF receptor impairs the process of callus

formation in a mice model (9).

Endochondral bone formation is a highly organized process that leads to longitudinal bone growth during vertebrate development where chondrocytes undergo phases of proliferation, maturation and apoptosis. During this process, blood vessel invasion from the metaphysis coincides with mineralization of the ECM, apoptosis of hypertrophic chondrocytes, ECM degradation, and bone formation (10). Gerber et al. (4) inactivated VEGF through the systemic administration of a soluble receptor chimeric protein to mice. Blood vessel invasion was almost completely suppressed, concomitant with impaired trabecular bone formation and expansion of the hypertrophic chondrocyte zone. Interruption of the anti-VEGF treatment was followed by capillary invasion, restoration of bone growth, resorption of the hypertrophic cartilage and normalization of the growth plate architecture (4).

The crucial role of VEGF in cartilage vascularization and bone development has been supported by different studies. Zelzer et al. (11) provided further *in vivo* evidence for the important role of VEGF in blood vessel invasion into hypertrophic cartilage during bone development. They described for the first time a connection between VEGF and chondrocyte survival during skeletal development. Using VEGF knockout mice they showed a delayed invasion of blood vessels into primary ossification centers and delayed removal of terminal hypertrophic chondrocytes (11). VEGF may also play a role in bone development in humans as its expression has been found in human neonatal cartilage (12).

Pathological role of VEGF in articular cartilage

VEGF level of expression is crucial in neonatal endochondral bone development. Contrary, adult human AC does not express VEGF under physiological conditions (13, 14). However, recent analyses have shown expression of VEGF and its receptors in OA cartilage and synovium (13-15). In several studies, chondrocytes and cartilage tissue obtained from OA patients had higher levels of VEGF and its receptors compared to healthy individuals (16, 17). Moreover, numerous studies showed the

correlation between OA and an increased expression of VEGF by chondrocytes (17, 18) in both traumatic and inflammatory conditions. Recently, Tibesku et al. (15) showed a significant correlation between the percentage of VEGF positive chondrocytes and histological and macroscopic grade of OA (15).

VEGF plays also a pivotal role also in inflammatory joint condition such as rheumatoid arthritis. Mouold et al. (19) showed the reduction of inflammation associated with synovial angiogenesis in VEGF knockout mice with induced inflammatory arthritis (19).

Numerous pathological stimuli can increase VEGF synthesis, such as hypoxia and mechanical stress (compression or shear stress) (20). Several other factors such as IL-1, IL-17, TNF, nitric oxide (NO) and reactive oxygen species (ROS) are able to induce VEGF expression in cultured chondrocytes through catabolic factors induction, including MMPs (21). Hypoxia can be considered the former stimulus for angiogenesis.

More specifically, reduced oxygen tension induces expression of VEGF through induction of a transcription factor, HIF-1 α (22). The target genes of HIF-1 are related not only to angiogenesis but also to cell proliferation/survival that could alter the chondrocyte metabolism. HIFs are transcription factors regulated post-transcriptionally by oxygen levels in the cell through oxygen sensors (hydroxylation on specific aminoacid residues by prolyl-hydroxylases, PHD). When oxygen levels are limited, HIF-1 α expression activates transcription of target genes such as VEGF (23). In OA, the expression of VEGF and HIF-1 are increased (24).

In OA the structures most affected by the progression of angiogenesis are mainly the sinovia tissue and, afterwards, blood vessels grow from the subchondral bone to occupy the osteochondral channels accompanied by extensions of sympathetic and sensory nerves. Giatromanolaki et al. (25) proved a significant overexpression of HIFs related to a high microvessel density and VEGF/KDR receptor activation, suggesting HIF- α -dependent synovial angiogenesis in OA (25).

Nevertheless, Ludin et al. (26) performed intra-articular injections of exogenous VEGF in healthy

mice demonstrating the possibility to induce the typical OA progression sequence in VEGF-treated animals. They noted progressive osteoarthritic changes in VEGF treated knee joints such as tidemark multiplication, marked subchondral sclerosis, progressive cartilage degradation, synovial membrane hyperplasia, and osteophyte formation (26).

In summary, these data corroborate the crucial role of VEGF by highlighting its importance in physiological angiogenesis, growth plate development and endochondral ossification as well as pathological conditions.

VEGF signal modulation in OA

OA is the most common joint disease leading to severe disability and pain with consequent decline of quality of life. Growing evidence is showing that the development of antiangiogenic treatments for patients with OA could represent a novel powerful therapy.

Chondroitin sulfate (CS) is a symptomatic slow-acting drug for OA widely used in the clinic and its properties are mainly due to an anti-angiogenic effect on synovial membrane and chondrocytes. Calamia et al. (27) showed its antiangiogenic, antiinflammatory, and anticatabolic properties (27). The observed antiangiogenic action of CS was due to a strong increase of the angiogenesis inhibitor TSP-1. Lambert et al. (28) confirmed these data suggesting that the beneficial role of CS in OA management could in part, derive from its anti-angiogenic properties by counteracting the IL-1b-dependent TSP-1 and Vascular Endothelial Growth Inhibitor (VEGI) down-regulation at both gene and protein levels (28).

Ashraf et al. (29) showed that angiogenesis inhibitor PPI 2458, an antiangiogenic fumagillin analog, reduced synovial and osteochondral angiogenesis, synovial inflammation, osteophyte formation, structural damage of AC and pain-related behaviors in a rat animal model of OA. This antiangiogenic factor arrest the growth of endothelial cells (EC), thus inhibiting their proliferation and angiogenesis (29).

Recently, Nagai et al. (30) evaluated the

effects of both intravenous and intra-articular administration of bevacizumab, a humanized anti-VEGFA monoclonal antibody, in a rabbit model of OA. These authors demonstrated that knees treated with bevacizumab exhibited a reduction in articular cartilage degeneration, osteophyte formation, and synovitis. In AC, expression levels of aggrecan, collagen type II and chondromodulin-1 were higher in the treated group. Histological evaluation and assessment of pain behavior showed a superior effect in the intrarticular administration group compared to the systemic administration of bevacizumab, even though the total dosage given to the intrarticular group was half that received by the systemic group (30).

In the future, it may be possible to use a wide range of angiogenesis inhibitors for the treatment of OA. Inhibiting inflammation, delaying osteophyte formation, reducing cartilage and meniscus innervation and maintaining the integrity of the OJ could provide direct pain relief for patients affected by OA.

VEGF signal modulation in the repair of chondral lesion

AC lesions represent a major concern in the field of orthopedic surgery due to a limited regenerative capacity of mature AC. Successful regeneration of any tissue requires the presence of reparative cells with the potential to differentiate into the phenotype required to restore the damaged site, supported by a microenvironment that guides their differentiation (31). Creating an avascular environment, through the inhibition of VEGF signaling, could be beneficial for the healing of a cartilage lesion through one of the clinically relevant repairing techniques mentioned above. In this prospective, Nagai et al. (32) demonstrated that a systemic administration of bevacizumab, a humanized monoclonal antibody anti-VEGF, in an osteochondral defect rabbit model leads to a complete cartilaginous filling in the treated group compared to a fibrous and bone tissue filling in the control group. They showed that intravenous administration of bevacizumab contributes to a better repair of articular cartilage in an osteochondral defect model (32).

A variety of scaffolds and cell types have been combined to optimize cartilage growth, maintaining chondrocyte phenotype, preventing vascularization of implanted tissues in order to maintain their biomechanical properties and prevent endochondral ossification. Centola et al. (33) developed and tested a clinically relevant fibrin/hyaluronan scaffold functionalized with bevacizumab for cartilage repair. The scaffold seeded with human nasal chondrocytes was implanted in the subcutaneous space of the back in nude rats. They showed that the sustained bevacizumab release from the scaffold efficiently blocked host vessels ingrowth and enhanced chondrocytes, improving survival rate. Therefore, this anti-angiogenic approach may support the formation of long-term stable and high-quality cartilaginous engineered tissue without any *in vitro* preconditioning (33).

According to these studies, angiogenesis control and in particular VEGF inhibition could actually play a critical role in tissue engineering mediated cartilage repair.

Modulation of VEGF signaling in chondrogenic differentiation of progenitor cells: applications in cartilage repair.

The repair process of large cartilage defects requires the presence of reparative cells with the potential to proliferate and differentiate into phenotypes that are required to restore the damaged site. There must also be a proper microenvironment that supports those cells. Progenitor cells, such as mesenchymal stem cells (MSCs), are a more attractive cell source than primary chondrocytes (34, 35). Among MSCs, muscle-derived stem cells (MDSCs) have been tested in experimental models for cartilage repair modulating angiogenic signals to mimic the proper microenvironment. Recently, the effect of blocking VEGF in chondrogenic differentiation of MDSCs has been investigated. Kubo et al. (36) tested the direct effect of both VEGF and sFlt-1 (Soluble Fms-like tyrosine kinase-1, a VEGF antagonist) on chondrogenic differentiation potential of mouse MDSCs *in vitro* and *in vivo* in a rat osteochondral defect model. Chondrogenic potential

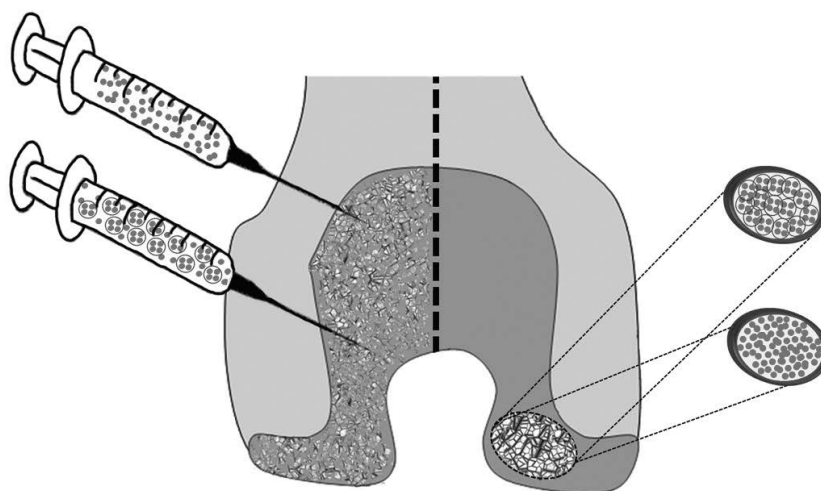


Fig. 1. Schematic representation of the proposed angiogenesis modulation strategies for the treatment of AO and cartilage lesion. On the left side an osteoarthritic knee treated with an intraarticular injection of antiangiogenic signals as proposed by Nagai et al. (2014) (**dots**) or injecting cells transduced with antiangiogenic genes as proposed by Matsumoto et al. (2009) (**circles with dots inside**). On the right side, a cartilage lesion treated with a scaffold releasing an antiangiogenic drug as proposed by Centola et al. (2013) (**dots**) or a scaffold engineered with cells transduced with antiangiogenic genes as proposed by Kubo et al. (2009) (**circles with dots inside**).

of MDSCs was significantly improved by the expression of the VEGF antagonist and decreased by VEGF (36). Accordingly with these data, Matsumoto et al. (5) investigated the effect of VEGF modulation in a rat model of OA. They performed intra-articular injections of murine MDSCs expressing BMP-4, in combination with MDSCs expressing either VEGF or sFlt-1. OA knees treated with MDSCs expressing sFlt-1 demonstrated reduced OA changes of cartilage matrix, less osteophyte formation, higher differentiation and proliferation and lower levels of chondrocyte apoptosis compared to the VEGF group (5).

Therefore, incorporation of plasmid vectors encoding endogenous angiogenesis inhibitor, into MSCs and chondrocytes used to seed cartilaginous constructs, is a potential strategy for maintaining the engineered cartilage phenotype.

DISCUSSION

There is a strict relationship between impaired angiogenesis and OA and VEGF might play a key role in the pathophysiology of cartilage degeneration.

The modulation of these signals may be beneficial in the treatment of OA. It may result in the reduction of inflammation and cartilage matrix degradation through setting up an appropriate environment that prevents chondrocyte hypertrophy, apoptosis and osteophyte formation. Moreover, cartilage repair strategies would benefit of a bioactive scaffold which is able to inhibit the angiogenic response mediated by VEGF in the cartilage lesion site.

Although an anti-VEGF antibody, such as Bevacizumab, is an attractive drug for the treatment of neovascular disease as largely used in the treatment of different oncological diseases, several complications are associated with its intravenous administration, such as haemorrhage, thromboembolism, proteinuria, delayed wound healing, and hypertension. However, scaffold release systems and local administration strategies, such as intrarticular administration, would be able to reduce the administration doses thus avoiding the reported side effects. Figure 1 schematically shows the different therapeutic strategies based on

angiogenesis modulation for either OA treatment or cartilage repair.

Targeting VEGF may open up future perspectives and horizons in the field of regenerative medicine, although further studies are needed to evaluate VEGF as a target for the treatment of OA and cartilage lesions.

REFERENCES

1. Hunziker EB, Quinn TM, Hauselmann HJ. Quantitative structural organization Of normal adult human articular cartilage. *Osteoarthritis Cartilage* 2002; 10:564-72.
2. Patra D, Sandell LJ. Antiangiogenic and anticancer molecules in cartilage. *Expert Rev Mol Med* 2012; 14:e10.
3. Franes RE, McWilliams DF, Mapp PI, Walsh DA. Osteochondral angiogenesis and increased protease inhibitor expression in OA. *Osteoarthritis Cartilage* 2010; 18:563-71.
4. Gerber HP, Vu TH, Ryan AM, Kowalski J, Werb Z, Ferrara N. VEGF couples hypertrophic cartilage remodeling, ossification and angiogenesis during endochondral bone formation. *Nat Med* 1999; 5:623-8.
5. Matsumoto T, Cooper GM, Gharaibeh B, Meszaros LB, Li G, Usas A, Fu FH, Huard J. Cartilage repair in a rat model of osteoarthritis through intraarticular transplantation of muscle-derived stem cells expressing bone morphogenetic protein 4 and soluble Flt-1. *Arthritis Rheum* 2009; 60:1390-405.
6. Marrelli A, Cipriani P, Liakouli V, Carubbi F, Perricone C, Perricone R, Giacomelli R. Angiogenesis in rheumatoid arthritis: a disease specific process or a common response to chronic inflammation? *Autoimmun Rev* 2011; 10:595-98.
7. Suri S, Gill SE, Massena de Camin S, Wilson D, McWilliams DF, Walsh DA. Neurovascular invasion at the osteochondral junction and in osteophytes in osteoarthritis. *Ann Rheum Dis* 2007; 66:1423-8.
8. Neufeld G, Cohen T, Gengrinovitch S, Poltorak Z. Vascular endothelial growth factor (VEGF) and its receptors. *FASEB J* 1999; 13:9-22.
9. Street J, Bao M, deGuzman L, et al. Vascular endothelial growth factor stimulates bone repair by promoting angiogenesis and bone turnover. *Proc*

- Natl Acad Sci USA 2002; 99:9656-61.
10. Provot S, Schipani E. Molecular mechanisms of endochondral bone development. *Biochem Biophys Res Commun* 2005; 328:658-65.
 11. Zelzer E, Mamluk R, Ferrara N, Johnson RS, Schipani E, Olsen BR. VEGFA is necessary for chondrocyte survival during bone development. *Development* 2004; 131:2161-71.
 12. Kusafuka K, Hiraki Y, Shukunami C, Kayano T, Takemura T. Cartilage-specific matrix protein, chondromodulin-I (ChM-I), is a strong angiogenesis inhibitor in endochondral ossification of human neonatal vertebral tissues *in vivo*: relationship with angiogenic factors in the cartilage. *Acta Histochem* 2002; 104:167-75.
 13. Sakata R, Kokubu T, Nagura I, Toyokawa N, Inui A, Fujioka H, Kurosaka M. Localization of vascular endothelial growth factor during the early stages of osteochondral regeneration using a bioabsorbable synthetic polymer scaffold. *J Orthop Res* 2012; 30:252-9.
 14. Wang QY, Dai J, Kuang B, Zhang J, Yu SB, Duan YZ, Wang MQ. Osteochondral angiogenesis in rat mandibular condyles with osteoarthritis-like changes. *Arch Oral Biol* 2012; 57:620-9.
 15. Tibesku CO, Daniilidis K, Skwara A, Paletta J, Szuwart T, Fuchs-Winkelmann S. Expression of vascular endothelial growth factor on chondrocytes increases with osteoarthritis - an animal experimental investigation. *Open Orthop J* 2011; 5: 177-80.
 16. Enomoto H, Inoki I, Komiya K, et al. Vascular endothelial growth factor isoforms and their receptors are expressed in human osteoarthritic cartilage. *Am J Pathol* 2003; 162:171-81.
 17. Pfander D, Kortje D, Zimmermann R, Weseloh G, Kirsch T, Gesslein M, Cramer T, Swoboda B. Vascular endothelial growth factor in articular cartilage of healthy and osteoarthritic human knee joints. *Ann Rheum Dis* 2001; 60:1070-3.
 18. Zhou JL, Liu SQ, Qiu B, Hu QJ, Ming JH, Peng H. Effects of hyaluronan on vascular endothelial growth factor and receptor-2 expression in a rabbit osteoarthritis model. *J Orthop Sci* 2009; 14: 13-9.
 19. Mould AW, Tonks ID, Cahill MM, Pettit AR, Thomas R, Hayward NK, Kay GF. Vegfb gene knockout mice display reduced pathology and synovial angiogenesis in both antigen-induced and collagen-induced models of arthritis. *Arthritis Rheum* 2003; 48: 2:660-9.
 20. Pufe T, Lemke A, Kurz B, Petersen W, Tillmann B, Grodzinsky AJ, Mentlein R. Mechanical overload induces VEGF in cartilage discs via hypoxia-inducible factor. *Am J Pathol* 2004; 164:185-92.
 21. Murata M, Yudoh K, Nakamura H, Kato T, Inoue K, Chiba J, Nishioka K, Masuko-Hongo K. Distinct signaling pathways are involved in hypoxia- and IL-1-induced VEGF expression in human articular chondrocytes. *J Orthop Res* 2006; 24:1544-54.
 22. Maes C, Carmeliet G, Schipani E. Hypoxia-driven pathways in bone development, regeneration and disease. *Nat Rev Rheumatol* 2012; 8:358-66.
 23. Lafont JE. Lack of oxygen in articular cartilage: consequences for chondrocyte biology. *Int J Exp Pathol* 2010; 91:99-106.
 24. Pfander D, Gelse K. Hypoxia and osteoarthritis: how chondrocytes survive hypoxic environments. *Curr Opin Rheumatol* 2007; 19:457-62.
 25. Giatromanolaki A, Sivridis E, Maltezos E, Athanassou N, Papazoglou D, Gatter KC, Harris AL, Koukourakis MI. Upregulated hypoxia inducible factor-1alpha and -2alpha pathway in rheumatoid arthritis and osteoarthritis. *Arthritis Res Ther* 2003; 5:R193-201.
 26. Ludin A, Sela JJ, Schroeder A, Samuni Y, Nitzan DW, Amir G. Injection of vascular endothelial growth factor into knee joints induces osteoarthritis in mice. *Osteoarthritis Cartilage* 2013; 21:491-7.
 27. Calamia V, Lourido L, Fernandez-Puente P, et al. Secretome analysis of chondroitin sulfate-treated chondrocytes reveals anti-angiogenic, anti-inflammatory and anti-catabolic properties. *Arthritis Res Ther* 2012; 14:R202.
 28. Lambert C, Mathy-Hartert M, Dubuc JE, Montell E, Verges J, Munaut C, Noel A, Henrotin Y. Characterization of synovial angiogenesis in osteoarthritis patients and its modulation by chondroitin sulfate. *Arthritis Res Ther* 2012; 14:R58.
 29. Ashraf S, Mapp PI, Walsh DA. Contributions of angiogenesis to inflammation, joint damage, and pain in a rat model of osteoarthritis. *Arthritis Rheum* 2011; 63:2700-10.
 30. Nagai T, Sato M, Kobayashi M, Yokoyama M,

- Tani Y, Mochida J. Bevacizumab, an anti-vascular endothelial growth factor antibody, inhibits osteoarthritis. *Arthritis Res Ther* 2014; 16:427.
31. Caplan AI, Elyaderani M, Mochizuki Y, Wakitani S, Goldberg VM. Principles of cartilage repair and regeneration. *Clin Orthop Relat Res* 1997;254-69.
32. Nagai T, Sato M, Kutsuna T, Kokubo M, Ebihara G, Ohta N, Mochida J. Intravenous administration of anti-vascular endothelial growth factor humanized monoclonal antibody bevacizumab improves articular cartilage repair. *Arthritis Res Ther* 2010; 12:R178.
33. Centola M, Abbruzzese F, Scotti C, Barbero A, Vadala G, Denaro V, Martin I, Trombetta M, Rainer A, Marsano A. Scaffold-based delivery of a clinically relevant anti-angiogenic drug promotes the formation of in vivo stable cartilage. *Tissue Eng Part A* 2013; 19:1960-71.
34. Vadala G, Russo F, Ambrosio L, Loppini M, Denaro V. Stem cells sources for intervertebral disc regeneration. *World J Stem Cells* 2016; 8:185-201.
35. Vadala G, Russo F, Musumeci M. Clinically relevant hydrogel-based on hyaluronic acid and platelet rich plasma as a carrier for mesenchymal stem cells: Rheological and biological characterization. *J Orthop Res* 2017; 35:2109-16.
36. Kubo S, Cooper GM, Matsumoto T. Blocking vascular endothelial growth factor with soluble Flt-1 improves the chondrogenic potential of mouse skeletal muscle-derived stem cells. *Arthritis Rheum* 2009; 60:155-65.