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MALAR REGION REJUVENATION THROUGH NON-INVASIVE TECHNIQUES: HYALURONIC ACID FILLERS AND LIPOFILLING

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Contour enhancements of the midface make up a dominant aspect of aesthetic surgery. The goal of midface rejuvenation is to decrease the prominence of grooves and creases as well as to provide volume to atrophied tissue. There are various options, including autologous tissue grafts, allogenic tissue grafts and alloplastic materials. In turn, patients' needs have led to an increasing trend of less invasive treatments. In the present study, 82 implants were used for midface augmentation in 41 patients (38 women, 3 men) between January 2014 and January 2016. Twenty-five patients were treated with Hyaluronic acid implants while the remaining 16 patients were treated with lipofilling. For both groups and in all cases, good integration of the filled material was observed in the malar region, with no significant treatment complications and the last follow-up visit demonstrated good results and overall satisfaction. Hyaluronic acid fillers and lipofilling are therefore the ideal answer to patients who aim for a natural and immediate result with manageable complications, but, unlike Hyaluronic acid, autologous fat allows us to obtain a long-lasting effect over time, resulting the closest thing to an ideal facial filler.

Contour enhancements of the midface, and particularly the malar region, make up a dominant aspect of aesthetic surgery, as well as traumatic, congenital and extirpative defect corrections (1-11). The characteristics of the ideal material for this purpose include easy harvesting with minimal donor site morbidity, good biocompatibility at the recipient site, minimal risk of perforation, ease at remodeling, desired form maintenance and consistency *in situ* and in body tissue inertness with an easy reproducibility of the technique (12, 13).

There are various options to take into careful consideration for the reconstruction of a malar depression, including autologous tissue grafts, allogenic tissue grafts and alloplastic materials.

Alloplastic materials are readily available, lack

donor-site morbidity, decrease surgical time and cost, and have relatively good post-operative tissue tolerance (14). Various alloplastic materials have been used in facial cosmetic and reconstructive surgery including silicone, Gore-Tex, Medpor, and expanded polytetrafluoroethylene (ePTFE) (1,15).

In comparison, autogenous grafts, such as grafted adipose tissue, have the advantage of relative resistance to infection, ease of incorporation into new tissues, absence of a foreign body reaction and decreased incidence of extrusion. With lipofilling one can get a more natural reconstruction of the face contour although they are lowly invasive with minimal donor site morbidity (15).

Furthermore, a number of various Hyaluronic acid fillers can be used for midface rejuvenation

Key words: malar region, hyaluronic acid, rejuvenation, filler, lipofilling

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and malar enhancement due to their efficacy and durability. Hyaluronic acid, a glycosaminoglycan (GAG) that binds water molecules and is involved in tissue hydration, is markedly reduced in aged skin (16-25). Hyaluronic acid fillers are readily available, e.g. alloplastic material, and a lowly invasive strategy with an optimal tissue tolerance, e.g. lipofilling.

Alloplastic implants have been an attractive option for the augmentation of malar deficiency because of the unlimited amount of material, reduced surgical time, and easy handling. Among alloplastic materials, there is emphasis on high-density porous polyethylene-Medpor (Medpor, Porex Surgical Inc, Newman, GA) (26) extensively used on humans for the past several years and has proven stable for facial reconstructions. However, Medpor has disadvantages too as radiolucent on imaging studies, infection, hematoma, seroma, subcutaneous palpation, and deformation of the contours (27). The displacement of the implant may result in protrusions through the skin, compression of nerves and of other important structures (28).

The goal of midface rejuvenation is to decrease the prominence of grooves and creases as well as to provide volume to atrophied tissue. In turn, patients need to slow down post-operation retrieval times and at the same time, enjoy an optimal aesthetic outcome, which has led to an increasing trend towards less invasive treatments. What is more, the possibility, albeit minimal, of complications deriving from alloplastic prostheses implant has moved our sample towards a larger use of Hyaluronic acid fillers and lipofilling.

This study aims at evaluating different options as well as identifying and optimizing the right strategies, by analyzing different procedures and materials, in the management of midface enhancement in patients with malar depression.

MATERIALS AND METHODS

Patients with malar hypoplasia and midface retrusion with an unbalanced profile and over-projecting mouth are considered the best candidates for augmentation with a malar or submalar implant. The selection of the appropriate implant is based on the physical exam. Analysis with the

patient both smiling and in repose help identification of areas of volumetric deficiency, with particular attention paid to asymmetries. After critical analysis of the patient's facial features, implants are selected that enhance volume and give the appearance of full, youthful cheeks.

In the present study, 82 implants were used for midface augmentation in 41 patients (38 women, 3 men) between January 2014 and January 2016. The age range was 26-68 years (mean age 43.7 years). Twenty-five patients were treated with Hyaluronic acid implants, the remaining 16 patients, with lipofilling.

The implants were used for augmentation in the paranasal area, malar area and orbital area. Post-operatively, patients were asked to come back for follow-up at weeks 2 and 4 and at months 3.6 and 12. At each follow-up visit, surgical complications were documented and patients were photographed. Position, facial expression, focal distance and camera settings were standardized. They were also asked to rate their overall satisfaction with the post-surgical facial appearance on a five-point scale (1: poor; 2: fair; 3: good; 4: very good; 5: excellent).

Procedures

For patients treated with Hyaluronic acid fillers, a 2 mL of the product was injected in the subcutaneous layer of the right and left malar/submalar areas. Five injection sites for each side were identified detecting relatively avascular subdermal areas able to spread Hyaluronic acid along the interstitial spaces between lymphatic vessels. The product was administered with a 27-G needle using a bolus technique. Two sessions were performed, once a month, for 2 months.

For patients treated with lipofilling fat harvest, preparation and reinjection were performed in a standardized fashion in accordance with Coleman's recommendations (29) by the same plastic surgeon.

The donor region was infiltrated with Klein solution (500 ml normal saline, 1 ml 2% lidocaine, 1 ml 0.1% epinephrine). After adequate anesthesia, a suitable amount of subcutaneous fat was harvested from the abdominal wall using manual suction with a 10 cc syringe and a 3 mm blunt cannula. Centrifugation was carried out at 3000 rpm for 3 min. Subsequently, the blood/tumescent fraction was drained and the oil was removed. The resulting purified fat layer was transferred into multiple 1-ml syringes

and used for grafting the malar region, according to the principles of structural fat grafting. Multiple access sites and a fan-like pattern technique using 1.4 mm and 1.8 mm blunt cannulas were used to transfer small aliquots of fat into various depths of the soft tissue (from the dermis to the muscular fascia or muscle). A three-dimensional network of tunnels was created to improve the contact between the graft and the local adipose tissue, in order to maximize its harvest. The quantity of transplanted adipose tissue was determined by attempting to obtain symmetry with the contralateral side with 30% overcorrection. Overcorrection was performed with the realization that there would be some loss of volume over time.

RESULTS

For the Hyaluronic acid group, good integration of the filled material was observed in the malar region

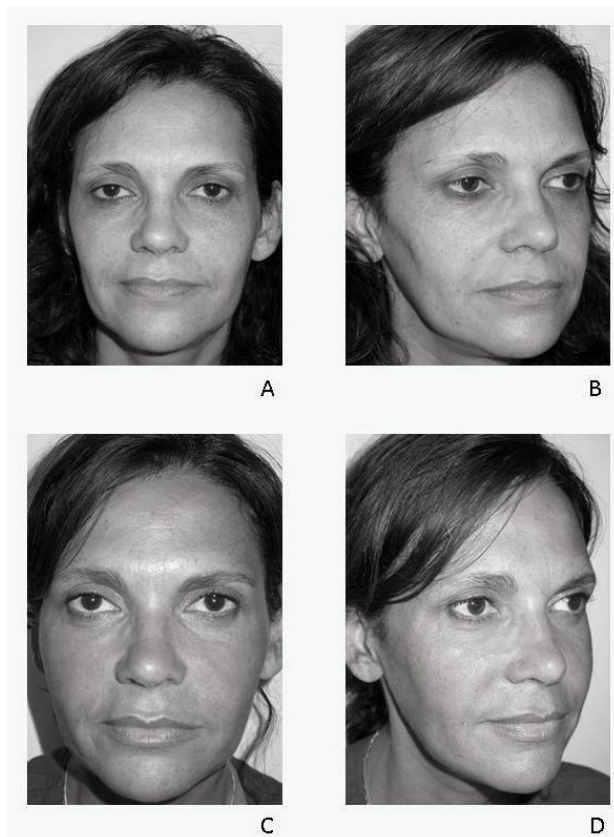


Fig 1. Patient with moderate midface ptosis treated with HA filler. **1 A-B:** Frontal view and 3/4 view, before treatment; **1 C-D:** Frontal view and 3/4 view 12 months after the treatment.

in all cases. Retrospective analysis of photographic documentation showed a progressive volumetric decrease in the first week after treatment, due to edema reabsorption; after that the volume obtained remained stable. At 12 months after treatment a decrease in the increase in volume of the malar region was observed, that might need further treatment. There were no significant treatment complications. Mild edema occurred frequently during the first postoperative week. No hematomas, infections, vascular or nervous injuries were recorded.

Analysis of patient satisfaction after the last follow-up visit clearly demonstrated better results with a 4.6 rate in their overall satisfaction.

For the lipofilling group, good integration of the grafted fat was observed in the recipient sites in all cases. Retrospective analysis of photographic documentation showed progressive volumetric decrease for up to approximately 6 months after surgery. After that, graft volume remained relatively stable. No further treatment was required 12 months after surgery.

There were no significant surgical complications, either from the fat harvest site or from the reconstructed site. Mild edema and bruising were frequent during the first post-operative week. No hematomas, infections, vascular or nervous injuries were recorded. Four patients felt the need to have a second procedure while none needed a third one.

Analysis of patient satisfaction after the last follow-up visit clearly demonstrated better results with a 4.5 rate in their overall satisfaction.

DISCUSSION

Volume restoration is not a new concept in midface rejuvenation. A number of authors believe that malar volume deflation actually precedes gravitational descent in facial aging, and consequently, optimal rejuvenation of the aging midface and malar area should involve repositioning of ptotic soft tissues, as well as correction of volume deficiency where present (29-43).

Surgical procedures involve mobilization of the midface flap and their repositioning according to an accurate pre-surgical planning. The midface flap, after

being adequately released, can be anchored either to the strong temporal aponeurosis with a long suture that authors have termed a “suspender-like” suture, or directly to the lower orbital rim bone with a suture that authors have termed a “belt-like” suture. Results are encouraging, but complications may be possible.

The need of patients to have good cosmetic results with early return to daily activities has increased the search for additional strategies. Use of alloplastic implants for facial augmentation has been well documented (44, 45) for several years. There remains some conflicting ideology as to the safety profile and utility of aesthetic facial augmentation with alloplasts.

Solid implant materials commonly used in facial reconstruction include silicone (polydimethylsiloxane), Silastic (solid silicone elastomer; Michigan Medical Corporation, Santa Barbara, CA), GoreTex (expanded polytetrafluorethylene; W. L. Gore, Associates

Inc., Flagstaff, AZ), Medpor (high-density porous polyethylene; Porex Industries, Fairburn, GA), and Mersilene (nonresorbable polyester fiber; Ethicon, Somerville, NJ). Other less common implant options include Supramid (polyamide nylon mesh; Ethicon), PMMA (polymethylmethacrylate), and Permacol (porcine dermal collagen; Tissue Science Laboratories PLC, Aldershot, United Kingdom).

Thus, among allografts, the porous high-density polyethylene (Medpor) has been extensively used in humans for many years and proved to be stable for craniofacial, orbital floor and ear reconstructions. It is a pure polyethylene with only one manufacturing process and pore size (150-200µm). Medpor is an alloplastic, biocompatible, flexible, resilient and nonabsorbable biomaterial and one of its main advantages are its pores, which permit vascularization through the implant installed.

Despite the possible benefits resulting from its use, Medpor has several disadvantages too, such as: radiolucent (46) on imaging studies, infection, hematoma, seroma, subcutaneous palpation, and deformation of the contour (27). Still, the displacement of the implant may result in skin protrusion, the compression of nerves and other important structures (28).

Infection in the setting of alloplastic implantation has always been an issue of concern as these materials lack the vascular ingrowth seen in autologous grafts and additionally, serve as a surface for bacterial colonization and biofilm formation. It remains controversial whether porous materials, like Medpor, have a greater risk of infection when compared to silicone, which has smooth counterparts.

Walker and Toriumi documented the presence of biofilms on explanted alloplastic facial implants (47) concluding that both silicone and Medpor are able to promote and harbor biofilm formation, although biofilms appear to grow more readily and more densely on implants with rougher surfaces such as Medpor.

Similarly, Berghaus and Stelter (48) have demonstrated that porous implants may have a higher risk of infection early on by providing scaffolding for bacterial growth.

Possible complications from Medpor implants



Fig 2. Patient with severe midface ptosis treated with lipofilling. 2 A-B: Frontal view and 3/4 view, before treatment; 2 C-D: Frontal view and 3/4 view 12 months after the treatment. It can be also noted a local improvements in skin quality.

are well documented in literature by de Moraes Ferreira, et al, in their review which includes diplopia, hypoesthesia, enophthalmos, limited eye movement, hematoma, infection, swelling, pain, displacement of implant, fistula formation, palpability of the implant, scar formation, seroma and dystopia.

Ridwan-Pramana, et al, looked at the outcomes from 69 Medpor facial implants placed in their institution and found an overall complication rate of 31.8% although it is correct to specify that the use of Medpor was not limited to the malar region only (49).

Finally, overcorrection must be avoided as this can promote extrusion of material, especially when skin is under tension and the soft-tissue mantle is damaged (48). What is recommended is fixation of the implant in place with osteosynthetic material that increases surgery time, cost and complexity (50). Because of likely complications deriving from the use of alloplastic material, we observe a higher and higher trend in the use of allogenic materials.

Midface rejuvenation with injectable Hyaluronic acid fillers have become an outstanding option for treatment of midface aging since it offers a versatile, safe and effective means of restoring a youthful appearance while being less invasive and disruptive than surgery (51, 52).

Correction with Hyaluronic acid fillers of the latest generation can last for up to 1 year, many patients choose to repeat the treatment in order to maintain their appearance.

Adverse events attributable to treatment with Hyaluronic acid are edema, erythema, and bruising although mild and resolve spontaneously within 14 days.

Furthermore, Hyaluronic acid is biodegradable filler. Fillers like these are generally preferred over permanent fillers. A major reason for this is safety; if complications occur they will generally disappear spontaneously. Biodegradable fillers also allow the possibility of surgical correction in the future, whereas with permanent fillers surgery may no longer be an option.

To patients who prefer a less invasive treatment, such as Hyaluronic acid fillers, even more stable and long-lasting, lipofilling of the malar region can be indicated. The benefits of this technique include a

readily available and autologous source of permanent filler, simultaneous body contouring in the process of fat harvesting, and relative ease of execution, negligible morbidity, low cost, with predictable and reliable results.

In addition, unlike synthetic fillers, autologous fat has the ability to change in structure with the patient's physiologic changes and adverse reactions are extremely uncommon.

Local improvements in skin quality (29) at the graft location are another benefit of lipofilling and may add to satisfaction with the post-operative result. Recent investigations on multipotent adipocyte-derived stem cells may enable thin patients to benefit from lipofilling (53-55).

CONCLUSIONS

Complications found with midface solid implants include inadequate correction or overcorrection, malposition, implant migration, infection, extrusion, nerve hypesthesia/anesthesia, and facial nerve injury. For this reason, we cannot consider alloplastic implants as a valid non-invasive alternative to surgery.

On the contrary, Hyaluronic acid fillers and lipofilling turn out to be the ideal answer to patients who aim at obtaining a natural and immediate result with manageable complications.

In our view, autologous fat is the closest thing to an ideal facial filler. It is readily available, inexpensive, and simple to obtain. There is no risk of host rejection or other immunologic response. Finally, unlike Hyaluronic acid, it allows us to obtain a long-lasting result over time.

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HYALURONAN: AN OVERVIEW

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Hyaluronic acid (HA) is a polyanionic natural polymer occurring as a linear polysaccharide composed of glucuronic acid and N-acetylglucosamine repeats. Hyaluronic acid has a wide range of applications with its excellent physicochemical properties such as biodegradability, biocompatibility, non-toxicity, non-immunogenicity and serves as an excellent tool in biomedical applications such as osteoarthritis surgery, ocular surgery, plastic surgery, tissue engineering and drug delivery. This work provides an overview on hyaluronic acid, its chemistry and biochemistry and its medical applications.

Hyaluronic acid is a linear polysaccharide that is abundant in several connective tissues. Due to its essential role as tissue lubricant and shock absorber, hyaluronic acid (HA) finds important medical applications in joints, skin and eye conditions (1), osteoarthritis (2-4), cartilage regeneration (5), skin regeneration (6) and retinal repair (7). Various authors have reported the numerous functions of HA and its fragments in wound healing (8, 9). Within the epidermis, HA represents an integral part of the ECM that surrounds keratinocytes, fostering their proliferation and thus enhancing re-epithelialization. Accordingly, the latter property of HA has been successfully leveraged in wound healing and for supporting scarless tissue repair (8). Further medical applications of HA include visco-supplementation, drug delivery, tissue repair and regeneration (e.g., cardiovascular tissue engineering), inflammation, angiogenesis, as well as brain and neural regeneration (10-13).

The present review is organized as follows: a description of HA structure and its synthesis and degradation mechanisms are initially described. Distribution and functions of HA within the ECM

of different tissues are analyzed, followed by a description of the most important signaling pathways involving HA; finally, a description of chemical strategies for HA derivatization are presented, together with a set of representative HA formulations for different medical applications.

Chemistry of HA

Karl Meyer and John Palmer were the first investigators to biochemically purify HA in 1934 (14). In their work, they described a procedure for isolating HA from the vitreous humor of bovine eyes, initiating a huge amount of research on that substance. In 1954, years after that first discovery, Weissmann et al. (15) solved and described the HA chemical structure. Firstly isolated as an acid and hence the name, HA is composed of an uronic acid and an aminosugar. Considering its existence *in vivo* as a polyanion, HA is also referred to as *hyaluronan*. The term hyaluronan was introduced by Endre Balazs (16), who adopted the nomenclature of polysaccharides. The term was meant to include both the acid form and the salified one (e.g. sodium hyaluronate) (17). HA is a linear polysaccharide

Hyaluronic acid, extracellular matrix, hyaladherins, hyaluronan formulations.

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made of repeating disaccharide units of D-glucuronic acid (GlcUA) and N-acetyl glucosamine (GlcNAc) linked by β -1,4 and β -1,3 glycosidic bonds (Fig. 1) (1, 18, 19).

The structure of the disaccharide is energetically very stable. Indeed, both sugars are spatially connected to glucose which, when in its beta configuration, has all its bulky groups (i.e., the hydroxyls, the carboxylate moiety and the anomeric carbon on the adjacent sugar) in sterically favorable equatorial positions, whereas all the hydrogen atoms occupy less advantageous axial positions from the steric point of view (17, 20).

Even though HA is classified as a glycosaminoglycan (GAG), considering it is composed by glucuronate units carrying an anionic charge at physiological pH and bi-carboxylate groups associated to negative charges balanced by mobile cations (e.g. Na^+), it differs from other GAGs. In fact, while the wide majority of GAGs are smaller in size (<50 kDa), HA is a megadalton molecule with an extended length ranging from 2 μm to 25 μm and a typical molecular weight between $\approx 2 \times 10^5$ and $\approx 10 \times 10^8$ Da, according to different authors and depending on the enzyme that catalyzed its synthesis (21-23). Unlike all other GAGs of the ECM, which are attached to core proteins and undergo post-translational modifications giving rise to proteoglycans, hyaluronan is the only GAG that

is not bound to any core protein and that does not undergo any secondary modification. Moreover, conversely to the other GAGs, hyaluronan synthesis takes place in the plasma membrane, from which it can be readily secreted, by a class of integral membrane proteins called hyaluronan synthases instead of the Golgi apparatus of the cell where most GAGs are produced.

The high molecular weight of HA coupled with its strong intermolecular interactions, given by a combination of the internal hydrogen bonds, the chemical structure of the disaccharide and interactions with the solvent, explain why HA aqueous solutions are highly viscous and possess a shear-thinning behavior. In fact, in a physiological solution, the internal hydrogen atoms form a non-polar relatively hydrophobic area, while the side chains form a polar, hydrophilic zone. These situations lead to a twisted ribbon structure. Thus, in solution, the polymer chain of hyaluronan assumes a stiffened helical configuration, which can be explained by hydrogen bonding between the hydroxyl groups along the chain, resulting in a coil structure that can trap 1000 times its weight in water.

Entanglement of HA chains, occurring already at low concentrations, is responsible for its jelly behavior. At the same time, upon the application of a shear stress, HA chains align in the streamlines of flow, which results in a decrease in the viscosity.

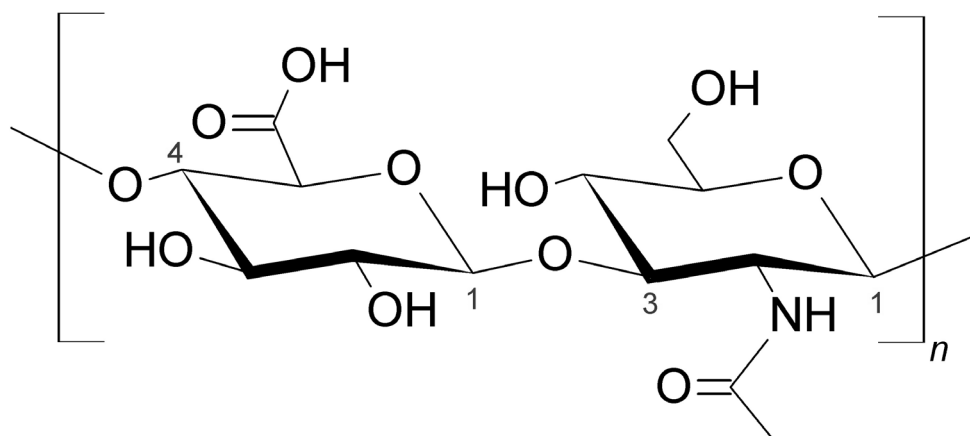


Fig. 1. Chemical structure of HA, composed of alternating residues of β -D-(1 $\rightarrow$ 4) glucuronic acid and β -D-(1 $\rightarrow$ 3) N-acetylglucosamine.

Hence, HA solutions are easily injectable. As such, HA is known as a “pseudo-plastic” material and its extraordinary rheological properties make it an ideal lubricant in several biomedical applications (24-27).

Synthesis and degradation of HA

Synthesis

Hyaluronic acid is a natural polymer biologically synthesized and secreted with a highly controlled continuous process by different type of cells in the body (e.g. fibroblasts, keratinocytes, or chondrocytes) by a unique enzymatic process. The biosynthesis of HA is regulated by three transmembrane glycosyltransferase isoenzymes called HAS1, HAS2 and HAS3. These isoenzymes possess two enzymatic components called glycosyltransferases. The mechanism of HA synthesis involves chain extension by addition of a monosaccharide, or better by alternating addition of GlcUA and GlcNAc to the reducing end of the chain (28). HAS3 is thought to be more active than HAS1 and HAS2, both of which produce high molecular mass HA molecules (3.9×10^6 Da), and it synthesizes shorter forms of HA molecules ($<3 \times 10^5$ Da).

Degradation

After injection into skin and joints, the half-life of HA is no longer than 24 hours, since HA is naturally degraded in the organism by a complex mechanism that can occur both via enzymatic or non-enzymatic reactions.

The enzymatic reactions involve three types of enzymes: hyaluronidase, β -D-glucuronidase, and β -N-acetylhexosaminidase. Throughout the body these enzymes are found in various forms, intracellularly and in serum (29). The mechanism of degradation changes in the three cases: hyaluronidase breaks high molecular weight HA (HMWHA) into smaller fragments, while β -D-glucuronidase, and β -N-acetylhexosaminidase degrade the fragments by removing non-reducing terminal sugars.

HA can also be naturally degraded in the organism by reactive oxygen species (ROS) with a mechanism that changes depending on the species involved (23,30,31).

HA degradation by non-enzymatic mechanisms also includes shear stress or thermal stress. The use

of ultrasonication showed that HMWHA chains degraded slower than the low molecular weight (LMWHA) ones. Rheological studies showed that HA solution degradation and decrease of viscosity are linked exponentially as a function of temperature. Other non-enzymatic degradation systems are chemical reactions such as acidic or alkaline hydrolysis. Acidic hydrolysis degrades the glucuronic acid moiety while alkaline hydrolysis occurs on GlcNAc units. Both these types of degradation often lead to the production of disaccharide fragments (23,32).

Distribution and functions within the ECM

Hyaluronan is widely distributed within epithelial, neural and connective tissues of vertebrates, and is one of the main constituents of the ECM in the human body. Long considered just as an inert molecule of the extracellular space, HA has been gradually revalued as a versatile and active molecule, playing several biological roles within the ECM, which encompass cell proliferation, differentiation, migration, adhesion and signaling (33,34). Interestingly, depending on its fragment size (in terms of molecular weight) and location, HA shows opposing biological roles within a certain tissue, such as pro- or anti-inflammatory effects, activation or inhibition of cell division, differentiation and migration (35). For example, HMWHA possesses space-filling, anti-angiogenic and anti-inflammatory activities, and is able to inhibit cell differentiation contrary to LMWHA, which is a pro-inflammatory, immunostimulatory and angiogenic polymer (9,36), which can be detected particularly in tumors. It has been reviewed that hyaluronan chains are involved in ovulation, embryogenesis, protection of epithelial layer integrity, wound repair and regeneration (9), thus showing the extraordinary range of biological activities of this glycosaminoglycan polymer. In virtue of its high molecular weight and ability to retain great amounts of water, HA is centrally involved in water homeostasis. Therefore, hyaluronan main role in the body consists in maintaining tissue architecture and hydration, creating loose hydrated matrices that permit cells

to divide and migrate during development (37) and the adhesion of immune cells (37,38). In fact, HA keeps the extracellular space in an open and hydrated state allowing cells and other ECM components to be supported and firmly held in the surrounding microenvironment (20). In addition, in the ECM microenvironment HA is known to interact with and retain several growth factors, cytokines, cell-surface receptors and to help the absorption of bioactive molecules at mucosal tissue interfaces (36, 39).

Several types of vertebrate cells can synthesize hyaluronan during the life of many mammalian species. This polysaccharide is indeed present in all organs and body fluids of different species, from rats, rabbits and humans. In the latter, it is distributed at diverse concentrations in various tissues and organs (Table I), such as umbilical cord, synovial fluid, dermis and epidermis (being a major component of the skin), vitreous fluid of the eye, the brain, thoracic lymph and at very low amounts ($\mu\text{g/g}$), within urine and plasma (serum) (40). In adult tissues, synthesis of HA is particularly active in derivatives of the mesodermal lineage, including joints and articular cartilage, where it mediates viscoelastic properties of synovial fluid; in connective tissues throughout the body, as an essential structural component, such as loose connective tissue surrounding muscle bundles (thus providing a lubricant on the surface of muscles and in the endomysium surrounding individual muscle fibers) and in perivascular and perineural

connective tissue (40-45). For instance, into both joints and eyes, specialized HA-secreting fibroblast-like cells of monocyte/macrophage origin have been identified, termed synoviocytes and hyalocytes, as the source of HA released in the synovial and the vitreous fluid, respectively (44, 46).

Cell response to HA

Hyaluronan regulates several physiological processes depending on its interaction with cell surface receptors types. In particular, HA binds cell surface receptors called hyaladherins, such as cluster determinant 44 (CD44), receptor for hyaluronic acid mediated motility (RHAMM), lymphatic vessel endothelial hyaluronan receptor (LYVE-1), hyaluronan receptor for endocytosis (HARE) and toll-like receptor 4 (TLR4) (47).

Cluster determinant 44 (CD44)

CD44 is a transmembrane cell surface receptor subject to extensive alternative splicing. All CD44 isoforms contain a hyaluronan binding site on the extracellular domain while intracellular domain interacts with cytoskeletal protein (48). The transduction pathway of the CD44 receptor signaling involves Ca^{2+} mobilization (49), Rho signaling (50), PI3-kinase/AKT activation (51) and cytoskeletal function (49-52). The CD44 interaction with hyaluronan is implicated in a several physiological events like cell-cell and cell-ECM adhesion, cell migration, cell proliferation, hyaluronan uptake and degradation and tumor progression (8).

Hyaluronan is also implicated in embryonic development by interaction with CD44 receptor. HMWHA promotes CD44 association with MEK kinase 1 (MEKK1), inducing MEKK1 phosphorylation and activating ERK-dependent and NF κ B-dependent pathways, promoting epithelial mesenchymal transition and differentiation of epicardial cells (53).

HA-CD44 signaling pathway is associated with ERK signaling transduction pathway and induce activation of downstream molecules such as cyclin D1 and E2F, regulating cell cycle progression (54).

Hyaluronan oligomers are involved in angiogenesis by interaction with CD44 and induce endothelial cell proliferation and tube formation *via* phosphorylation

Table I. Relative amount (ppm, i.e. μg per gram tissue) of HA in different human tissues, organs or body fluids.

Tissue/Organ/Fluid	Concentration (ppm)
Umbilical cord	4100
Synovial fluid	1400 – 3600
Dermis	200
Epidermis	100
Vitreous fluid (eye)	140 – 338
Brain	25 – 115
Thoracic lymph	8.5 – 18
Urine	0.1 – 0.3
Plasma (serum)	0.01 – 0.1

Adapted from (22, 40).

of FAK and ERK1/2 proteins, resulting in upregulation of c-Jun and c-Fos (55).

Interaction of fragmented hyaluronan with CD44 receptor is also implicated in inflammatory regulation. CD44-HA signaling plays a role in host defense through activation, homing, rolling, and extravasation of lymphocytes into inflammatory sites (56). Alveolar macrophages uptake hyaluronan *via* CD44 receptor and induce HA endocytosis and delivery to the lysosomes and subsequently its degradation (57, 58). HA fragments and HA oligomers can interact and stimulate dendritic cells to produce proinflammatory cytokines (59, 60).

Receptor for Hyaluronic Acid Mediated Motility (RHAMM)

RHAMM is an ubiquitous intracellular and cell plasma membrane protein (61, 62). Hyaluronan can bind cell surface RHAMM and induces kinase activity of c-Src, FAK and PKC, probably as a co-receptor for integral membrane proteins (63). On the other hand, intracellular RHAMM can bind kinases, calmodulin or cytoskeletal components (actin filaments, podosomes, microtubules and mitotic spindle) (48, 64) inducing cytoskeletal rearrangement (48, 61, 64). Intracellular RHAMM can bind directly to ERK1 and it can form complexes with ERK2, MEK1 and ERK1/2 substrates controlling activation and targeting of ERK1/2 to specific substrate. Proteins that are phosphorylated on tyrosine as a result of hyaluronan-RHAMM interactions include paxillin, cortactin, FAK and MAPK ERK1/2 (65). RHAMM expression can be downregulated by tumor suppressor p53 (66).

Hyaluronan promotes spreading of cell lamellae and focal adhesion turnover in fibroblasts through cell surface RHAMM. In this way cell surface RHAMM co-regulates migration of thymocytes on fibronectin together integrin receptor (67).

Cell surface RHAMM is essential for the activation of protein tyrosin kinase cascades in endothelial cells in response to hyaluronan (65).

Lymphatic Vessel Endothelial Hyaluronan Receptor-1 (LYVE-1)

LYVE-1 is an endocytic receptor of hyaluronan and is expressed in the lymphatic endothelium.

LYVE-1 is a homolog of CD44 receptor, it is a type I membrane protein containing a link domain similar to the proteolytic hyaluronan binding domain of the link protein superfamily (68) and played an important role in LMWHA induced lymphangiogenesis. Yu et al. demonstrate that LMWHA induces an increase of the colocalization of S1P3 protein (sphingosine 1-phosphate receptor) and LYVE-1 on the cell surface. These results raising the possibility that S1P3 may interact with LYVE-1 and participate in LMWHA mediated lymphangiogenesis (69). Du et al. demonstrate that LYVE-1 ligands, such as HA, induce opening of lymphatic intercellular junctions, allowing the macromolecules or cells transition from interstitium into lymphatic vessels, suggesting that LMWHA interaction with LYVE-1 facilitates tumor cell invasion into lymphatic vessels (70).

Hyaluronan Receptor for Endocytosis (HARE)

HARE, also known as stabilin-2, is a transmembrane receptor protein containing a C-type lectin-like HA binding module. HARE is highly expressed in the sinusoidal endothelial cells of liver, lymph nodes, spleen and bone marrow, and it is involved in the normal turnover of hyaluronan and other GAGs. HARE can bind and endocytose hyaluronan *via* clathrin-coated pit pathway inducing hyaluronan degradation in the lysosome (71). Pandley et al. found that HARE binds only to some sizes of HA (40-400 kDa) and can estimate cell signaling by activation of MAPK ERK1/2 and NF- β mediated gene expression (72).

Layilin

Layilin is a type I transmembrane protein with an extracellular domain homologous to C-type lectins and binds hyaluronan through its lectin-like domain (73). After layilin interaction with HA, intracellular domain of layilin interacts with merlin (protein of the Ezrin/Radixin/Moesin (ERM) superfamily) and modulates cell cytoskeletal structure (74). Layilin binds specifically to HA, but not to heparin or chondroitin sulfate, suggesting a role for layilin in processes in which HA is known to be involved, including cell adhesion and motility. The identification of HA as a ligand for layilin defines

this talin-binding partner as a novel member of the diverse family of HA-binding proteins and should facilitate attempts to find the true biological role for layilin (73).

Toll-like receptors 4 (TLR4)

In addition to binding with CD44, HA also binds TLR4, which is a component of the innate immune system (75, 76). TLR4 is widely distributed in the gastrointestinal tract and participates in mediating the host response to commensal and pathogenic bacteria. HA binding to TLR4 promotes epithelial proliferation and homeostasis in radiation-induced colitis model in mice (75), where HA-TLR-4 interaction induces an increase of cyclooxygenase-2 expression and prostaglandin synthesis. HA, as well as TLR4 and TLR2, is necessary to sustain basal NF- κ B activation and prevent epithelial cell apoptosis (77). TLR4 signaling occurs through the activation of NF- κ B protein by myeloid differentiation factor 88 (MyD88) dependent pathway or through TIR-domain-containing adapter-inducing interferon- β (TRIF), which leads to interferon regulatory factor 3 activation (78).

In the joints of rheumatoid arthritis (RA) patients, exogenous and endogenous TLR ligands have been identified, and in synovial fibroblast cells TLR3 and TLR4 are highly expressed. HA suppresses the expression of matrix metalloproteinases (such as MMP-1 and MMP-3) in RA synovial fibroblasts (RSF) *via* TLR 4 binding (79).

Synthesis of HA derivatives

Thanks to its chemical structure composed of repeating disaccharide units of D-glucuronic acid and N-acetyl glucosamine linked by β (1,4) and β (1,3) glycosidic bonds, HA offers numerous strategies for the synthesis of its derivatives (20). In particular, the chemical modifications of HA can be performed by targeting the two available functional moieties of HA: the carboxylic acid group and the hydroxyl group. While in the first case it is well known the position of the targeted site, in the second case this is not obvious due to the presence of three secondary and one primary hydroxyl groups. However, it is reasonable to assume that the primary hydroxyl

group in C6 of the N-acetyl glucosamine unit of HA is more reactive compared to the others because of the better accessibility of reagents to primary alcohols.

So far, several methods for the synthesis of HA derivatives and HA-based hydrogels have been published and reviewed (20, 80-85). Most of functionalization reactions are performed in an aqueous environment. However, when the reagents used for the modification are sensitive to hydrolysis, the derivatization reaction can be conducted in polar organic solvents such as dimethyl sulfoxide (DMSO) and dimethylformamide (DMF). In this case, the pristine HA sodium salt is often transformed into the tetrabutylammonium (TBA) salt to be solubilized in such solvents (86-88).

Modification of the -COOH

One of the most widely employed method for the modification of carboxylic group consists in the use of water soluble carbodiimides (89). This class of compounds catalyzes a coupling reaction with primary amine to form stable amide groups. Among the available carbodiimides, the most frequently used is 1-ethyl-3-[3-(dimethylamino)-propyl]-carbodiimide hydrochloride (EDC·HCl) thanks to its high solubility in water. In order to improve efficiency or create dry-stable (amine-reactive) intermediates, *N*-hydroxysuccinimide (NHS) or its more water-soluble analogue (Sulfo-NHS) is often included in EDC coupling protocols. This reaction is rather delicate and pH-sensitive and proceeds through two main steps: firstly, the reaction starts with the activation by EDC of the HA carboxylic acid, which forms an O-acyl isourea intermediate. Then, the second step consists of the nucleophilic attack by the amine on the activated HA, which leads to the formation of the amide bond. This reaction is generally conducted in slightly acidic buffer (pH = 3.5÷4.5) devoid of extraneous carboxyl and amine groups. For this reason, MES buffer (4-morpholinoethanesulfonic acid) has become the first-choice buffer to carry the EDC/NHS catalyzed coupling reaction. Due to its simplicity and the mild conditions required, this strategy has been used for the synthesis of a large number of HA derivatives. Primary amines (86, 90) (e.g. hexamethylene diamine

or cystamine), vinyl groups (91) (e.g. amino ethyl methacrylate – AEMA), azide and alkyne groups (92-94) and thiol groups (95,96) are the most common moieties introduced along HA chains using the EDC/NHS reaction. Such derivatives are then generally employed (using different chemical approaches such as thiol-ene reactions (97), click-chemistry (98-100), UV photocuring (101,102), etc.), as precursors for the synthesis of HA based hydrogels in which living cells, drugs, micro/nano-particles and bioactive compounds can be easily encapsulated.

The EDC/NHS coupling reaction can be also used to promote the so called auto-crosslinking reaction in which the carboxyl groups of HA react with its own hydroxyl groups to form intermolecular ester bonds (103, 104). The auto-crosslinking reaction is conducted in a non-solvent (generally mixtures of acetone or ethanol and water 8:2 v/v) in which pre-formed, dried HA scaffolds are immersed and left to react for 1 or 2 days. Compared to other methods of HA hydrogel synthesis, the auto-crosslinking reaction is particularly interesting as the final hydrogel network is composed exclusively of chemically linked pristine HA chains.

Modification of the –OH

The hydroxyl groups of HA can be also targeted to prepare value-added HA derivatives. Epoxides and anhydrides are the most common classes of compounds that readily react with –OH groups to form respectively new ether and ester bonds. These reactions are generally conducted in strong alkaline conditions (pH>10) to deprotonate the hydroxyl groups (pKa ~ 10) which thus become more nucleophilic compared to the deprotonated carboxylic groups.

So far, epoxides such as glycidyl methacrylate (105, 106) have been used to introduce vinyl moieties along HA chain enabling further UV photocrosslinking of the HA derivative, while bis-epoxides such as poly(ethylene glycol) diglycidyl ether (PEGDE) (107,108) or butanediol-diglycidyl ether (BDDE) (109-111) have been successfully used as crosslinking agents to directly create stable hydrogels. It is worth mentioning that BDDE-based crosslinking approach is FDA approved and is used in most of HA hydrogels currently on the market as the HA–BDDE degradation

products have not demonstrated any cytotoxicity and epoxide compounds are hydrolyzed into simple diols (110).

Another common reaction that targets the hydroxyl groups of HA is the esterification with anhydrides, in particular with methacrylic anhydride (112, 113). This reaction is generally conducted in ice cold water at pH = 8÷10 and leads to a HA derivative bearing methacrylic moieties that, as in the aforementioned case with glycidyl methacrylate, can be further use to prepare UV photo-crosslinkable hydrogels. An interesting feature of this reaction consists in the fact that by simply tuning the amount of methacrylic anhydride, it is possible to tune the final degree of substitution and consequently, the physical, chemical and mechanical properties of the resulting hydrogels can be precisely tailored (113).

In addition to the reactions with epoxides and anhydrides, the proximal hydroxyl groups of D-glucuronic acid unit of HA can be oxidized with sodium periodate to form dialdehydes, thereby opening the sugar ring (114,115). This interesting method has been used mainly for grafting peptides onto the aldehyde groups in order to improve the properties of the final materials in terms of cell adhesion, spreading and differentiation (116). The oxidized derivatives of hyaluronic acid have been also tested together with dihydrazide compounds as injectable, in situ forming hydrogels for *nucleus pulposus* regeneration (115).

Hyaluronic acid formulations

Thanks to its unique physicochemical properties and biological functions, HA and HA derivatives have been extensively investigated and widely used for pharmaceutical and medical applications, such as the treatment of arthritis (117, 118), ophthalmic surgery (119) and drug delivery (120). The successful results obtained by its medical use have led to the production of several commercial HA products, which have been extensively described elsewhere (121).

Dermal fillers

HA's peculiar features, such as its ability to bind to large amounts of water, its natural presence in the skin and its low potential for adverse reactions, have

made it an attractive candidate for dermal filler use (4). Starting from this basis, several HA dermal fillers have been produced by modifying: type of crosslinker, degree of crosslinking, gel hardness, viscosity and total HA concentration, with the aim to combine ease of injection with long life and efficacy as a filler.

In December 2003, the first HA product was approved in the United States (Restylane; Medicis Aesthetics Holdings Inc.) for the use in cosmetic surgery, and was soon followed by other commercial dermal fillers (e.g. Hylaform®, Restylane® and Dermalive®), either in a stabilized form or in combination with other polymers (122). The injection of such products into the dermis, is reported to reduce facial lines and wrinkles in the long term with fewer side-effects and better tolerability compared with other commonly used fillers (e.g. collagen) (123).

Drug delivery

HA has been investigated as a drug delivery agent for various routes of administration both parenteral and non-parenteral. The latter application mainly includes ocular (124) and nasal (125,126) delivery systems. Thanks to its muco-adhesive properties, HA forms a film at the cornea surface protecting the loaded drug and creating a reservoir that releases the drug slowly, thus increasing drug retention and efficiency (127). On the other hand, its use in parenteral systems has reached relevance for the sustained release of protein drugs through subcutaneous injection (120). In addition, more novel applications including the specific intracellular delivery of genes or anticancer drug have also been suggested (128). Indeed, when injected intravenously, HA targets specific receptors including CD44, thus selectively carrying drugs to areas where the concentration of these receptors is elevated (129).

Tissue engineering

On the basis of these encouraging results, HA and its derivatives have been extensively used as medical products on the clinical practices and, more recently, have become recognized as an important building block for the development of new biomaterials for tissue engineering and regenerative medicine applications (4). In this framework, HA has been the focus of active research especially as coating of

biomedical devices as well as in the form of scaffolds or cell-laden hydrogels for *in vitro* cell culture or *in vivo* cell carrier.

Hyaluronic acid offers great practical potential as a scaffolding material since a range of crosslinking techniques can be adopted, both to enhance the material residence time as well as to control its mechanical properties (130). Furthermore, since the viscosity of the injectable derivatives can be easily controlled by the molecular weight of the HA used (131), the recent discovery of HMWHA in naked mole rats has opened a new arena in HA-based tissue engineering. In addition to chemical modifications, new compositions such as colloid mixtures of micro/nanoparticles with HA macromers or dual network hydrogels consisting of HA macromers and other polymers—are also expanding the multiplicity of HA hydrogel formulations (132). For example, HA solutions have been recently mixed with ceramic compounds (e.g. bioactive glass nano-particles or β -tricalcium phosphate) to create novel paste-like bone-substitute materials. Furthermore, beside their use in hydrogel formulations, hyaluronic acid derivatives have been also manufactured into many other forms such as highly porous and fibrillar sponges, electrospun fibers, non-woven meshes and flexible sheets (103, 135). A complete overview of therapeutic applications of HA-based scaffolds in tissue engineering and regenerative medicine has been reported in some notable review articles (127, 130).

CONCLUSIONS

Due to its unique combination of favorable physico-chemical and biological properties, HA shows a plethora of applications in the surgical, tissue engineering, and drug delivery fields. Indeed, this intriguing naturally occurring biomaterial is involved in numerous cell signaling pathways, concerning regenerative processes of different tissues and organs. The easily handleable chemistry of HA has opened the way to several derivatization routes which, in turn, have led to the development of different HA formulations for applications spanning from fillers to tissue engineering scaffolds and drug delivery systems.

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THE USE OF HYALURONIC ACID IN THE TREATMENT OF SHOULDER CAPSULITIS: A SYSTEMATIC REVIEW

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Adhesive capsulitis (AC) is a common pathological condition of the shoulder characterized by painful restriction of range of motion (ROM) of the glenohumeral joint. Currently, no consensus has been reached regarding the best treatment. Hyaluronic acid (HA) injection is a safe procedure that can result in significant improvement in active and passive ROM, alleviating pain and improving shoulder function. We systematically reviewed current literature in order to evaluate the best evidence about the effectiveness of intra-articular HA injection for the treatment of primary AC. We searched Medline, CINAHL, Embase, Google Scholar and Cochrane Library. We selected studies comparing clinical outcomes of patients treated with HA in association or not with conventional therapy. Seven studies were evaluated: 2 uncontrolled randomized studies and 5 prospective randomized clinical trials with level of evidence I. Clinical outcome measures used included, among other, ROM, Visual Analogic Scale (VAS) pain scores, Constant score, Activity of daily living, Shoulder Pain and Disability Index (SPADI), American Shoulder and Elbow Surgeons (ASES) and Japanese Orthopedic Association Score (JOA score). Improvement was noted in terms of ROM, constant scores and pain in patients affected by AC treated with intra-articular HA injections. When compared with cortisone intra-articular injection, HA has equivalent clinical outcomes and ROM. The heterogeneity of treatments used in the studies reviewed, makes it difficult to draw a definite conclusion on the subject. HA injections do not seem to determine the final outcomes directly compared with conventional treatments. However, they could play an important role for early mobilization in the initial stages, during which, due to pain and inflammation, the patient keeps the shoulder immobilized for a long time, determining the direct cause of AC. Numerous variables, including use of lidocaine, different HA and AC stages, could influence the results and deserve to be accounted for in future investigations.

Adhesive capsulitis (AC) can be defined as a pathological condition of the shoulder characterized by painful restriction of range of motion (ROM) of the glenohumeral joint, mainly in external rotation (1). Despite the disease progresses through fibrosis and terminate in joint contracture (2), it is generally considered as a self-limiting process (3) with an etiology that remains still unclear (2).

About 2% to 5% of the population suffer from AC, with women (4) aged between 40- and 60-years-old most commonly affected (5) and the non-dominant hand more frequently involved (6, 7). About 20% to 30% of patients are likely to develop the condition in the opposite shoulder (2, 4).

The pathogenesis of primary AC is still poorly understood, but idiopathic inflammation of the lining

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of the shoulder joint may reasonably be ascribed as the primary cause (8, 9). Beyond the primary AC, there are a variety of diseases that may be associated with this pathological condition, including diabetes mellitus (10), thyroid dysfunction (11) and autoimmune diseases, breast cancer treatment (12), stroke, or myocardial infarction (2).

Many treatments have proven to be effective in relieving pain and restoring shoulder ROM and function (13, 14). Non-operative measures include pharmacological treatment of the synovitis and inflammatory mediators and physical modalities aimed to prevent or modify capsular contracture, among these: NSAIDs, oral and injected corticosteroids, hyaluronic acid (HA) injection and physiotherapy (15, 16). In patients refractory to conventional therapies, more invasive procedures such as capsular distention, manipulation under anesthesia and arthroscopic capsular release have been carried out (8). Currently, no consensus has been reached regarding the best treatment (13, 17).

HA is an integral component of the synovial fluid and is crucial for the chondroprotection of joints (18). When HA is injected into a joint, cytokine-induced responses and synovial inflammation decrease, ultimately mitigating pain and increasing joint function. Also lubrication, chondro-protection and improved synovial fluid characteristics have been observed (19). In addition to the reduced inflammation, injection of HA into AC affected glenohumeral joint is associated with subsequent improvement of shoulder ROM (20). The treatment of knee osteoarthritis with intra-articular HA injection can also prove the potential efficacy of the visco-supplementation (21-30). Nevertheless, the advantages of HA injection in the treatment of AC have not been universally accepted, due to controversial evidence (31, 32).

The aim of this systematic review is to evaluate the best evidence about the effectiveness of intra-articular HA injections in the treatment of primary AC. We could conclusively hypothesize that glenohumeral HA injections could result in significant improvements in active and passive shoulder ROM, alleviating pain and improving shoulder function.

MATERIALS AND METHODS

The systematic literature review was formatted and based on guidelines suggested by "The PRISMA Statement for Reporting Systematic Reviews and Meta-Analyses of Studies", which seeks to provide a method of producing more standardized and complete systematic reviews (33).

Search protocol

A first search was performed through online databases, to retrieve articles concerning the research topic. Medline, PubMed (<http://www.ncbi.nlm.nih.gov/pubmed>), Cochrane (<http://www.thecochranelibrary.com/view/0/index.html>) and Google scholar (<http://scholar.google.it/>) were searched through to the 24th of February 2017. Search-terms and combinations of these were: "adhesive capsulitis," "frozen shoulder," "stiff shoulder," "periarthritis," "Duplay's disease," "hyaluronic acid," "sodium hyaluronate" and "viscosupplementation" as keywords.

All type of studies published in peer reviewed journals were firstly retrieved. Unpublished trials were searched using the ClinicalTrials.gov registry (<https://clinicaltrials.gov/>). After consulting the title and the abstract of the articles, only clinical randomized trials, prospective and retrospective cohort studies, cross-sectional studies on live human subjects were selected for the review.

Study inclusion criteria

Inclusion criteria were: English, Spanish, French and German language studies, Human subjects, publication dates from the last 30 years, studies investigating treatment of primary AC or with diabetes mellitus, studies investigating intra-articular glenohumeral or joint HA injection, studies reporting clinical outcomes following intra-articular glenohumeral joint HA injection and level I, II, III, IV evidence studies.

Exclusion criteria were: Level V evidence; non-English, Spanish, French and German language studies; non-human or cadavers subjects; studies investigating treatment of nonadhesive capsulitis causes of shoulder pain or mixed diagnosis; studies investigating treatment of secondary causes of AC (ex-diabetes mellitus).

Study selection protocol

The whole bibliography of the studies eligible for

inclusion was accurately screened to identify any studies missed through online searching. Of these, the full-text articles were carefully read and evaluated by two different reviewers (A.T. and R.P.) who discussed whether a study should be included or not. Relevant data reported by the studies were extracted and summarized into tables in order to provide reviewers with a systematic overview of the papers included. The studies inclusion process is shown in Fig. 1.

RESULTS

Election of the studies

One hundred and twenty two studies were found during the first electronic search. Six more studies were identified as relevant by searching the bibliography of the eligible articles by hand. Finally, 7 studies were included for the present review.

Five articles (1, 20, 32, 34-37) reported on HA injection in patients with AC, 5 articles (1, 32, 35-37) were randomized controlled trials with an evidence level of I, while 2 articles (20, 34) were prospective uncontrolled study with an evidence level of IV. Ten

eligible full-text articles were assessed and excluded because mixed diagnosis in the experimental group was found (osteoarthritis, Duplay's disease, rotator cuff tear) or primary aim of the study was not the treatment of AC or reported poor outcomes.

Study size and follow up

A total of 429 shoulder (in five patients of the Calis et al. (1) study, the disease was bilateral) were treated and enrolled to take part into studies reviewed by our research team. The reported males were 146 (35.5%) and the reported females 278 (64.5%). the mean age at intervention was 56.6 years, ranging from 53.6 (35) to 65.4 (34). All other demographic data are summarized in Table I.

Treatment of adhesive capsulitis

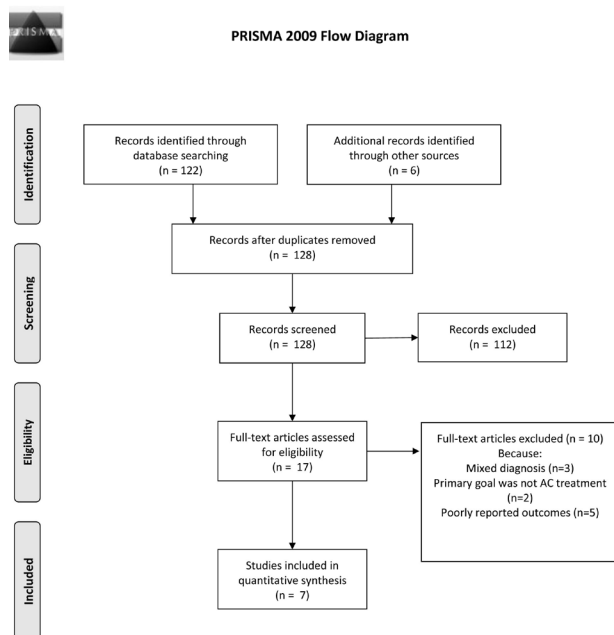
The injection site (intra-articular) was the same for all patients but different types of HA with different molecular weight were used in the enrolled studies. While the major part of studies used high molecular weight HA, Lee et al utilized one with a low molecular weight. Park et al (37) and Hsieh et al (32) didn't specify the type of molecular weight used (Table II).

All studies, except for a subgroup of Itokazu et al. (34) that used subacromial bursa as site of infiltration, used intra-articular as injection site but they differ from the anterior (36) or posterior (1, 20, 32, 34, 36, 37) approach to the shoulder and the use of ultrasound or anatomical technique (1, 20, 32, 34, 36).

In the randomized controlled trials analyzed, the control group was composed by physical therapy, corticosteroid injection or only home stretching and Codman's exercise (1). Details are provided in Table III.

Outcome measures and data

To evaluate the clinical outcomes of the best treatment of AC, most studies used validated functional scores (Table IV). The most utilized in the reviewed papers was ROM, assessed in 6 studies (1, 32, 34-37) evaluated in terms of degrees of flexion, abduction, internal and external rotation. While VAS was analysed in 3 studies (1, 35-37), Shoulder Pain and Disability Index (SPADI) was evaluated by Park et al. (37) and Hsieh et al. (32). Other used



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Fig. 1. *Studies inclusion process.*

scores were: Constant score, American Shoulder and Elbow Surgeons (ASES) and Japanese Orthopaedic Association shoulder score (JOA score). A detailed outcome data is represented in Table V.

Complications

From the data obtained in the analyzed papers, we can assume that HA injection could be considered a safe procedure, indeed no adverse events or complications occur after injection in all studies.

Only Park et al. (37) reported 12 patients that felt pain during capsular distention after procedure but not during the injection.

DISCUSSION

The aim of this systematic review is to evaluate the best evidence about the effectiveness of intra-articular HA injections in the treatment of primary

Table I. Demographic data.

Study	Year	Level of evidence	Number of shoulders	Male / Female	Mean age (years)	Duration of symptoms (months)	Follow-up duration (weeks)
Calis et al. ¹	2006	I	95 (5 bilateral)	33/57	56.9	>1	12
Itokazu et al. ²⁰	1995	IV	62	28/34	65.4	4.7	9.2
Lee et al. ²⁷	2009	I	40	19/21	53.6	9.5	6
Tamai et al. ²⁰	2004	IV	11	5/6	56	7	6
Hsieh et al. ²⁴	2012	I	63	PT group: 8/23 HAPT group: 12/20	PT group: 56.4 HAPT group: 52.6	4.5	12
Lim et al. ²⁸	2014	I	68	19/49	53.8	7.3	26
Park et al. ²⁹	2013	I	90	Group A: 12/33 Group B: 10/35	Group A: 55.23 Group B: 56.33	Group A: 5.31 Group B: 5.33	6

GH: Glonohumeral; **SH:** sodium hyaluronate; **SS:** Saline Solution; **HA:** Hyaluronic acid; **IA:** intra-articular; **nr:** not reported.

Table II. HA preparation.

Study	HA preparation
Calis et al, 2006 ¹	30 mg (HMW; 1000–2900 kDa)
Itokazu et al, 1995 ²⁰	25 mg HMW (800-900 kDa)
Lee et al, 2009 ²⁷	25 mg LMW (molecular weight not reported)
Tamai et al, 2004 ²⁰	HMW (800 kDa)
Hsieh et al, 2012 ²⁴	20 mg (molecular weight not reported)
Lim et al, 2014 ²⁸	10 mg (3000 kDa)

LMW: Low molecular weight; **HMW:** High molecular weight; **mg:** milligrams; **kDa:** Kilodaltons.

Table III. *Type of treatment.*

Author and year	Diagnostic Tools	Treatment groups	Injection(s)	Injection guidance	Injection site
Calis et al, 2006 ¹	History + PE	GH joint HA injection versus GH joint triamcinolone injection versus Physical therapy only versus Control (home stretching. Codmans)	HA weekly (2) versus one-time triamcinolone (1)	Anatomy (posterior approach)	IA
Itokazu et al, 1995 ²⁰	History + PE	GH joint SH injection	Weekly HA (5)	Anatomy	Glenoid cavity (42) Subacromial bursa (20)
Lee et al, 2009 ²⁷	History + PE + RX + US	Ultrasound-guided GH joint injection versus Blind GH joint injection technique	Initial triamcinolone (1), followed by weekly HA (5)	US (posterior approach)	IA
Tamai et al, 2004 ²⁰	History + PE	GH joint HA injection	Weekly HA (5)	Anatomy (posterior approach)	IA
Hsieh et al, 2012 ²⁴	History + PE	Group 1 GH joint injections of HA, 20 mg, and also PT program for 3 months. Group 2 received PT alone	Weekly HA (3)	Anatomy (posterior approach)	IA
Lim et al, 2014 ²⁸	History + PE	Blind GH joint injection technique	One shot of methylprednisolone acetate + lidocaine VS 3 times weekly HA GH injection	Anatomy (anterior approach)	IA
Park et al, 2013 ²⁹	History + PE + US	Group A: 0.5% lidocaine plus triamcinolone 40mg IA injection Group B 0.5% lidocaine plus hyaluronic acid 20 mg and capsular distension	Every 2 weeks (3)	US (posterior approach)	IA

CS: corticosteroid; **HA:** hyaluronic acid; **IA:** intra-articular; **F:** female; **M:** male; **PE:** physical examination; **PT:** physical therapy; **US:** ultrasonography.

Table IV. *Outcome measures.*

Study	VAS	ROM	ASES	SPADI	SF-36	JOA	ACTIVITY OF DAILY LIVING	CONSTANT
Calis et al. ¹		X						X
Itokazu et al. ²⁰		X						
Lee et al. ²⁷	X	X						
Tamai et al. ²⁰						X		
Hsieh et al. ²⁴		X		X	X		X	
Lim et al. ²⁸	X	X	X					X
Park et al. ²⁹	X	X		X			X	

AC. We identified 7 studies (1, 20, 32, 34-37) that reported clinical outcomes following treatment of AC with glenohumeral HA injection. Even though corticosteroid injection is one of the most commonly used method for management of adhesive capsulitis,

we suppose that glenohumeral HA injections, a relatively new method for the treatment of AC would result in significant improvements in active and passive ROM, alleviating pain and improving shoulder function.

Table V. *Outcome data.*

STUDY	OUTCOME	RESULTS		
Calis et al. ¹ 90 patients, 95 shoulders HA 27 shoulders CS 26 shoulders PT 22 shoulders Control 20 shoulders	ROM			
	Abduction	HA	Pre: 109.6±17.8	3 month:145.9±21.0
		CS	Pre: 117.8±23.6	3 month:150.3±19.6
		PT	Pre: 116.8±16.4	3 month:158.4±18.3
		Control	Pre: 114.2±22.1	3 month:133.5±15.3
	External Rotation	HA	Pre: 43.7±10.5	3 month:63.3±11.4
		CS	Pre: 47.3±12.5	3 month:63.0±10.8
		PT	Pre: 50.0±10.6	3 month:73.8±10.4
		Control	Pre: 45.7±9.0	3 month:55.0±8.1
	Constant	HA	Pre: 50.1±8.9	3 month:70.1±10.3
		CS	Pre: 54.6±9.7	3 month:70.3±9.9
		PT	Pre: 55.3±9.4	3 month:70.3±9.9
		Control	Pre: 51.2±12.1	3 month:61.2±10.8
Itokazu et al. ²⁰ HA 70 patients	ROM	Extension	Pre:33.4±14.7	Post:35.7±14.5
		Flexion	Pre:118.2±37.8	Post :130.0±35.8
		Adduction	Pre:14.6±16.0	Post :17.9±18.8
		Abduction	Pre:100.2±36.8	Post :116.0±35.6
		Internal rotation	Pre:63.8±26.8	Post:68.9±22.2
		External rotation	Pre:39.3±32.1	Post :47.9±31.9
Lee et al. ²⁷ HA 40 patients 20 US-guided injection 20 blind injection	VAS	US-guided	Pre:5.4±0.5	Week 6:0.9±0.3
		Blind	Pre:6.0±0.4	Week 6:0.9±0.3
	ROM Flexion	US guided	Pre:112.9±5.6	Week 6:160.2±5.3
		Blind	Pre:110.7±6.8	Week 6:157.2±5.9
	Abduction	US guided	Pre:106.7±5.9	Week 6:151.2±3.5
		Blind	Pre:101.1±6.3	Week 6:148.4±3.8
	External rotation	US guided	Pre:48.9±7.7	Week 6:79.2±4.6
		Blind	Pre:46.4±5.3	Week 6:77.6±4.5
	Internal rotation	US guided	Pre:31.2±3.5	Week 6:72.2±3.4
		Blind	Pre:28.7±3.4	Week 6:69.1±5.4
Tamai et al. ²⁰ HA 11 patients	JOA score (Japanese Orthopaedic Association shoulder scoring system)	Pre: 27±10	Post:29±12	
	CE (coefficient of enhancement)	Pre: 2.5±1.5	Post:2.1±1.0	

Hsieh et al. ²⁴ 32 patients HA plus PT 31 PT	Active ROM	Flexion=69.25, abduction =55.14, external rotation=25.79, and internal rotation=21.55.
	Passive ROM	Flexion=70.75, abduction=65.26, external rotation=34.57, and internal rotation =24.02.
	SDQ score	HAPT Pre:67.43±23.38 3 month:40.20±26.62 PT Pre:63.05±18.99 3 month:33.59±27.51
	SPADI score	HAPT Pre:54.06±20.47 3 month:32.47±19.75 PT Pre:54.84±21.67 3 month:26.61±20.60
Lim et al. ²⁸ 34 patients HA 34 CS	VAS	HA Pre:7.2±2.2 Week 12:3.9±2.4 CS Pre:7.2±1.8 Week 12:3.6±2.0
	ASES	HA Pre:29.2±15.3 Week 12:53.8±19.4 CS Pre:30.7±13.5 Week 12:58.4±17.3
	Constant	HA Pre:35.8±12.2 Week 12:52.3±18.7 CS Pre:39.9±14.9 Week 12:58.7±15.2
	ROM	HA Forward elevation Pre:98.6±5.1 Week 12:119.0±17.6 External rotation Pre:8.6±11.2 Week 12:26.6±16.1 Internal rotation Pre:L3-L4 Week 12:L1-L2 CS Forward elevation Pre:97.1±5.8 Week 12:122.4±18.2 External rotation Pre:13.2±7.7 Week 12:22.4±14.6 Internal rotation Pre:L3-L4 Week 12:T12-L1
Park et al. ²⁹ 90 patients HA 45 patients CS 45 patients	SPADI score	HA Pre:61.2±9.8 Week 6:21.90±6.80 CS Pre:61.7±9.4 Week 6:20.04±5.80
	VNS	HA Pre:6.1±1.0 Week 6:2.3±0.5 CS Pre:6.2±0.9 Week 6:2.2±0.4
	ROM Flexion	HA Pre:104.0±17.0 Week 6:144.30±15.20 CS Pre:103.6±17.0 Week 6:145.80±12.80
	Abduction	HA Pre:77.2±14.8 Week 6:115.00±12.90 CS Pre:76.5±14.7 Week 6:113.85±12.90
	External Rotation	HA Pre:23.7±8.8 Week 6:56.00±8.00 CS Pre:23.2±9.9 Week 6:44.50±7.70

HA has been playing an outstanding role in the last years in the treatment of countless diseases. It is a polyanionic, non-sulfated glycosaminoglycan distributed widely in the human body and has a wide range of applications with its excellent properties and characteristics and could be used even more

in medical applications. Its anti-inflammatory properties on synovitis have been proven by Tamai et al. (20) in his randomized uncontrolled study on 11 patients affected by AC. Moreover, Blaine et al. (38) and Leardini et al. (39) in their studies assert that HA is well tolerated and brings clinical

improvements for the treatment of osteoarthritis and persistent shoulder pain that is not solved by conventional therapy. However, it was not possible to include these studies in our review because of the heterogeneity of diagnoses and it was not possible to extrapolate data from individual groups analyzed.

In regards to the role of the ultrasonography (US) during the injection, Park et al. (37) and Lee et al. (35) compared the efficacy of US-guided intra-articular injections with blind technique. They showed that the US-guided technique provides reduction of pain, a better ROM improvement and general shoulder function compared to the blind technique.

Several studies evaluated conventional therapies and their relationship with HA injections in the management of AC. Hsieh et al. (32) and Rovetta et al. (40), tested the efficacy of HA as adjunct to Physical Therapy (PT). They compared the efficacy of intra-articular HA/corticosteroid injections plus physical therapy with that of physical therapy alone for the treatment of AC of the shoulder. These studies show an improvement in alleviating pain, disability, and quality of life after the injections of HA/steroid or only physical therapy; furthermore, the active and passive ROM improved with the duration of the therapy.

Calis et al. (1) and Park et al. (37), compared the efficacy of HA injections as alternative to other conventional treatment methods (steroid injections, physical therapy or home stretching). The results of both studies show that all the treatment groups (HA) improved significantly compared to the control group. While Park et al. (37) assert that HA injection gives the same effect as the steroid injection alone in alleviating pain and functional improvement, Calis et al. (1) underlined the importance of physical therapy as first line of treatment and the HA injection could be administered only as an alternative to physical therapy and steroid injections in AC treatment.

Lim et al. (36), carried out the only randomized controlled trial that compared the early clinical results of intra-articular injection of HA or corticosteroid in patients with idiopathic AC without the limitation of other substances (lidocaine and saline solution) or physical therapy in 68 patients. The study shows that intra-articular injection of HA and corticosteroid

produce a clinical improvement in alleviating pain and in the functionality of the ROM. They concluded that HA injection can be considered as an alternative treatment, and could be considered as a substitute of corticosteroid injection.

Three studies (20, 34, 38) investigated intra-articular HA injection in association with lidocaine, demonstrating an improvement of the patient conditions after injection. Although association with lidocaine could represent a limitation of these studies because the improvement of clinical conditions cannot be ascribed to lidocaine or HA injection.

Our review can be considered an implementation of the knowledge regarding the effectiveness of intra-articular HA injections in the treatment of primary AC. Contrary to previous reviews on this specific topic (18, 31), we included the first prospective randomized clinical trial (36) that compared early clinical results only of intra-articular injection of HA or corticosteroids in patients with idiopathic AC, without the limitation of other substances (lidocaine and saline solution) or physical therapy.

In our experience, we believe that HA injection does not seem to directly determine the outcomes compared with conventional treatments in the management of AC. We hypothesize that it could play an important role for the early mobilization in the initial stages of the disease during which, because of pain and inflammation, the patient has the shoulder immobilized for a long time; this specific condition could be the direct cause of adhesive capsulitis of the shoulder.

Various limitations need to be highlighted: enrolled participants differ with stage and severity of AC and age and duration of symptoms. Furthermore, different volumes/types of lidocaine, of HA (10, 20 or 30 mg) and corticosteroids were adopted and different physical therapy regimens were chosen. The approach is different among studies with US guided or not. Follow-up duration is very short considering that the maximum of follow-up in only one study (36) is 26 weeks.

CONCLUSION

The results of studies investigating HA injections

in patients affected by AC are encouraging, given the effectiveness in suppressing cytokine-induced responses and reducing synovial inflammation, alleviating pain and improving shoulder function. These specific features of HA could be detrimental in the initial stage of AC in which the patients keep for a long time the shoulder immobilized. However, currently available evidence is not strong enough to support the routine clinical use of HA injections in patients affected by AC.

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MENISCAL EXTRUSION AS BOOSTER OF OSTEOARTHRITIS

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Meniscal extrusion (ME) has shown to play a critical but still unclear role in osteoarthritis (OA) development. ME has been described as an important risk factor in the progression of knee OA, as it is involved in the thinning of articular cartilage, joint space narrowing, spontaneous osteonecrosis of the knee and subchondral bone marrow lesions. Meniscal damage of any degree of severity could cause ME in both compartments, but it is commonly associated with severe meniscal tears or root tears mainly in the medial meniscus. Magnetic resonance imaging is the most commonly used imaging modality in the assessment of ME, while ultrasonography may represent a valid alternative with high sensitivity and specificity. Conservative treatment for ME includes physical therapy and rehabilitation to maintain range of motion, corticosteroid injections and intra-articular injections of hyaluronic acid to provide short-term relief of knee pain. The goal of this study is to review standards of current diagnosis and treatment of ME and its relationship to knee OA.

Knee osteoarthritis (OA) is a major public health issue and causes chronic pain and disability among elderly patients in most of the developed countries. Epidemiologic studies have shown that knee OA affects over 250 million people worldwide, with relevant impact on health care and society, and its incidence is showing a growing trend with the increasing life span (1-3). OA is a common chronic disease characterized by capsule thickening, hyaline cartilage damage, inflammation of the synovium, degeneration of ligaments, hypertrophy of the subchondral bone, formation of osteophytes and meniscal tears and extrusion (4-6).

Meniscal extrusion (ME) has shown to play a critical but still unclear role in OA development (7-11). It is defined as significant medial displacement of the peripheral edge of medial meniscus beyond the central margin of the medial tibial plateau. It occurs when the external margin of the meniscus exceeds

the margin of the tibial plateau by >3 mm (12-17). The degree of extrusion is significantly related to meniscal degeneration and the most common extrusion is in the medial meniscus.

A review of the anatomy of the menisci is important to understand the mechanism of ME. The medial and lateral menisci are two fibrocartilaginous discs located between the surfaces of the femur and tibia in the medial and lateral compartments of the knee. It has been shown that medial ME is more common than lateral ME. In addition to the fact that the medial meniscus is the point of weight-bearing, can be explained by the difference in attachment to the capsule between the two menisci, that leads medial meniscus to be more prone to subluxation due to displacement of the medial capsule by fluid or osteophytes (18).

The main functions of menisci are absorption and transmission of the loads across the knee joint,

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and lubrication for joint movement preventing cartilage damage. Indeed, the axial load from the body weight is transferred by femoral condyles to the menisci, yielding their motion in the radial direction. Biomechanical studies have shown that at least 50% of the compressive load of the knee joint is transmitted through the meniscus in extension, whereas approximately 85% of the load is transmitted in 90° of flexion, reducing the mechanical stresses on cartilage surfaces and subchondral bone (18, 19).

It is well accepted that the loss of meniscal integrity reduces radial stiffness, causes radial displacement and increases the peak pressure in the knee joint. Significant meniscal extrusion may alter the weight-bearing capacities of the tibiofemoral compartments, leading to damage in the articular cartilage, as well as in the subchondral bone, ultimately contributing to progression of knee OA (13, 20, 21). Moreover, ME occurs more often in patients with knee OA than in normal control individuals, but it is important to demonstrate if it causes OA due to the overloading of the joint articular surface or the laxity of supporting meniscal structures associated with OA predisposes these patients to meniscal extrusion (20, 22).

The goal of this study is to review standards of current diagnosis and treatment of ME and its relationship with knee OA.

Risk Factors

Kenny was the first to quantify meniscal displacement, using the ratio of meniscal overhang to meniscal width, and noticed that radial displacement of the medial meniscus could be related to a loss of meniscal function (23, 24). Indeed, ME is commonly associated with severe meniscal tears or root tears that disrupt the hoop tension of the meniscus (12, 25, 26).

The posterior horn of the medial meniscus has a strong attachment to the tibia near the tibial spine, known as the meniscal root, which contributes to an increased frequency of tears in this region. Tears resulting in extrusion include meniscal horn/root tears, radial tears of more than 50% of meniscal width in size and large complex tears (11, 14-16). These result in loss of the ability to resist hoop strain and biomechanically overload the joint articular surface.

Several other factors, such as tibiofemoral cartilage

damage and knee malalignment, may be related to ME, even when no other meniscal pathologic findings are detected. Crema et al. (11) in a total of 2131 knees showed strong and significant associations between meniscal damage of any degree of severity and ME in both compartments ($P < 0.0001$), with higher grades of meniscal tears having stronger associations with ME in both compartments ($P < 0.0001$). Also, they demonstrated that root tears were strongly associated with ME in the medial compartment. ($P = 0.0002$). They proved that varus and valgus alignment are independent factors associated with, respectively, medial ($P = 0.005$) and lateral ($P < 0.0001$) ME. They found no evidence of an independent association between the presence of knee effusion nor increasing body mass index (BMI) and ME.

On the other hand, Ding et al. (22) proved that BMI, weight, tibial bone area at baseline, medial tibiofemoral cartilage defect score, prevalence of radiographic OA, medial joint space narrowing and osteophytes at baseline, loss of medial tibial cartilage volume and progression of tibiofemoral cartilage defect score, were greater in patients with medial ME (MME) compared with patients without ME (all $P < 0.0001$). They also showed that past knee injury could be associated with ME. Specifically, ME occurred in 13% of those with past knee injury versus 6% of those without past knee injury.

Choi et al. (15) evaluated 248 patients retrospectively, whom underwent arthroscopic meniscectomy for tears of the medial meniscus. The mean age was 53.5 (range 15 to 81 years). On a preoperative knee coronal magnetic resonance imaging, meniscal tears were classified by type (longitudinal, horizontal, radial, oblique, complex, and root tear). Of the 121 knees with minor extrusion (< 3 mm), 16 (13.2%) had a medial meniscal root tear, while 127 knees with pathologic extrusion (> 3 mm), 50 (39.4%) had a root tear. Thus, they found an association between pathologic ME and root tear ($P < 0.001$). They also showed that ME was significantly correlated with the severity of the chondral lesion ($P < 0.001$).

In a study by Costa et al. (16) magnetic resonance imaging (MRI) had been used as a diagnostic tool for measuring the extrusion of the medial meniscus,

quantified in millimeters. They showed that medial meniscus extrusion (>3 mm) is strongly associated with severe meniscal degeneration ($p=0.003$), extensive tear, complex tear ($p<0.001$) and tear involving the meniscal root ($p<0.001$), while longitudinal (non-radial) and horizontal tears were not associated with extent of extrusion ($p=1.0$).

Muzaffar et al. (14), in their retrospective study in a group of 102 patients with ME of 3 mm or more, determined the morphology of tears that are most likely to be associated with significant extrusion, showing that medial meniscal posterior horn tears occurred in 63 cases (64.26%), 21 cases were medial meniscal body tears (21.42%), 5 medial meniscal root tears (5.1%), 9 lateral meniscal body tears (9.18%) and 4 lateral meniscal posterior horn tears (4.08%).

Rennie et al. (18) compared the rate of ME between 40 knees of young athletes and 36 knees of the control subjects. In their study, 48% of the athletes' knees presented evidence of meniscal extrusion, that showed significant associations with joint effusion ($p=0.004$), meniscal tears ($p=0.01$) and anterior cruciate ligament (ACL) tears ($p=0.04$). On the other hand, 78% of the control subjects' knees had ME, but it did not present associations with joint effusion, meniscal tear, or ACL tear ($p=1.00$, 0.99 and 0.99 , respectively). Therefore, this significant correlation between meniscal tears and subluxation in athletes may be explained by the elimination of hoop stresses and greater or more sustained compressive forces generated by athletes within the knee due to meniscal tears.

Diagnosis

An early diagnosis of ME is critical for a proper treatment. Its detection is important not only because it is associated with underlying tear but also because ME itself is related to development of osteoarthritis. Indeed, it represents a significant finding, showing the inability of the meniscus to protect the underlying articular cartilage and it has shown to precede cartilage loss and onset of bony degenerative joint disease within the knee (4, 8). Moreover, it was found that medial ME reflects cartilage damage more clearly than X-ray findings (24).

Currently, MRI is the most important imaging

modality in the assessment of knee pathologic findings, exhibiting excellent diagnostic performance in the detection of meniscal pathologic abnormalities, with an accuracy of diagnosing greater than 90% (17,27). In addition, MRI has proved to be a useful tool in the assessment of ME, typically with the patient's knee in supine, neutral position. However, MRI maintains its own set of limitations, because it is a very expensive and time-consuming investigation and represents a static, non-weight bearing exam (27).

In their study, Magee et al. (17) reviewed 300 knee MRI exams (in coronal, axial and sagittal planes) of patients who had undergone knee arthroscopy. Forty-two of the MR exams demonstrated medial ME >3 mm from the tibial margin, and 34 of these patients had meniscal degeneration, complex tear, or a large radial tear involving the meniscal root. On the other hand, 33 of these patients had meniscal degeneration, complex tear, or radial tear near or at the meniscal root on arthroscopy. Thus, all MRI findings were arthroscopically confirmed, proving the importance of MRI as the preferred diagnostic tool for diagnosis of ME and meniscal pathology.

Meniscus extrusion is evaluated with MRI, typically with the patient's knee in supine, neutral position, but loaded MRI may more accurately determine the extent of MME. In a study by Patel et al., 143 healthy volunteers and patients with OA underwent MRI of the knee under unloaded and loaded conditions. Previously, the subjects were positioned on the MRI scanner table in the supine position with their knees in neutral position, fully extended, to acquire unloaded imaging sequences. Then, a device applied an axial load to the leg of 50% body weight for 10 minutes, and the same MR sequences were repeated. In the results, differences in medial ME between loading and unloading were significant in the entire cohort and especially across Kellgren-Lawrence scores 0, 1 and 3 ($p<0.0001$). On the other hand, no significant differences in extrusion with loading and unloading were found for the lateral meniscus ($p=0.07$).

Paparo et al. (28), introduced Δ MME, a new meniscal parameter correlated with several important features of medial tibiofemoral osteoarthritis which consists in the difference between orthostatic MME

and clinostatic MME. In their study, they used an open-configuration, rotating MR scanner, which made it possible to obtain a vertical rotation of the patient table from 0° (clinostatic position), up to 90° (orthostatic position). The MRI study consisted of two phases: imaging in the orthostatic position and imaging in the clinostatic position. They proved that MME measured on orthostatic images was significantly higher than that measured in clinostatic position ($p < 0.0001$). Finally, the Kellgren-Lawrence score, WOMS Global Score, cartilage loss, meniscal damage and osteophytes were significantly correlated to Δ MME ($p < 0.005$).

Ultrasonography may represent a valid alternative to MRI since it is widely available and it has been used, with high sensitivity and specificity, for the assessment of meniscal tears, providing inexpensive, quick and dynamic information of the meniscus (24, 29). Ultrasound also may offer a relatively simple way to assess ME with the patient both supine (at rest) and standing (weight bearing).

In their study, Nogueira-Barbosa et al. (29) included 93 patients with chronic knee pain that underwent an MRI and an ultrasound examination of the knee on the same day, to determine extrusion measurements of the medial meniscus. Ultrasound images were acquired at the medial aspect of the knee using longitudinal sections parallel to the medial collateral ligament. The comparison between semi-quantitative assessments of medial ME on ultrasound and MRI by the same reader showed moderate-to-substantial agreement. Inter-observer agreement was substantial for MRI and almost perfect for ultrasound. Thus, ultrasound showed excellent diagnostic performance for the detection of medial ME when taking MRI as the reference. Moreover, it showed that cartilage evaluation with ultrasonography is related to cartilage evaluation on arthroscopy.

Kijima et al. (24) measured cartilage thickness of the femoral medial condyle and MME of bilateral of 76 knees of 38 patients who presented unilateral knee pain with ultrasonography. The cartilage of femoral medial condyle was depicted by putting the ultrasound probe on the femoral medial condyle with the knee flexed, whereas the MME was depicted by

putting the ultrasound probe on the medial joint space with the knee extended. They showed that in knees with cartilage with the same amount of thinning, MME was significantly greater in knees with pain than in those without pain ($P = 0.0005$). As a result, the amount of MME could become an index related to the pain of knee OA.

ME as a Predictor of Osteoarthritis

The presence of severe ME is strongly correlated with the structural progression of knee OA, and the thinning of articular cartilage over time (20, 22, 30, 31).

ME is an independent predictor of tibiofemoral cartilage loss, and is associated with joint space narrowing, chondral lesions and meniscal tears.

Adams et al. (7) compared thinning of cartilage and degree of ME in patients with and without osteoarthritis. In this study, ME, without thinning of articular cartilage, was observed in all patients with Kellgren 1-2 narrowing, while it was accompanied by significant loss of articular cartilage in all patients with advanced disease (Kellgren 4). Thus, they showed that early narrowing of the joint space observed on conventional radiographs appears to be a function of ME rather than loss of articular cartilage. Conversely, narrowing of the joint space in advanced disease appears to predominantly reflect progressive loss or changes in articular cartilage.

Lerer et al. (8) described the association between MME and degenerative joint disease (DJD) in the progression of knee OA. They found that 20% of their patients with no or minimal DJD had pathologic MME. These results suggest that MME likely precedes and contributes to the development of medial femorotibial DJD, due to an increased load per unit area at the femorotibial articular surfaces. On the other hand, 75% of knees with significant DJD had >3 mm MME, endorsing the hypothesis that the unimpeded impaction of articular cartilage that leads to changes of DJD likely follows MME.

Berthiaume et al. (32) measured difference in cartilage volume loss of different knee regions in 32 patients with knee OA associated with mild to moderate or severe meniscal damage (tear or extrusion). At MRI knee acquisitions at 24 months, the patients with medial ME showed a significantly

increase in loss of medial compartment cartilage volume rather than patients without MME ($p < 0.001$). Moreover, they reported that patients with severe medial meniscal tear had greater loss of medial compartment cartilage volume than did those with no medial meniscal tear ($p = 0.002$). The relationship between the arthritic knee and ME is well known, but extruded menisci are also seen in non-pathologic knees. Puig et al. (33) determined that ME is much more common in non-arthritic knees than previously estimated. They prospectively studied knee MRI of 100 non-advanced arthritic patients that were operated on arthroscopically for any disorder other than a meniscal pathology. In the study, 68.5% of cases showed some degree of medial ME, while only 15% of cases presented lateral ME, proving the important prevalence of MME also in non-arthritic patients.

Driban et al. (9) conducted a descriptive study in order to understand the underlying etiopathogenesis and describe MRI findings that are associated with incident accelerated knee OA. Of a total of 38 patients, the cases were knees with incident accelerated knee OA, and the comparison groups were knees with incident common knee OA and knees that did not develop knee OA over 48 months of observation, based on baseline and change in radiographic osteoarthritis severity. They showed that among the 18 knees with accelerated knee OA 13 (72%) had incident medial meniscal pathology with extrusion and 5 (28%) knees had subchondral damage. As a result, recognizing ME and/or change in shape, lateral meniscal tear, or acute subchondral damage may be vital for identifying individuals at risk for accelerated knee OA.

ME and Spontaneous Osteonecrosis of the Knee

Spontaneous osteonecrosis is characterized by a sudden onset of acute knee pain, which is commonly unilateral, and the predilection for the load-bearing segment of the medial femoral condyle. It typically occurs in individuals older than 50 years, predominantly female, and has a rapidly developing course (34-36). Its etiology is almost unclear, but it has been demonstrated that certain factors such as steroids, trauma and medial meniscal tears or extrusions may

play a role in the pathogenesis (37-39).

Robertson et al. (37) evaluated 30 patients with spontaneous osteonecrosis of the medial femoral condyle by radiographs and MRI. They found tears of the posterior root of the medial meniscus in 24 of these patients (80%), of which 15 complete and 9 partial tears. Moreover, all complete posterior medial root tears had >3 mm of ME. They also showed that volume of osteonecrotic lesion is associated with ME. Indeed, in the presence of ME of 1.5 mm, the volume of osteonecrotic lesions ranges from 1770 to 7941 mm³, whereas it ranges from 1584 to 19899 mm³ in the presence of complete posterior medial root tear and ME of 3.8 mm.

Nelson et al. (38) evaluated 32 patients (35 knees) with a subchondral insufficiency fracture of the knee (SIFK). They proved the importance of ME in the progression of this pathology, showing that 31 knees presented a meniscal tear, and there were 23 knees with extrusion of 3 mm or more. As a result of the main role of ME and articular cartilage changes, they concluded that spontaneous osteonecrosis is very likely associated with OA.

SIFK has been described as an event leading to osteonecrosis and OA. In a study by Wilmont et al. (39) MRI of 74 patients with spontaneous insufficiency fracture of the knee were retrospectively reviewed for soft tissue edema presence and location and meniscal tears and extrusion. Medial meniscal tears were present in 64% of the knees, and they demonstrated a trend in further increasing the likelihood of a concomitant medial sided SIFK ($p = 0.03$). Follow-up imaging was available in 25 patients, and it showed progression of SIFK in 17 patients (68%), 3 of whom underwent arthroplasty. Moreover, 13 of 14 knees with ≥ 3 mm of extrusion progressed, compared to 2/7 patients without ME. As important findings, they found that SIFK had a strong association with medial meniscal tears and extrusion.

Bone marrow lesions (BML) and bone cysts are subchondral bone lesions that tend to progress over time in established OA. Wang et al. (21) showed the relationship between ME and subchondral bone changes in a cohort of participants with mild to moderate knee OA. In univariate analysis, they

found that ME is associated with reduced tibial cartilage volume, increased tibial plateau bone area and increased prevalence of subchondral BMLs and bone cysts in the medial and lateral tibiofemoral compartments (all $P < 0.001$). Over the 2-year follow-up, baseline medial ME predicted the increases in bone marrow lesions and bone cysts and the expansion of tibial plateau bone in the medial tibiofemoral compartment (all $P < 0.001$). As a result of ME, impact at the femoral and tibial bone surface is likely to increase the susceptibility of subchondral bone to trauma during dynamic movements of the knee.

Teichtahl et al. (10), in a 72-month longitudinal study examined whether compartmental baseline ME and BMLs were associated with incident and progressive knee OA (independent of each other). In particular, they demonstrated that people with radiographic OA had a higher prevalence of baseline MEs ($p < 0.001$) and tibial BMLs ($p < 0.001$) than people without radiographic OA. Moreover, among people with radiographic OA, the presence of a medial or lateral ME was associated with significantly greater rates of cartilage volume loss in that respective tibial compartment ($p < 0.001$), with an increased risk of progressive radiographic OA ($p = 0.02$) and knee joint replacement surgery ($p = 0.001$).

Predictive factors for the development of ME

- Five studies associated root meniscal tears to ME (11, 14-16, 18) demonstrating strong evidence of association.
- Three studies did not show significant association between radial tears, horizontal tears, vertical longitudinal tears, oblique tears and meniscal extrusion (14-16).
- One study reported a positive association between varus and valgus alignment and respectively, medial and lateral ME (11).
- Given the reported positive association in one study (18) and no association in another study (11), there is an inconsistent association between knee joint effusion and ME.
- Only one study showed the relationship between past knee injury and ME (22).
- One study evaluated the association between

ACL tears and ME (18).

- Because of the presence of positive association in one study (22) and no association in another study (11), there is an inconsistent association between body mass index and ME.
- Eight studies associated ME to thinning of articular cartilage and progression of OA, demonstrating strong evidence of association (7-10, 21, 32, 38, 39), whereas one study (33) showed the prevalence of ME also in non-arthritic knees.
- Two studies found that ME is associated with an increased prevalence of subchondral bone marrow lesions and bone cysts in the medial and lateral tibiofemoral compartments (10, 21).
- Three studies reported a positive association between ME and spontaneous osteonecrosis of the knee (37, 39).

Treatment

Meniscal tears, which represent the main cause of ME, are the most common pathology of the knee with a mean annual incidence of 66 per 100000 (40). Because degenerative meniscal tears are part of the same disease process as osteoarthritis, the treatment approach is similar.

Non-operative treatments for meniscal injuries includes activity modification, weight loss, nonsteroidal anti-inflammatory medications (NSAIDs), supervised exercise and physical therapy programmes, unloading braces, and injections (40-42).

The initial focus of non-surgical therapy is the relief of knee pain. Conservative treatment is composed of physical therapy and rehabilitation in order to maintain range of motion (ROM), improve knee and hamstring flexibility, increase quadriceps and knee strength, and retain knee proprioception.

Østerås et al. (43), compared the effectiveness of medical exercise therapy (MET) versus arthroscopic surgery in patients with degenerative meniscus injury and knee pain. They showed that after 3 months, both the groups exhibited improvements compared to baseline, and that conservative therapy is equally effective as surgery in pain and function scores. Moreover, anxiety and depression were significantly reduced ($p < 0.05$) in the MET group compared with

the patients receiving arthroscopic surgery.

In a study by Katz et al. (44) 351 patients with a meniscal tear and mild-to-moderate OA were randomly assigned to surgery and postoperative physical therapy or to a standardized physical-therapy regimen.

At the 12-month follow-up, it showed that the two groups had similar changes from baseline in the WOMAC physical-function and KOOS pain scores, proving that arthroscopic meniscectomy provided no additional benefit over a standardized physical therapy program.

Herrlin et al. (45) compared arthroscopic meniscectomy followed by supervised exercise with supervised exercise alone in a total of 90 patients with knee pain, medial meniscal tear and no or minimal osteoarthritis. In the conservative group, each patient followed a standardized exercise programmer, twice a week for 8 weeks, for improving muscle strength and endurance, muscle flexibility, balance and proprioception. They showed that there were not significantly differences between the two groups in terms of reduced knee pain, improved knee function and improved quality of life.

In unicompartmental OA, knee alignment plays an important role for disease progression and functional decline. In these cases knee braces may alter the alignment of the lower extremity, applying an external varus or valgus force to the knee to shift loads towards the non-affected compartment. Thus off-unloader braces represent a mechanical intervention designed to reduce pain, improve physical function and possibly slow disease progression of knee OA (46, 47).

In their study Kirkley et al. (47), randomized 119 patients into 3 groups: medical treatment only (control), neoprene sleeve, and unloader brace. It demonstrated that both bracing groups showed a substantially greater improvement in quality of life scores compared to the control group ($p < 0.001$). Moreover, compared to neoprene sleeves, the valgus-unloader brace group demonstrated significantly less pain on the 6-min walking test ($p = 0.021$) and a strong trend towards a significant improvement on the total WOMAC score ($p = 0.06$).

The role of glucosamine/chondroitin sulfate for the treatment of ME associated with knee osteoarthritis is still unclear. Raynauld et al. (30) examined the long-

Table I. Results of evidence synthesis (57)

Factors associated with meniscal extrusion	Study	Association	Evidence (direction)
1. Root meniscal tears	Crema et al. (11), Choi et al. (15), Costa et al. (16), Muzaffar et al. (14), Rennie et al. (18)	Positive	Strong (positive)
2. Radial tears, horizontal tears, vertical longitudinal tears, oblique tears	Choi et al. (15), Costa et al. (16), Muzaffar et al. (14)	No	Strong (No)
3. Varus and valgus alignment	Crema et al. (11)	Positive	Inconsistent
4. Knee joint effusion	Rennie et al. (18), Crema et al. (11)	Positive [Rennie et al. (18)], no [Crema et al. (11)]	Inconsistent
5. Past knee injury	Ding et al. (22)	Positive	Inconsistent
6. ACL tears	Rennie et al.	Positive	Inconsistent
7. Body mass index (BMI)	Ding et al. (22), Crema et al. (11)	Positive [Ding et al. (22)], no [Crema et al. (11)]	Inconsistent
8. Thinning of articular cartilage and OA progression	Berthiaume et al. (32), Adams et al. (7), Lerer et al. (8), Driban et al. (9), Wang et al. (21), Teichtahl et al. (10), Nelson et al. (38), Wilmont et al. (39)	Positive	Strong (positive)
9. Subchondral bone marrow lesions and bone cysts	Wang et al. (21), Teichtahl et al. (10)	Positive	Strong (positive)
10. Spontaneous osteonecrosis of the knee	Robertson et al. (37), Nelson et al. (38), Wilmont et al. (39)	Positive	Strong (positive)

term (6-year) effect of combined glucosamine (Glu) and chondroitin sulfate (CS) treatment on cartilage volume in 429 participants with knee osteoarthritis, medial ME at baseline and at least 1 mm of medial compartment joint space width at baseline. They presented that the progression of cartilage volume loss improved over time with Glu/CS exposure for the global knee associated with the lateral compartment (2-3 years $p=0.006$; 4-6 years $p=0.002$), but the medial compartment and its sub-regions were devoid of such Glu/CS effect. On the other hand, in a study by Roubille et al. (48), 600 participants with knee OA were stratified based on whether or not they received analgesics/NSAIDs and/or Glu/CS for 24 consecutive months and on the presence or absence of medial ME at baseline. It was demonstrated that participants with ME who took Glu/CS had significantly less cartilage volume loss in the medial plateau at T24 ($P<0.010$) and showed a statistical trend toward less cartilage volume loss in the medial tibiofemoral compartment compared to those who did not take Glu/CS.

Corticosteroid injections for the treatment of meniscal tears may provide short-term relief of knee pain due to their anti-inflammatory and immunosuppressive effect, which leads to interrupt the inflammatory and immune cascade at several levels (49, 50).

In a study by Vermesan et al. (49), 120 patients with degenerative medial meniscus tears were randomized to either intra-articular steroid injection or arthroscopic debridement. At one year, the differences between the two groups in terms of the Oxford Knee Score were not statistically or clinically significant. Thus, degenerative medial meniscus tears in the presence of OA can only marginally benefit from arthroscopic debridement over intraarticular steroid injections in the short term.

The intra-articular (IA) injections of hyaluronic acid (HA) have to be considered among non-operative treatments for meniscal injuries. They are used to restore normal viscoelastic properties of the pathologically altered synovial fluid, reduce synovial inflammation, protect against cartilage erosion, and promote intra-articular HA production, in order to prevent the progression of chondral lesion and knee OA (41,50).

Zorzi et al. (51), investigated the efficacy of intra-articular injection of hyaluronic acid in the

management of meniscal tears and in meniscal tear repair. Fifty patients, diagnosed with single meniscal tear were allocated in two groups: the non-operative group was treated with ice applications, rest and knee off-loading combined with the intake of paracetamol for 2 weeks; patients in the second group received two IA injections of a hydrogel formulation obtained from a HA derivative (HYADD4®) two weeks apart in addition to the conservative treatment. At 60-day follow-up a decrease in the perception of pain in both groups was demonstrated, but the VAS pain score showed that the reduction in patients treated with HYADD4® was greater than that observed in patients belonging to the control group ($p<0.001$). Moreover, both the WOMAC total score and the sub-scores, considering pain, stiffness and physical functioning of the knee, decreased over time, and they were always lower in the HYADD4® group than the control group ($p<0.017$). Thus, they concluded that IA HA might be effective in the conservative management of pain related to meniscal tears.

Unfortunately, the benefits of non-operative therapy in the reduction of the painful knee symptoms in the long-term tend to decrease due to the worsening of meniscal degeneration, and these patients are eligible for arthroscopic meniscectomy, in order to improve their quality of life.

The fundamental principle of meniscus surgery is to remove only the torn piece of meniscus while retaining as much normal meniscus. Tears with a high probability of healing with surgical intervention are repaired, but in most cases, tears are not repairable and a meniscectomy becomes necessary. The indications for performing a meniscus repair consist in: peripheral tears, because only the peripheral 10%-25% of the meniscus benefits from a blood supply, shorter tears and vertical longitudinal tears and acute tears rather than chronic. For these reasons, especially in older patients, meniscectomy is more performed (40, 52, 53).

The disadvantage of meniscectomy is that it alters joint biomechanics, leading to chondral wear and subchondral fracture, furthering joint and meniscal degeneration (19, 42, 54). Indeed, total meniscectomy is a significant risk factor for

accelerated knee OA and it is associated with early knee total replacement (55, 56).

In a study by Roos et al. (55), 107 patients who underwent open meniscectomy due to an isolated meniscal tear were followed up 21 years later, in order to study the presence of advanced radiographic changes and signs of OA in those patients. It demonstrated that surgical removal of a meniscus represents a significant risk factor for radiographic tibiofemoral OA, with a relative risk of 14.0.

In a study by Brophy et al. (56), of the 1286 patients undergoing total knee arthroplasties for OA, nearly one-third (29%) had a history of knee surgery and the most common type of surgery was arthroscopy, followed by meniscectomy. Moreover, patients who had undergone previous meniscectomy underwent total knee arthroplasty at an average interval of 12.6 years after meniscectomy and 14.3% of patients underwent total knee arthroplasty within one year after arthroscopic meniscectomy.

CONCLUSION

ME represents an important risk factor for knee OA. It is often associated with severe meniscal tears or root tears mainly in the medial meniscus and spontaneous osteonecrosis of the knee.

MRI is the most commonly used imaging modality in the assessment of ME, while ultrasonography may represent a valid alternative with high sensitivity and specificity.

Conservative treatments rely on physical therapy and rehabilitation to maintain range of motion, corticosteroid injections and intra-articular injections of hyaluronic acid to provide short-term relief of knee pain. Surgical management may be necessary when conservative treatment fails and meniscal lesions are present.

Further research on the diagnosis and treatment of this pathology should be pursued to prevent and better manage knee OA.

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INTRARTICULAR INJECTIONS OF HYALURONIC ACID FOR TRAPEZIO-METACARPAL OSTEOARTHRITIS: A SYSTEMATIC REVIEW

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Osteoarthritis (OA) of the base of the thumb, also known as Trapezio-Metacarpal (TM) OA, is a disabling condition, which mainly affects women and manual workers. When TM OA is not adequately treated, patients develop deformity and loss of function of the thumb. The surgical approach is a widespread strategy to treat this condition, but there is still no consensus on the most effective procedure. Therefore, several conservative strategies are commonly used, such as nonsteroidal anti-inflammatory drugs (NSAIDs) administration, thumb strengthening exercise, splinting, steroid (CS) and hyaluronic acid (HA) intrarticular injections. The present review of the literature aims to summarize the available scientific evidence on the treatment of TM OA with injections of HA. Thirteen studies were included: 7 randomized controlled trials, 5 case series and a case-control study. Among these, 5 studies compared HA versus CS injection. Results from most of them reported better outcomes with HA injections in terms of function (strength) and joint motion, while CS injections had greater effect on pain; moreover, CS action was faster but shorter, while HA required more time to obtain a therapeutic benefit and lasted longer. In non-comparative articles, this trend was also confirmed. Indeed, the authors reported an improvement in pain relief up to six months. Similarly, all studies indicated hand function improvement over time, measured through DASH score, pincher and grip strength tests. Available data from included studies show that there is no clear evidence to suggest a treatment with HA injections as the best advisable non-operative treatment for TM OA. However, promising potentials were shown by the randomized controlled trials, suggesting that there is some benefit and less comorbidities with the administration of HA. Further research, such as trials evaluating larger cohorts with validated scores for long-term follow-up, is still necessary.

Trapezio-Metacarpal (TM) joint osteoarthritis (OA) is a degenerative condition affecting the base of the thumb, causing instability, pain at rest or during activity and functional impairment of the hand. It particularly affects women, in whom it is especially common during postmenopausal age (1). When TM OA is not adequately treated, patients develop a deformity of the thumb, due to the contracture in adduction, leading to the subluxation of the joint

(2). The most common radiological classifications used for TM OA are Eaton and Littler classification, specific for TM joint, and Kellgren and Lawrence classification, common to others joints too. Several surgical procedures are widely used to treat TM OA (3, 4), however there is still no consensus on the most effective procedure (5) and, at the same time, there is a higher risk of complications. Therefore, conservative strategies represent a valid alternative

Key words: trapezio-metacarpal, osteoarthritis, hand, hyaluronic acid, injections

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and should be considered as first approach.

Common conservative procedures includes nonsteroidal anti-inflammatory drugs (NSAIDs) administration, thumb strengthening exercises, splinting, corticosteroid (CS) intra-articular injections, that provide pain relief and slightly improve function (6,7). Injections with hyaluronic acid (HA) have been proposed recently for the treatment of TM OA. HA is a glycosaminoglycan normally present in the synovial fluid and cartilage matrix (8-15). However, during OA its levels considerably decrease (16). HA articular injections have proved to relieve pain and improve function in many osteoarthritic joints, mostly in knee, but also in hip and ankle (17, 18). Intra-articular HA injection for TM joint is a relatively new practice that seems to be a good alternative to CS injections. Although CS injections can provide pain relief (19), it is known to cause local side effects such as depigmentation, peri-articular soft tissue calcification, tendon and ligament weakening (20, 21) as well as general side effects in specific populations, such as diabetic patients. Since HA is naturally present in the joint, most of the available formulations do not cause systemic side effects (22). It has been reported that an avian origin preparation (sodium hyaluronate, Hyalgan, Hylan G-F 20: Synvisc) led to allergic reaction and transient asthenia in a few and rare cases. However, this procedure presents some disadvantages, such as pain during injection, swelling, necessity of more than one infiltration and high costs (23).

However, functional results reported in literature are heterogeneous and still unclear. The purpose of this study is to systematically review the available literature regarding the treatment of TM OA using HA intraarticular injections, to understand the efficacy in terms of pain and functional improvement, and provide suggestions for future studies.

MATERIALS AND METHODS

Study inclusion criteria

In this systematic review, we searched papers concerning HA injections for the treatment of OA of TM joint. Main inclusion criteria were: peer reviewed studies of each level of evidence according to Oxford

classification; clinical outcomes of patients affected by TM OA treated with HA injections; validated outcome measures; English, Spanish, French or Italian languages; adult subjects included; published in the last 15 years. Randomized Controlled Trials (RCT), Case Series (CaS) prospective or retrospective, Case-Control Studies (C-CS) were considered for inclusion, while case reports, technical notes, letters to editors, instructional course, systematic reviews and meta-analyses were excluded. Furthermore, *in vitro* studies and cadaver investigation were excluded from the review process.

Search protocol

A literature search was performed using the isolated or combined keywords including: “hyaluronic acid AND trapezio-metacarpal AND arthritis”, “hyaluronan AND trapezio-metacarpal AND arthritis”, “hyaluronic acid AND trapezio-metacarpal AND injection”, “hyaluronic acid AND carpo-metacarpal AND arthritis”. The online research was completed on February 24th 2017 and was carried-out consulting main online databases: PubMed-Medline, Google Scholar, CINAHL, Cochrane Central and Embase Biomedical.

Study selection protocol

The published literature was screened. All bibliographies of the articles potentially relevant for inclusion were screened by hand, in order to enrich the search with more related studies, which might have been missed through online research. The abstract of all the papers retrieved was analysed separately by two reviewers (L.D.B. and G.T.) and studies considered eligible for review process were retrieved in full-text and read completely. Study selection process is shown in Fig. 1. Data considered relevant to be extrapolated were summarized into tables and discussed in duplicate (R.P. and L.D.B.) before final inclusion for manuscript writing.

RESULTS

Included studies details

A total of 13 studies were included for detailed evaluation. Of these, 7 were RCT of I level of evidence (LOE), 5 were CaS of IV LOE and one was a C-CS of III LOE. Studies were published between 2005 and 2014. All the studies regarded intrarticular

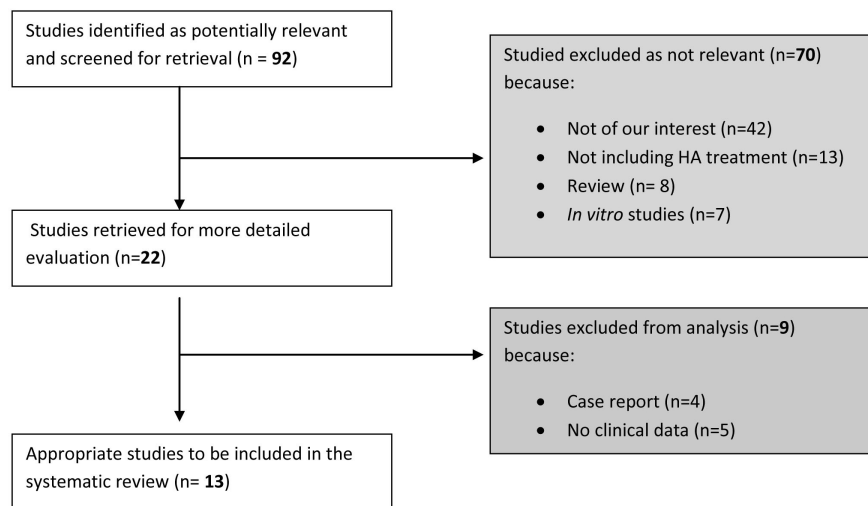


Fig. 1. Flow diagram of the study selection process.

injections of hyaluronic acid and 5 compared this treatment with CS injections, 2 with placebo saline. Details of the included studies are summarized in Table I.

Sample features and follow-up

Based on data of the included studies, a total of 542 patients were treated for TM arthritis (males: 110; females: 416). The mean age of patients at the time of treatment was 62.2 years and ranged between 37 (24) and 92 (16) years. The mean time of the follow-up was 24.3 weeks ranging from 4 (25) weeks to 1 (26) year. Only one study (23) did not report any follow-up since clinical assessment was limited to the time of injection. Demographic data of patients are summarized in Table I.

Type of treatment

Different types of commercially available HA were utilized in the included studies. Two studies (26) utilized Ostenil 5mg/0.5mL and one (27) utilized Ostenil mini 1mL; 3 studies (28-30) utilized Hylan G-F 20 1mL. Other HAs used were Sinovial 1 mL (31), Orthovisc 15mg/1mL (24), Hyalgan 0.8mL (32), and Hyalubrix 0.5mL (33). Control groups in included RCTs were patients treated with injections of betamethasone acetate 1 mL (29) or 3 mg/0.5mL

(16) triamcinolone (Volon A10) 1 mL (27), methylprednisolone acetate (Depomedrol) 1 mL/40 mg (24), saline 1mL (28). Details on interventions are summarized in Table II.

Outcome measures

The most important and used score for clinical assessment among included studies was the Visual Analogue Scale (VAS) for pain. This was measured either at rest or during joint motion in two studies (25, 32). One study (23) measured VAS during injection procedure. Furthermore, Duruoz Hand Index (DHI) was measured in two studies (26, 34) for the assessment of hand function; Dreiser's Index was measured in two more studies (25, 33). Other outcome measures were Disability of Arm Shoulder and Hand (DASH) score (30), Functional Index for Hand OsteoArthritis (FIHOA) (16) and Purdue Pegboard Test (24). Furthermore, pinch strength was evaluated in 6 studies (24-29).

Outcome data

Non-comparative studies

VAS for pain was the main outcome assessed in the included studies. VAS significantly improved in one study by Salini et al. (25), showing the following score decrease: 1.8 ± 1.07 to 0.5 ± 0.68 ($p < 0.001$) and

Table I. Demographic data of included studies.

Study	Year	Type of study	Level of evidence	N. Patient recruited	M	F	Mean follow up (weeks)	Mean age at intervention
				Tot= 542	Tot= 110	Tot= 416		
				Av = 41.7	Av = 9.2	Av = 34.7		
				SD= 20.9	SD= 14.0	SD= 13.4		
Bahadir et al.	2009	RCT	I	40	0	40	52	61.8
Di Sante et al.	2011	CaS	IV	31	3	28	24	n.a.
Figen et al.	2009	RCT	I	33	0	33	24	62.6
Frizziero et al.	2014	CaS	IV	58	8	50	24	57
Fuchs et al.	2006	RCT	I	56	11	45	26	60.25
Heyworth et al.	2008	RCT	I	60	8	52	26	63
Ingengoli et al.	2011	CaS	IV	16	1	15	24	62.5
Karalezli et al.	2007	C-C S	III	16	n.a.	n.a.	n.a.	54.5
Mandl et al.	2009	CaS	IV	32	10	22	26	64
Monfort et al.	2014	RCT	I	88	52	36	24	62.8
Roux et al.	2007	RCT	I	42	4	38	12	64.8
Salini et al.	2009	CaS	IV	18	7	11	4	70.6
Stahl et al.	2005	RCT	I	52	6	46	24	62

M: Male; **F:** Female; **Av:** Average; **SD:** Standard Deviation; **RCT:** Randomized Control Trial; **CaS:** Case Series; **C-C S:** Case-Control Study.

Table II. Type of intervention and product used in each trial included.

Study name	Year	Grade of OA	Side	Main intervention	Placebo/ Control
Bahadir et al.	2009	Eaton-Littler II and III	23 R, 17 L	Sodium hyaluronate (Ostenil , 5 mg/0.5 mL): 3 weekly injections	Triamcinolone acetone (Kenacort-A, 20 mg/0.5 mL): 1 injection
Di Sante et al.	2011	Eaton and Gleackel I to IV	n.a.	1mL solution sodium hyaluronate: 1 injection weekly for three weeks, US guided.	-
Figen et al.	2009	Eaton-Glickel I to IV	n.a.	HA- Hylan G-F 20, 1 ml: 1 injection	Control (contralateral hand) - saline, 1 ml: 1 injection
Frizziero et al.	2014	KL grade 2-3	n.a.	HA: Hyalgan 500-730 KDa: 1 injection 0.8 mL once weekly for 3 weeks	-
Fuchs et al.	2006	Kellgren II	n.a.	Ostenil mini, 1 ml: 3 weekly injections	Triamcinolone (Volon A10), 1 ml: 1 injection
Heyworth et al.	2008	n.a.	51 dominant	Hylan G-F 20, 1 ml: 2 weekly injections	I control group: Betamethasone acetate 1 ml: First week, 1 mL saline; second wk, 1 mL II control group: saline 1 ml: 2 weekly injections
Ingengoli et al.	2011	KL grade 3-4	n.a.	Hyalubrix 3.2kDa (Fidia pharmaceuticals), 1 injection 0.5 mL once weekly	-
Karalezli et al.	2007	Eaton grade 3-4	n.a.	Sodium hyaluronate (Ostenil , 5 mg/0.5 mL): 3 weekly injections	-
Mandl et al.	2009	KL grade 2-4	n.a.	HA: Synvisc (Hylan G-F 20), one injection 1mL weekly for 3 weeks	-
Monfort et al.	2014	KL grade 1-3	n.a.	Sodium Hyaluronate 5 mg/0.5 ml: 3 weekly injections	Betamethasone acetate, 3 mg/0.5ml: 3 weekly injections
Roux et al.	2007	Kellgren II to IV	n.a.	Sinovial, 1 ml: 1 injection	I control group: Sinovial, 1 ml: 2 weekly injections II control group: Sinovial, 1 ml: 3 weekly injections
Salini et al.	2009	KL grade 2-3	n.a.	Sodium hyaluronate 0.8-1.2 KDa, 1 injection 1mL 0.8%	-
Stahl et al.	2005	Eaton-Littler II	n.a.	Sodium Hyaluronate (Orthovisc) 1 ml/15mg: 1 injection	Methylprednisolone acetate (Depomedrol), 1 mL/40 mg: 1 injection

from 8.05 ± 0.94 to 4.15 ± 1.42 ($p < 0.001$), at rest and during motion, respectively. Similar results were obtained by Frizziero et al. (32), for both rest and motion pain ($p < 0.001$). In a similar fashion, several studies reported VAS improvement for pain at rest. Ingegnoli et al. (33) reported an improvement from baseline (median 68.8 mm) after 2 and 24 weeks (median 40 mm and 55 mm, $p = 0.0003$ and $p = 0.009$ respectively). Di Sante et al. (34) reported significant VAS reduction at 1 and 3 months follow-up ($p < 0.01$), while no significance was achieved at 6 months ($p = 0.6$); Mandl et al. (30) reported a VAS improvement from average baseline value of 62mm to an average value of 46.3mm ($p = 0.006$) at 26 weeks of follow-up. In a study by Karalezli et al. (23), VAS was also evaluated during the injection procedure under fluoroscopy guidance and not, showing that the first group of patients reported significantly lower pain during injection (4.1 vs 5.6, $p < 0.005$).

Dreiser Hand Index (DrHI) was also evaluated in several studies to assess hand function after HA injections. Salini et al. showed a significant improvement (+11.59%; $p < 0.004$) (25). However, two studies showed that no significance was achieved in mean decrease and long-term results did not maintain the decreasing trend. In fact, Ingegnoli et al. (33) reported that DrHI decreased from 9.0 to 8.0 at week 2 but after 6 months, the median of Dreiser's index remain stable at 8.0, but these differences were not statistically significant (33). Similarly, no statistically significant difference was found by Di Sante et al. on DrHI improvement at 1 month ($p = 0.08$), 3 months ($P = 0.1$) and 6 months ($P = 0.9$) (34). DASH score reported by Mandl and colleagues (30) faced a significant improvement at 26 weeks of follow-up (from baseline to follow-up: 29.7 to 17.1, $p < 0.001$). Salini et al. reported an increase in pinch strength of 24.07 % ($p < 0.001$) (25).

Comparative studies

Stahl et al. (24) carried out a RCT to compare the use of intrarticular HA and CS. At final follow up (6 months) they found a significant pain improvement ($P < 0.001$) in both groups, but there was not a significant difference between HA and CS treatment. However, patients treated with HA showed a

progressive improvement in pinch strength and function at 3-month follow-up. Similarly, in a study by Heyworth et al. (29) there were no significant differences in average VAS pain, grip strength and DASH score between groups, although each group had significant improvement compared to baseline ($p < 0.05$). However, only the group treated with Hylan G-F 20 reported a durable pain relief and improvement in grip strength at last follow up, but was not significant compared to other groups.

Pinch strength showed the only significant difference ($p < 0.05$) between groups at week 12 in patients treated with HA compared to CS and control group. Evaluation of ROM showed no significant difference.

In their RCT, Fuchs et al. (27) reported that patients treated with CS injection experienced a faster pain relief (2-3 weeks) while patients treated with HA reached pain relief more gradually reaching its maximum at 26 weeks. However, 88% of patients in HA group and 79.1% of patients in CS group referred pain improvement after the last follow up ($P = 0.3624$). Furthermore, lateral pinch was significantly higher in patients treated with HA than in patients treated with CS ($P = 0.0226$).

The RCT conducted by Bahadir, et al (26), showed that VAS score decreased significantly for both HA and CS groups after treatment but the decrease was significant at 12 months only for the CS group ($P = 0.013$). Furthermore, the VAS score was significantly lower in the CS group compared with the HA group at 1 to 6 months of follow up ($P < 0.001$). Grip strength improved significantly in both groups during the study period. This improvement was significant at 3 months ($P = 0.006$) for the CS group and 6 months for the HA group ($P = 0.038$). The only difference in grip strength between groups was at 1 month when grip strength was significantly higher in the CS group than the HA group ($P = 0.034$). Moreover, hand function, evaluated using the DHI, showed significant better results in CS group compared to HA group at 1, 3, and 6 months. However, at last follow up, the DHI score was significantly lower in both groups.

Conversely to the aforementioned results, Monfort et al. (16) showed that Functional Index

for Hand Osteoarthritis score, had a trend towards greater clinical improvement in the HA group ($P=0.071$) compared to that treated with CS at 3 months, though VAS difference was not significant. A sub analysis of patients with Functional Index score ≥ 5 and VAS ≥ 3 at baseline showed a significantly higher median functionality score in the HA group ($P=0.005$ at 3 months and $P=0.020$ at 6 months). Further subgroup analysis showed significantly greater improvement in functionality score ($P=0.004$ at 6 months) in a group with baseline VAS ≥ 5 , which was already evident after the second injection at 14 days ($P=0.028$). Figen-Ayhan's study (28) studied 33 patients bilaterally treated with a HA injection on a hand and a CS injection on the contralateral side (considered control group). Statistically significant improvements were detected in the HA group in terms of function ($p=0.001$), VAS pain ($p=0.002$), and pinch strength ($p=0.004$) at the 24 weeks. However, only VAS pain scores decreased temporarily in control hands at 6 weeks ($p=0.02$).

Roux et al. (35) compared 1, 2 or 3 injections of HA in 3 groups of patients and showed that no VAS difference at 1 month ($p=0.075$) and 3 months ($p=0.382$). Intra group differences between baseline and 3 months was significant in groups of patients treated with 2 and 3 injections ($p=0.012$ and $p=0.002$ respectively).

Details on advantages of imaging techniques and adverse reaction reported are showed in table III and table IV.

DISCUSSION

We performed a systematic review of the literature to provide an overview of the treatment with HA injections of TM OA. Prevalence of TM OA in Western populations varies from 8% to 12% in the general population, with a peak of 33% in post-menopausal women (36). It causes pain and hand disability, mostly in grip, pinch strength and fine movement, and can be associated to OA of other joints. The first approach to achieve pain relief and improve functionality of the hand is the conservative treatment, but available therapies are often ineffective, making surgical procedure

necessary (30). Although there is strong evidence that intrarticular HA injections are effective to treat knee OA (22), there is an overall lack of a consistent number of studies regarding the use of HA for the management of TM OA.

Furthermore, there is still no consensus among the scientific community and opinions are different among official scientific societies. The European League Against Rheumatism (EULAR) and the Italian Society of Rheumatology (ISR) agree in considering HA injections beneficial for TM OA, even though a low level of evidence is associated to this recommendation (IIb and III, for EULAR and ISR respectively) (37, 38). Conversely, according to the American College of Rheumatology, intra-articular HA or CS injections should not to be recommended, because of the lack of strong evidence and the potential occurrence of side effects (39).

In this systematic review, we included 7 RCTs with a good level of evidence. Among these, 5 studies compared HA versus CS injections. Results from most of them (16, 24, 27, 29) indicated a better recovery of hand functionality after HA treatment, while CS had greater effect on pain; furthermore CS action was faster but shorter, while HA required more time for therapeutic benefit, but lasted longer. On the contrary, in the study by Bahadir et al. (26) it was shown that CS injections were more effective in pain relief, lasted longer and provided a better function of the hand, compared to HA injections.

In the remaining 6 non-comparative articles, this trend was confirmed: the authors reported an improvement in pain relief of up to 6 months, except in the study conducted by Di Sante and colleagues (34), in which the improvement lost significance at 24 weeks. Similarly, all studies indicated hand function improvement, but Ingegnoli et al. and Di Sante et al. (33, 34) analyzed the evolution of instability over time, reporting that improvement was not significant at 1 and 6 months respectively.

The wide heterogeneity of products used in injections makes the comparison very difficult. Studies reporting the use of similar product (in different formulation) were Bahadir, Karazezli and Fuchs (23, 26, 27), who used Ostenil, and Figen-Ayhan, Heyworth and Mandl (28, 30) who used

Table III. *Advantages of imaging assisted techniques.*

Study name	Main intervention	Imaging assessment	Advantages reported
Di Sante et al.	Sodium hyaluronate 1mL	US	Accurate individuation of joint space line, visualization of the needle tip inside the joint
Ingengoli et al.	Hyalubrix 0.5 mL	US, PWD	Visualization of the needle tip within the joint, evaluation of inflammatory state over time
Karalezli et al.	Ostenil , 5 mg/0.5 mL	Fluoroscopy	Reduction of pain for more accurate position of the needle
Salini et al.	Sodium hyaluronate 0.8-1.2 KDa, 1 injection 1mL 0.8%	US	Evaluation of OA joint changes, visualization of the needle tip within the joint

US: Ultrasound; **PWD:** Power Doppler.

Table IV. *Adverse reactions.*

Study name	N.er of patients	Pain during injection	Pain after injection	Local swelling	Skin flushing	Systemic effects	Total of events
Bahadir et al.	40	-	-	-	-	-	None
Di Sante et al.	31	-	-	-	-	-	None
Figen et al.	33	-	-	-	-	-	n.a.
Frizziero et al.	58	3	7	-	2	2	14
Fuchs et al.	56	-	-	-	-	-	n.r.
Heyworth et al.	60	-	-	-	-	-	None
Ingengoli et al.	16	-	-	-	-	-	n.a.
Karalezli et al.	16	-	-	-	-	-	n.a.
Mandl et al.	32	-	3	-	-	-	3
Monfort et al.	88	-	5 CS, 5 HA	2 CS, 3 HA	-	-	15 (7 CS, 8 HA)
Roux et al.	42	-	-	-	-	-	~ 30% (*)
Salini et al.	18	2	-	-	-	-	2
Stahl et al.	52	-	-	-	-	-	None

None: reported no adverse event; **n.a.:** data not available in the paper; **n.r.:** events occurred were not related to treatment; **CS:** group treated with corticosteroids; **HA:** group treated with hyaluronic acid; *****: the study reported an equal percentage of adverse events (about 30%) in all groups of treatment.

Hylan G-F 20. However, there are no data according to molecular weight. Furthermore, timing and number of injections were inhomogeneous, as there was not a unique treatment protocol. In 7 out of 13 studies (16, 23, 26, 27, 30, 32, 34) patients underwent 3 weekly injections while in the study by Heyworth et al. (29), patients underwent 2 weekly injections. In 4 studies (24, 25, 28, 33), only one injection was performed and

in the study by Roux et al. (35), 3 weekly injections protocol was compared to 2 weekly injections and a single injection.

Follow-up was also very variable, ranging from 4 (25) to 52 (26) weeks, which is a possible risk of bias.

In included studies, the diagnosis of TM OA was assessed by X-ray and 3 different classification systems were used (Eaton-Littler, Eaton-Glicker,

Kellgren Lawrence), except for Heyworth et al. (29) who did not report any radiologic classification.

Finally, some studies (33, 34) focused on the injection technique and radiologic support, without reporting compared results or having a control group; in the study by Karalezli et al. (23) the outcomes were only those collected during the procedure, without results over time.

CONCLUSION

The data from included studies showed that there is no clear evidence to advise HA injections as the best advisable non-operative treatment for TM OA. However, promising results were shown by the RCT, suggesting that there are some benefits from the administration of this therapy and less comorbidities, though data are insufficient to confirm the real additional advantage. Further and homogeneous trials, studying large cohorts through standardized scores and longer follow-up, are still necessary to provide a stronger statistical analysis.

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TOPICAL HYALURONIC ACID IN RHINITIS MEDICAMENTOSA: COULD OUR PERSPECTIVE BE CHANGED?

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This study was designed to prospectively evaluate the role of nebulized hyaluronic acid (HA) administered for 10 days as treatment for patients with rhinitis medicamentosa (RM). RM is a pathological condition of the nasal mucosa induced by prolonged, excessive or improper use of topical decongestants. It is characterized by persistent nasal congestion that can lead the patient to increase the frequency of application and the quantity of the substance being applied, resulting in dependence on topical nasal decongestants. Twenty-five patients were treated with HA nebulized via Spray-sol twice a day for 10-days (T1) (HA Spray-sol treatment group). Subsequently, after 3 days of washout, patients were treated with physiological saline nebulized via Spray-sol twice a day for 10 days. (T2) (saline Spray-sol treatment group). The HA Spray-sol treatment group (tp) significantly improved visual analogue scale (VAS) scores ($T0=6.25\pm1.64$ vs $T1=3.91\pm1.30$; $p<0.05$), whereas there was no statistically significant difference in the saline Spray-sol treatment group (tp) ($p>0.05$), results confirmed by the anterior active rhinomanometry (AAR) data (HA Spray-sol tp $T0=1.193\pm0.83$ vs $T1=0.44\pm0.25$, $p<0.05$; saline Spray-sol tp ($p>0.05$). An improvement in the Global Rhinitis Score (GRS) was recorded in both groups ($T0=15.37\pm5.16$ vs $T1=5.54\pm3.23$, $p<0.05$; saline Spray-sol tp $T0=15.37\pm5.16$ vs $T2=10.7\pm5.43$; $p<0.05$). Both groups showed a significant reduction in mucosal oedema and nasal secretions. Patients treated with HA Spray-sol reduced or even eliminated (11/25 patients) the use of topical decongestant within 10 days of treatment with HA. The results of this study suggest nebulized topical 9-mg sodium hyaluronate plays a pivotal role in the management of RM.

Nasal decongestants are commonly used to relieve congestion in patients with nasal obstruction such as in acute and chronic rhinitis, rhino-sinusitis and nasal polyposis. Topical nasal decongestants operate through the stimulation of α -adrenergic receptors causing vasoconstriction, reducing congestion of the mucosa and providing rapid reduction of obstructive symptoms (1). Vasoconstriction induced by topical decongestants is usually followed by rebound congestion (or vasodilatation) and tachyphylaxis

(reduced efficacy of a drug dosage after repeated use) (2).

RM is a pathological condition of the nasal mucosa induced by prolonged, excessive or improper use of topical decongestants and characterized clinically by vasodilation “rebound”, mucosal oedema and “tachyphylaxis” with progressive loss of efficacy of topical decongestants with a reported incidence in otolaryngology clinics from 1% to 7% (3). The first criteria for the diagnosis of RM were proposed in

Key Words: sodium hyaluronate, rhinitis medicamentosa, nasal decongestants, nebulization

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1952 and included: 1. A history of prolonged use of nasal medication 2. Constant nasal obstruction 3. Poor narrowing of the nasal mucosa. Since then, several criteria have been used to try to characterize RM, however, to date no validated set of criteria has been created. Clinical rhinoscopic examination is non-specific, showing areas of red, thickened mucosa and dull zones. Microscopic examination demonstrated epithelial metaplasia with epithelial hyperplasia, loss of ciliated cells, arteriolar dilatation and an increase in mucus-secreting cells and perivascular oedema of the submucosal glands in patients with RM (1). Patients with RM typically complain about severe nasal congestion with associated itching, burning, epistaxis, mucosal dryness (with crusting), nocturnal snoring, xerostomia, predisposition to pharyngitis, insomnia and daytime sleepiness (4). The “ischaemic state” of RM, due to the rapid reduction of the blood supply to the nasal mucosa, is responsible for damage to the nasal mucosa, including loss of cilia, ulceration, mixed inflammatory cell infiltration, increased swelling of the submucosal glands and goblet cell hyperplasia (5).

Moreover, nasal decongestants can be absorbed by the nasal mucosa and cause systemic side effects (1), primarily: cardiovascular, central nervous, endocrine and ocular side effects (6). The goal of RM treatment is the cessation of topical decongestants with control of symptoms, although abrupt and immediate suspension is poorly tolerated by the patient. There is no standard treatment for RM and the patient often needs to concurrently take various drugs such as topical corticosteroid, systemic decongestants, alone or in combination with antihistamine in order to stop the abuse of decongestants (7).

Recently, both our group (8) and other researchers (9) published several works which outline the therapeutic properties of hyaluronic acid (HA) administered topically at the level of the upper respiratory tract, thanks to its hygroscopic properties, non-antigenicity, viscoelastic and anti-inflammatory (10) properties and an ability to adjust the fluidity of secretions (11-19).

The aim of the study is to evaluate the efficacy of HA nebulized using a new device, specifically

developed for the nasal cavities, called “Spray-sol” in patients diagnosed with RM.

MATERIALS AND METHODS

Thirty adult patients (mean age of 43.1 years; range 26-73) with RM were prospectively enrolled from November 2015 to June 2016 at the department of Otolaryngology of Campus Bio-Medico University. Based on scientific literature, we considered patients with RM if they had two or more of the following symptom conditions:

- Patients taking nasal decongestants three times or more a day for at least 5 weeks;
- Patients who had shown during fibroendoscopy evaluation the presence of: vasodilation “rebound” or mucosal oedema;
- Patients with “tachyphylaxis” (progressive loss of efficacy of topical decongestants);
- Perennial nasal obstruction.

Five patients declared that they were taking nasal decongestants twice daily, 17 patients 3 times a day, 4 patients 4 times a day, 3 patients 5 times a day and 1 patient 6 times a day. The study, as defined in the Helsinki Convention, was approved by the Ethics Committee of the University Campus Bio-Medico in Rome (Protocol: 31.15 TS ComEt CBM) and all patients signed a written informed consent.

Patients treated with immunotherapy and corticosteroids or patients suffering from asthma, nasal polyposis, marked septal deviation, infections of the upper respiratory tract within the last 14 days, pregnant women and patients with signs and symptoms of systemic diseases were excluded.

Therapeutic schedule

After recruitment (T0) patients were treated with HA (Yabro: 3 ml of HA dissolved in 2 ml of isotonic solution IBSA farmaceutici Lodi, Italy) nebulized via Spray-sol twice a day (morning and evening) for 10-days (T1, HA spray-sol tp). After 3 days of washout the same patients were treated with saline solution nebulized via Spray-sol twice a day (morning and evening) for 10 days (T2, saline spray-sol tp) (Fig. 1). In order to evaluate improvement to quality of life, the groups were asked to answer 2

questionnaires:

- **Global Rhinitis Score (GRS):** each patient was asked to fill out a daily diary to estimate the severity of their main RM symptoms. The main evaluated symptoms were headache, nasal congestion, nasal secretion and xerostomia. Each symptoms' score ranges from 0 (no symptoms) to 10 (severe symptoms); all the main symptoms were grouped in the *Global Rhinitis Score (GRS)*. The mean GRS score was from 0 (most comfortable) to 30 (severe symptoms, dissatisfying situations). In the same diary, the patients were asked to mark the number of decongestants they administered daily, during HA or saline treatment. This questionnaire was created by our research group and differs from the universally recognized questionnaire (SNOT-22, VAS) because it brings together and highlights the most common symptoms typically referred to by patients with RM and assesses

their improvement after the treatment with HA (Fig. 2).

- **Visual Analogue Scale (VAS) questionnaire,** which includes questions about generic symptoms of nasal obstruction, in particular: headache, nose congestion, runny nose, secretion from the nose into the throat, feeling of fullness in the face and face pain. The VAS scale range was from 0 (most comfortable) to 10 (severe symptoms, dissatisfying situations).

The nasal fibroendoscopic examination was performed using a flexible nasal fiberendoscope 3.5 mm Rhino-Pharyngo-Laryngo-Fiberscope, STORZ, Tuttlingen, Germany).

The findings scored were related to mucosa oedema, congestion and nasal secretions (0: none; 1: mild; 2: moderate; 3: severe).

Anterior active rhinomanometry (AAR) measures trans-nasal pressure and airflow separately in each nostril during quiet, tidal breathing in the

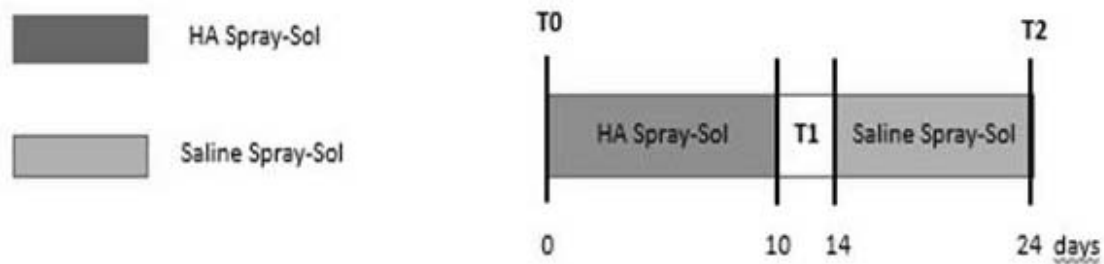


Fig. 1. Therapeutic schedule.

Please get a score from 0 (no symptoms) to 10 (severe symptoms).

How many times a day do you use nasal decongestant?

HEADACHE	
NASAL CONGESTION	
NASAL SECRETION	
XEROSTOMIA	
GLOBAL SYMPTOMS	

Fig. 2. Global rhinitis score (GRS).

sitting position. AAR was performed according to the International Committee for the Standardization of Rhinomanometry guidelines (20) using a GM Instrument (Glasgow, U.K.) NR6 rhino-manometer. A small plastic catheter was inserted through a pierced piece of sponge connected to a flexible silicone tube leading to the pressure port of the meter. The sponge was placed across the contralateral nostril to measure the nasopharyngeal pressure, taking careful not to interfere with the nostril while being tested. The tube comes out of a transparent mask covering the patient's nose and mouth tightly during the test. For each nostril, inspiratory nasal resistance is calculated over four inspiratory–expiratory cycles at a fixed pressure of 150 Pa. Resistance of >0.35 Pa/cm³ per second was considered pathological (21).

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences Software (SPSS 10.0 for Windows, SPSS Inc., Chicago, IL, USA). Data are shown as mean and standard deviation.

Parametric (Student's *t*-test) and non-parametric (Wilcoxon test for paired data and Mann-Whitney

U test for non-paired data) tests were used to compare different values. Our criterion for statistical significance was set at *p* values of less than 0.05 (2-tailed).

RESULTS

Of the 30 patients included in the study, 25 patients completed the study (14 male and 11 female; mean age of 44 years; range 30–70). Five patients dropped out of the study: 2 were not contactable at T1 and 3 did not take part in the study due to the onset of allergic rhinitis. No side effects were recorded.

VAS score

The VAS score was statistically significantly lower in HA Spray-sol tp ($T_0=6.25\pm1.64$ vs $T_1=3.91\pm1.30$; $p<0.05$), unlike the saline Spray-sol tp, which showed the mean VAS score similar to baseline ($T_0=6.25\pm1.64$ vs $T_2=5.062\pm1.45$; $p>0.05$ $p=0.05$) (Fig. 3a).

Global rhinitis score

HA Spray-sol tp significantly and quickly improved nasal complaints as shown in the GRS questionnaire

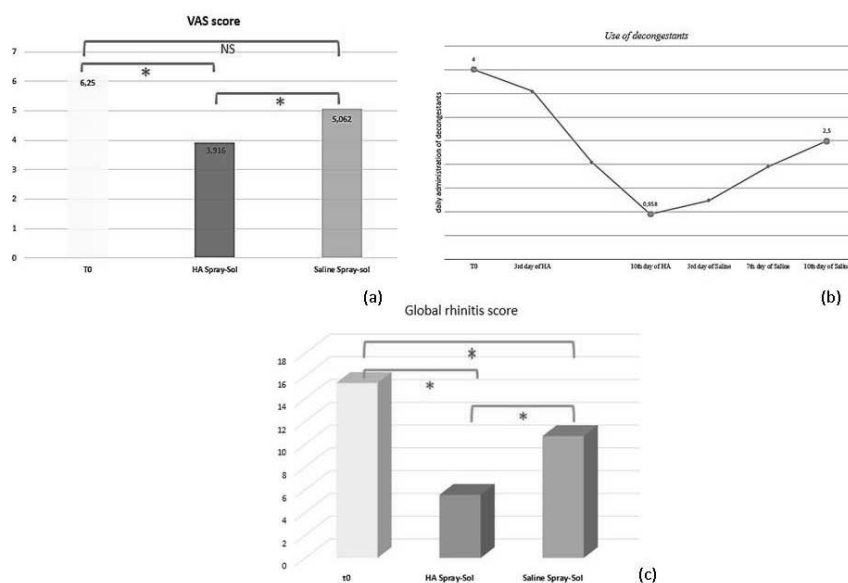


Fig. 3. Subjective evaluation: a): VAS score; b): Use of decongestants; c): Global rhinitis score.

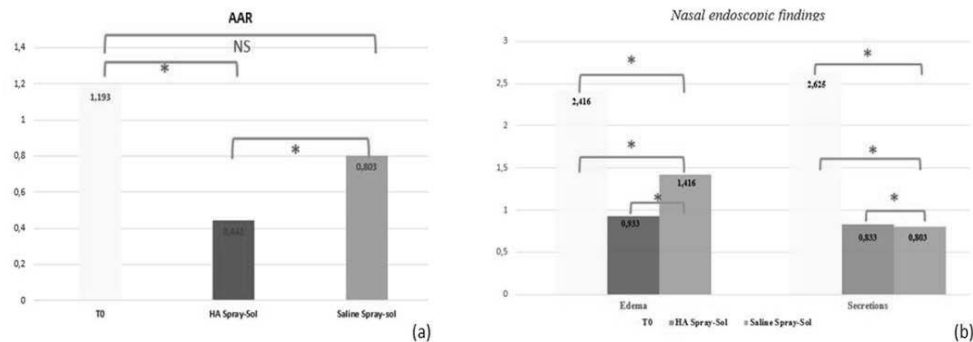


Fig.4. Objective evaluation: a): nasal endoscopic findings; b): AAR.

score ($T0=15.37\pm5.16$ vs $T1=5.54\pm3.23$, $p<0.05$ $p=0.000045$). We noted a significant improvement also in the patients treated with saline spray-Sol tp ($T0=15.37\pm5.16$ vs $T2=10.7\pm5.43$; $p<0.05$ $p=0.00006$), but in the group treated with HA spray-sol tp the improvement was significantly greater ($T1=5.54\pm3.23$ vs $T2=10.7\pm5.43$; $p<0.05$ $p=0.004$) (Fig. 3b).

Use of decongestants

Patients treated with HA Spray-sol tp reduced or even eliminated (11/25 patients) the use of topical decongestant within 10 days of treatment ($T0=4\pm1.53$ vs $T1=0.958\pm0.806$, $p<0.05$ $p=0.0000000003$).

Eleven out of 25 patients stopped the use of topical decongestants, in particular, two of these were taking vasoconstrictor twice a day, and eight, three time a day.

The use of topical decongestants was lower at T2 in comparison with T0 ($T0=4\pm1.53$ vs $T2=2.5\pm0.884$; $p<0.05$ $p=0.000000009$), but the reduction in HA Spray-Sol tp was statistically significantly greater ($T1=0.958\pm0.806$ vs $T2=2.5\pm0.884$; $p<0.05$) (Fig. 3c $p=0.0001$).

Endoscopic score

The reduction of mucosal oedema of the middle turbinate showed a statistically significant improvement in the HA Spray-sol tp group ($T0=2.41\pm0.65$ vs $T1=0.833\pm0.56$, $p<0.05$ $p=0.0000000001$), the same goes for nasal secretions ($T0=2.62\pm0.64$ vs $T1=0.833\pm0.63$; $p<0.05$ $p=0.0000000001$).

Endoscopic score indicated an improvement in

the saline Spray-sol group, even though it was less than T1 (Fig. 4a).

AAR

AAR showed that there was a significant reduction in the resistance of the nasal passages in the HA Spray-sol tp group ($T0=1.193\pm0.83$ vs $T1=0.44\pm0.25$; $p<0.05$ $p=0.00001$); the resistance improvement was also noted in the saline Spray-sol tp group, although not significantly ($T0=1.193\pm0.83$ vs $T2=0.80\pm0.45$; $p>0.05$ $p=0.05$) (Fig. 4b).

DISCUSSION

Patients with nasal obstruction frequently find subjective relief with the use of topical decongestants. The abuse of these agents can lead to RM, with rebound congestion and decreased efficacy of the decongestant. There is no consensus on RM treatment and the patient is often forced to undergo a number of long and wasteful medical treatments,



Fig. 5. Spray-sol nebulizer.

often with only partial benefit and multiple side effects, both local and systemic (22).

RM patients, refractory to medical treatment, that decide to undertake nasal surgery must undergo aggressive medical treatment, including nasal steroid and oral steroid burst prior to surgery to reduce the known risk of intraoperative bleeding due to nasal mucosa changes and RM associated tachyphylaxis (23).

HA is a natural constituent of many human tissues and it can be administered either topically or through injections, these features make it a product characterized by the high level of safety. Today HA is widely used in many branches of medicine, especially in aesthetic medicine and reconstructive surgery, without any contraindications or negative interactions.

Topical HA, alone or in combination, was successfully used in upper airway inflammation in adults and children (24). It was also used as a coadjuvant treatment in acute and chronic tooth and gingival disease, in oral ulcers, to relieve symptoms in patients suffering from GERD (gastroesophageal reflux disease) and in the post-operative period after nasal and sinus surgery with encouraging results (10). The form of the drug that we used in our study was a mixture of saline solution and hyaluronic acid (25).

We thought HA had potential to treat patients with RM as, thanks to its properties, it is primarily involved in the regulation of vasomotor tone and gland secretion in the nasal mucosa, significantly contributing to mucosal host defense by stimulating ciliary clearance of foreign bodies and simultaneously retaining enzymes, which are important for homeostasis in the apical surface 3.

Ciliary motility was considered the primary study endpoint in view of the recognized role of ciliary beating as a first-line defense mechanism and of the known effects of HA in modulating ciliary activity.

HA can be delivered using a common spray mechanism but it is well known that the RM nasal changes not only affect the inferior turbinate, but all of the nasal mucosa. The spray (which dispenses particles of large diameter (about 50 μm), introducing small quantities of the substance into the nasal cavities) effectively reaches the head

of inferior turbinate and the nasal floor and only a small portion of the upper and deeper portions of the nasal cavity, therefore, not ensuring an adequate and homogeneous distribution of the substance to all the areas of the nasal mucosa, which generally appears to be completely congested in patients with RM (26).

Recently, a new device has been proposed called Spray-sol, which is able to atomize particles with a smaller diameter (around than 20 μm) than the traditional spray. This decreased diameter and the availability to modulate the amount of the substance administered allows for an adequate and homogeneous distribution of the substance throughout the entire nasal mucosa (Fig. 5).

Spray-sol is as practical and convenient as a common spray, but allows for the nebulization of smaller particles that can easily reach the entire nasal mucosa, even when administering substances with a high viscosity, such as HA. It has a very short administration time (seconds), is a single-patient product, portable, does not require an electrical power source and is easily disposable (27).

The efficacy of Spray-sol is well illustrated by our data, indicating a notable improvement also in the administration of saline solution alone in the saline Spray-sol tp. The possibility of delivering HA directly to the nasal mucosa makes its use in the medical treatment of RM more rapid and effective. The results obtained in our study showed that the administration of HA by Spray-sol can be used as a potential “weaning” therapy in patients with RM.

HA, nebulized using Spray-sol can also represent a viable therapeutic alternative to stop nasal vasoconstrictor abuse and place the patient in the “best condition” to treat the cause of chronic nasal obstruction with medical treatment, surgery or a combination of both, in the absence of side effects. The promising results from this study have lead us to initiate an investigation on the potential role of HA in combination with Spray-sol in other types of acute and chronic rhinitis, especially in gravidic rhinitis, when almost all of the drugs for rhinitis are contraindicated. Further studies on a larger scale will be needed to confirm the encouraging results obtained from this study.

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SYSTEMATIC REVIEW: THE EFFICACY OF TOPICAL HYALURONIC ACID ON ORAL ULCERS

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The management of oral ulcers is a challenge for clinicians. Whilst there is widespread use of topical corticosteroids, antibiotics and antimicrobial, there is only weak evidence for the effectiveness of any of the topical treatments. Hyaluronic Acid (HA) has been recently proposed for topical administration in the treatment of oral ulcers and other painful oral lesions. The aim of the study is to systematically review the published literature regarding all the therapeutic effects of HA on painful oral lesions such as oral ulcers and oral lichen planus. Relevant published studies were found in PubMed, Google Scholar and Ovid using a combined keyword search or medical subject headings. At the end of our study selection process, 4 relevant publications were included: two regarding oral lichen planus, one Behçet's Disease and Recurrent Aphthous ulcer and one in oral ulcers in general. Both subjective parameters such as healing period, VAS for pain and objective assessments such as number of ulcers, maximal area of ulcer and inflammatory signs, significantly improved after HA treatment. These data allow us to suggest that HA may play a pivotal role in the treatment of oral ulcers.

Oral ulcers typically manifest as a small erosion of the epidermal layer of the non-keratinized oral mucosa. They may be associated with significant pain that interferes with eating, speaking, talking, swallowing and typically persist for 7 to 14 days (1).

There are numerous causes of oral ulcerations: traumatic, metabolic, dermatological, allergic, immunological, infectious and neoplastic. However, causes may overlap and in some cases, the aetiology is unclear. In some cases, oral ulcers may be manifestations of systemic diseases such as skin, connective tissue, blood or gastrointestinal tract disorders. Oral Lichen Planus, a chronic immunological inflammatory mucocutaneous disorder, is characterized by erosive atrophic and ulcerative lesions (2). Oral or genital aphthous ulcers

are a main criterion for the Behçet's (BD) diagnosis and are often the initial sign. In addition, there is the recurrent aphthous ulcer (RAU), the most common form of recurrent oral ulcers, characterized by recurring painful ulcers of the mouth (3).

The management of oral ulcers is a challenge for clinicians, because the cause often remains unknown. The goal of treatment should be to eliminate atrophic and ulcerative lesions to alleviate symptoms and in selected cases to prevent the risk of malignant transformation (4).

Topical corticosteroids, antibiotics, antimicrobial agents and analgesics are highly recommended for patients with oral ulcers. Whilst there is widespread use of these agents, there is only weak evidence for the effectiveness of any of the topical treatments (5).

Key words: hyaluronic acid, oral mucosa, oral ulcers, oral lichen planus, aphtous ulceration and oral scars

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Longer treatment and frequent exposure to topical steroids and antibiotics may cause fungal infection and drug resistance, which may further lead to more severe adverse effects or even life-threatening complications (4). Moreover tachyphylaxis, defined as a rapidly decreasing response to a physiologically active agent after administration of a number of doses, it is not uncommon with the chronic use of the topical steroids (6).

Chlorhexidine gluconate is also frequently used for the recurrent ulceration. A recent review concluded that chlorhexidine mouthwash appears to play a role in the management of aphthous ulceration, possibly by reducing the prevalence of secondary infection, but it does not provide significant or immediate pain relief (7).

For many years natural herbal medicines have been frequently used as an alternative therapy for oral ulcers in many countries (8), but no evidence-based reviews regarding the efficacy and safety of the topical application of these medicines have been available in the literature to date (9).

Recently, HA has been developed in different formulations for topical administration in the treatment of oral ulcers and other painful oral lesions, such as in the dentistry field, where HA is already widely used in the treatment of gingivitis and periodontitis as evidenced by this recent review (10).

Hyaluronic acid (HA) is a linear polymer of glucuronic acid, N-acetylglucosamine disaccharide (10-18). It can be found in many tissues and body fluids of mammals, with the highest concentrations in connective tissue and skin (19).

It possesses various physiological and structural functions, which include cellular and extracellular interactions, interactions with growth factors and regulation of the osmotic pressure and tissue lubrication. All these functions help in maintaining the structural and homeostatic integrity of the tissue.

The hygroscopic and viscoelastic properties of HA plays a vital role in the wound healing process as confirmed by several studies such as in the healing of leg ulcers and the nasal mucosa after surgery (20). Indeed HA promotes re-epithelization via proliferation of basal keratinocytes and reduces collagen disposition and scarring (21). It plays

a pivotal role in inflammatory response, which typically characterizes the lesions of the oral mucosa.

High-molecular-weight HA suppresses the immune response preventing excessive exacerbations of inflammation. It is broken in low-molecular-weight fragments under the influence of reactive oxygen species (ROS), generated by infiltrating polymorphonuclear leukocytes and other inflammatory cells, performing a key role in signaling tissue damage and mobilizing immune cells (22, 23). Thus HA, due to its anti-inflammatory and reparative properties, seems to be useful for the treatment of oral ulcers.

The aim of the study is to systematically review the published literature regarding all the therapeutic effects of HA on painful oral lesions such as oral ulcers, oral lichen planus and other diseases related to oral ulcers, to clarify and classify the main application areas of HA, the pathophysiological basis and the schedule of HA application.

MATERIALS AND METHODS

Research and study selection

Relevant published studies were searched for in PubMed, Google Scholar and Ovid, using either the following keywords or, in the case of PubMed database, medical subject headings, such as: ("hyaluronic acid" and "oral ulcers"), ("sodium hyaluronate" and "oral ulcers"), ("hyaluronan" and "oral ulcers") and ("hyaluronic acid" and "oral lichen planus") ("sodium hyaluronate" and "oral lichen planus"), ("hyaluronan" and "oral lichen planus") ("hyaluronic acid" and "aphtous ulceration") ("sodium hyaluronate" and "aphtous ulceration"), ("hyaluronan" and "aphtous ulceration") ("hyaluronic acid" and "oral scars") ("sodium hyaluronate" and "oral scars"), ("hyaluronan" and "oral scars") with no limit selected for the year of publication .

Literature search was performed according to PRISMA guidelines with the following main eligibility criteria: only English, human controlled trials and effect size of HA evaluated clinically in patients with oral ulcers and in other common oral ulcerative lesions. Literature reviews, technical notes, letters to editors, and instructional course were excluded.

Two authors (CM and MA) independently assessed

the full-text version of each publication, making their selection on the basis of content and excluding papers that did not include the specifically required content. The reference lists of articles that meet the first criteria were fully reviewed to identify useful articles that were not included in the initial electronic search.

During the research phase in each of the journals, articles strictly coherent with the topic were identified first, while studies on animal models were excluded after the primary selection. At the beginning of our selection process, we excluded articles about the use of HA to manage chemo/radiotherapy-induced oral mucositis (n=2). Then we excluded articles about the use of HA combined with topical anti-inflammatory drug and or other drugs (n=1). At the end of our study selection process, 4 relevant publications were included (Fig. 1).

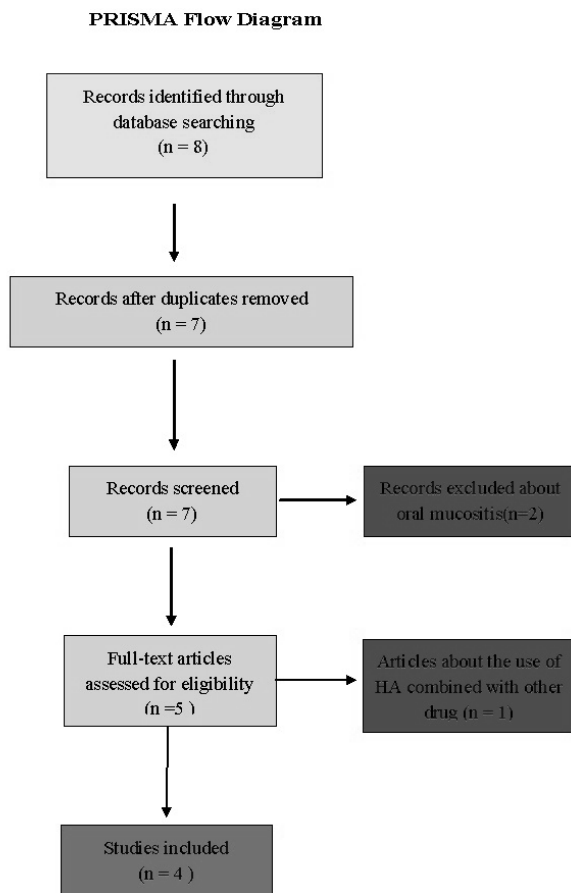


Fig. 1. *Prisma Flow Diagram.*

RESULTS

HA and oral ulcers

Nolan (6) et al. evaluated the efficacy of a topical HA gel in the management of RAU in 120 patients, 60 treated with HA gel and 60 patients with placebo gel. The patients applied the gel, two to three times per day for 7 days. On day 4, 5 patients in the study group had significantly fewer ulcers than those treated with placebo, such as the incidence of new ulcer occurrence.

Lee et al. (24) investigated the efficacy of topical HA gel on recurrent oral ulcers in 33 patients: 17 affected by BD and 16 patients affected by RAU. The patients used HA gel twice a day for 2 weeks and the subjective parameters from patients, consisting of the number of ulcers, healing period, level of pain and objective assessments consisted of the number and maximal area of ulcers and inflammatory signs (swelling, redness and local heat) were evaluated at baseline and after 2 weeks. In the RAU and BD groups, all the subjective parameters had significantly decreased statistically, while in the BD group, the number of ulcers after treatment decreased but the results had no statistical difference.

In regards to objective parameters, the number and maximal size of the ulcers had significantly decreased statistically between pre-treatment and post-treatment in the RAU group. In the BD group, only the reduction in maximal size of the ulcers was statistically significant. However, no significant differences were found between the two groups in number and maximal area of ulcers. The inflammatory signs of swelling and local heat significantly improved after treatment. The inflammatory sign of redness improved, but was not statistically significantly.

HA and Oral Lichen Planus

Nolan A. et al. (25) investigated the efficacy of a topical HA gel in 124 patients with erosive oral lichen planus; 62 patients were treated with HA gel and 62 patients with placebo gel. The patients were advised to apply the gel regularly 4 to 5 times daily for 28 days. Application of topical HA showed a significant reduction on soreness scores (valuated

Table I. *study selection and relevant publications.*

Authors	HA group	Control Group	Treatment	Parameters Evaluated	Results
HA and oral ulcers					
Nolan et Al. ¹	60 patients were treated with HA gel (0,2%)	60 patients were treated with placebo.	The patients apply two to three times per day for the next 7 days.	Patients were asked to record the discomfort arising from the ulcerated area with VAS at baseline and at 5, 10, 15, 20, 30, 45, 60, 120, 180 and 240 min after application. Also the patients wrote down in a long diary, the number of ulcers and the occurrence of any new ulcers every day during the treatment period.	Patients in both treatment groups reported a rapid reduction in their discomfort scores arising mainly for about 30 min. On day 5, patients in the HA group had significantly fewer ulcers than those treated with placebo. On day 4, the incidence of new ulcer occurrence was significantly lower in the HA group when compared with placebo treatment patients.
Lee et AL. ²	The patients were divided into two groups: a BD disease group (17 patients) and a RAU group (16 patients).		The patients used 0.2% HA gel (Gengigel®) twice a day for 2 weeks.	At baseline and after 2 weeks, the clinicians investigated the subjective parameters of patients (number of ulcers, healing period, visual analogue scale (VAS) for pain) and objective assessments (number of ulcers, maximal area of ulcer and inflammatory signs)	For the subjective symptoms, in RAU group, the number of ulcers, healing period and VAS of pain were statistically significantly decreased while in the BD group, only the healing period and VAS for pain were statistically significantly decreased. For the objective symptoms, in the RAU group, the number and maximal size of the ulcers were statistically significantly decreased while in the BD group, only the reduction in maximal size of the ulcers. Swelling and local heat were statistically significantly improved in the RAU group, but not in the BD group.
HA and oral lichen planus					
Nolan et Al. ³	62 patients were treated with HA gel.	62 patients were treated with placebo gel.	The patients were advised to apply the drug regularly 4-5 times daily for 28 days.	Recordings about soreness relief with VAS were made before the beginning of treatment and at the first visit at 5 min, 60 min, 2, 3 and 4 h post-applications. The patients completed a long diary, noting soreness scores at 5 and 60 min after application and oral function once a day. The size of erosive/ulcerative area was evaluated at the first visit and on day 15 and day 29.	Application of topical HA showed a significant reduction on soreness scores compared with baseline from 5 min after application to 4 hours when compared with placebo. Furthermore HA group showed a significant reduction in the size of the erosive/ulcerated area after 29 days of treatment when compared with baseline.
Shetty RR. et Al. ⁴	25 patients received topical 0.2% HA gel	25 patients received topical placebo gel	The patients were advised to apply the drug three times daily after food for 14 days.	Subjective symptoms: soreness score evaluated by VAS and Objective symptoms like degree of erythema and mean area of the lesion, were recorded preoperatively and on day 7, day 14, day 21 and day 28.	HA group showed a highly statistically significant reduction on soreness evaluated by VAS score than placebo group. In 8 patients of the study group the pain or the burning sensation disappeared and in 5 patients reduced substantially between 7 and 21 days. Also HA group showed a highly statistically significant reduction in degree of erythema and in the mean area than placebo group at the end of Day 14, Day 21 and Day 28, while no statistically significant difference was seen at the end of day 7 comparing the two groups.

with VAS) compared with baseline from 5 m after application to 4 h when compared with placebo.

Furthermore, HA group showed a significant reduction in the size of the erosive/ulcerated area after 29 days of treatment when compared with baseline. There was no difference between treatment groups with respect to oral function (difficulty in eating).

Shetty RR, et al. (26) evaluated the relief of symptoms and reduction in the size of oral lichen planus ulcers with HA (25 patients used HA gel and another 25 patients received topical application of placebo). The patients were advised to apply the gel 3 times daily after meals for 14 days.

The clinicians investigated subjective symptoms

with VAS and the objective symptoms (with degree of erythema and mean area of the lesion) at baseline and on day 7, 14, 21 and 28.

HA group showed a highly statistically significant reduction on soreness evaluated by VAS score than placebo group. In 8 patients of the study group, the pain or the burning sensation disappeared and in 5 patients it reduced substantially between 7 and 21 days. The HA group Also showed a highly statistically significant reduction in degree of erythema and in the mean area compared to placebo group at the end of day 14, 21 28, while no statistically significant difference was seen at the end of day 7 between the two groups.

DISCUSSION

Topical medications appear to be the first choice treatment for oral ulcers. Currently there are several topical agents for the treatment of oral ulcers such as steroids, antibiotics, analgesics and natural herbal medicines, but no strong evidence is available (27, 28). Moreover, there is no codified treatment about the drugs employed, the frequency of administration and the duration of treatment. The data, which have emerged from our analysis of the literature, allow us to suggest that HA may play a role in the treatment of oral ulcers.

Patients treated with topical oral HA experienced an immediate and sustained reduction of symptoms, such as pain from ulcerated area and an improvement in oral function particular in the eating. Both subjective parameters such as healing period, VAS for pain and objective assessments such as number of ulcers, maximal area of ulcer and inflammatory signs, significantly improved after HA treatment (6, 24-26).

HA is a non-sulfated glycosaminoglycan found in the extracellular matrix, which plays a multifunctional role in scar-free wound healing and in physiology in the oral cavity.

The main “anti-ulcers” mechanism of HA are:

- Reduction of inflammatory signs of ulcerated area (swelling, redness and local heat): HA suppresses the immune response preventing excessive exacerbations of inflammation performing a powerful antioxidant effect through

free-radical agents (23, 29);

- Reduction of the healing period: HA with its hygroscopic and viscoelastic properties plays a vital role in the wound healing process, promoting re-epithelization via proliferation of basal keratinocytes and reducing collagen disposition and scarring. In the skin and perhaps on the oral mucosa, this property is likely to be relevant in controlling tissue hydration during periods of change such as the inflammatory process or response to tissue injury;
- Lower frequency of ulcer recurrence: HA could be a barrier or protective mechanism from stimuli arising in the oral environment, indeed its highly viscous native contributes to retardation of viral and bacterial passage through the HA-rich pericellular zone (30, 31) .

The advantage of HA compared to steroids is that it is safe in all patients including infants and pregnant women, whom are reluctant to use steroids. There is no risk of overdose and can be safely recommended to individuals who may not follow instructions easily. It does not cause any discomfort, making it acceptable to children (6). Moreover, it is known that HA could be used in association to corticosteroid, topically enhancing the effects of corticosteroid as shown in the treatment of nasal chronic inflammatory diseases (32).

The schedule of the treatment still remains to be clarified, including time of treatment, number of times that the gel should be applied, the type of administration (spray, gel, crème, nebulization, and so on) and the therapeutic schedule.

Further laboratory-based research and large-scale randomized controlled clinical trials on a larger scale are advisable to confirm these promising results.

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THE EFFICACY OF TOPICAL HYALURONAN IN RHINOSINUSITIS: A SYSTEMATIC REVIEW

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Rhinosinusitis is one of the most common inflammatory conditions of the nasal cavity and paranasal sinuses and is one of the most common causes of absence from work and for visits to the family doctor. The treatment strategy in both acute rhinosinusitis (ARS) and chronic rhinosinusitis (CRS) is to reduce the severity of the symptoms, minimize the duration of the disease and prevent complications. Topical therapy has become an important tool in otolaryngologists' armamentarium for rhinosinusitis treatment. Recently, topical hyaluronic acid (HA), the major component of many extracellular matrices that promotes tissue healing, including activation and moderation of the inflammatory responses, cell proliferation, migration and angiogenesis, has been proposed for ARS and CRS adjuvant tool. The aim of the study is to systematically review the published literature regarding all the therapeutic effects of HA on the ARS and CRS. Relevant published studies were found in PubMed, Google Scholar and Ovid, using a combined keyword search or medical subject headings. At the end of our study selection process, 5 relevant publications were included: 2 of them investigated the potential role of HA in reducing symptoms and preventing exacerbations of CRS in adult population, two of them in paediatric patients affected by upper respiratory tract infections and one of them in cystic fibrosis patients with bacterial rhinopharyngitis. Data deriving from the present review of 5 clinical studies showed that the use of topical HA represents a relevant therapeutic advance in rhinosinusitis to minimize symptoms and prevent reactivation with a significant improvement of their quality of life, as it avoids systemic side effects and increases local drug activity. Further studies on larger populations and with new specific nebulization devices for upper airway are needed to confirm these encouraging results.

Rhinosinusitis is an inflammatory condition of the nasal cavity and paranasal sinuses and represents a heterogeneous group of diseases, with different underlying etiologic factors and with various clinical manifestations (1). This inflammation is associated with typical clinical pattern characterized by major and minor symptoms (Table I), which results in dramatic reductions in overall well-being and quality of life (QOL).

Based on the duration of symptoms, rhinosinusitis can be classified in acute rhinosinusitis (ARS), chronic (CRS) and recurrent (rCRS), which is differentiated from chronic rhinosinusitis (CRS) (Table II).

ARS has a relatively rapid onset and is normally of a four-week duration or less. Most cases are viral in origin and the resolution of symptoms usually occurs within 5 to 7 days without any specific

Key words: hyaluronic acid, acute rhinosinusitis, chronic rhinosinusitis, rhinosinusitis

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DISCLOSURE: THE FIRST AND THE LAST AUTHORS ARE INVENTORS
OF SPRAY-SOL DEVICE AND PARTNERS OF BRIO SRL. ALL OTHER
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medication (2). Bacterial ARS presents more severe symptoms including purulent drainage that worsens after 5 days or persists beyond 10 days and/or with symptoms that are out of proportion to those typically associated with a viral upper respiratory process. Recurrent ARS is defined as four or more episodes of acute disease within a 12-month period, with resolution of symptoms between each episode (each episode of a duration of at least seven day).

CRS is distinguished by symptoms that persist for 12 weeks or more and it has been classified into two different phenotypic forms: CRS with nasal polyps (CRSwNP) and CRS without nasal polyps (CRSsNP).

Rhinosinusitis is a significant health problem and one of the main reasons for a visit to primary care clinics. Sinusitis has a significant impact on quality-of-life measures (e.g. study SF-36) with decrements in overall perception of health, vitality and social functioning, comparable with those observed in patients who have angina or chronic obstructive pulmonary disease. Sinusitis is one of the main reasons for which patients take antibiotics and lose productivity in the workforce. Recent studies estimate that CRS results in 18 to 22 million office visits to physicians in the United States each year (3).

The treatment strategy in both ARS and CRS is to reduce the severity of the symptoms, minimize the duration of the disease, control infection especially bacterial and prevent complications and further development into a chronic disease in ARS (4). The available rhinosinusitis medical treatments comprise antibiotics, corticosteroids and topical therapy; however, their efficacy is still debated (5).

Oral antibiotics are considered therapeutic options in bacterial ARS and in selected CRS cases, whereas oral steroids are strongly recommended for short-time management of CRS with nasal polyps and in the perioperative period. Existing evidence suggests that topical therapies, such as sinonasal saline irrigations and topical nasal steroids, have become an integral component in the management of both ARS and CRS (6).

However, for patients with medically resistant CRS and nasal polyps and in selected complicated

ARS, the surgical approach (7), particularly functional endoscopic sinus surgery (FESS), represents the best treatment of choice (8).

Recently, among topical therapeutical tools, Hyaluronic Acid (HA) is a promising molecule that has been recently proposed as adjuvant treatment in the ARS and CRS in adult and children population to minimizing symptoms, promoting recovery and preventing exacerbations (9).

HA is a glycosaminoglycan, synthesised by the cellular plasma membrane and highly concentrated in the extracellular matrix (10-18). Its particular binding mechanisms and architectural configuration within the connective tissue have effects on stability, lubrication, water homeostasis, molecule filtering and cell behaviour modification (such as anti-inflammatory modulation) (19).

Thanks to its hygroscopic, viscoelastic and mucoadhesive properties, and its high immunological and toxicological safety, it is used in many branches of medicine such as cosmetic surgery, ophthalmology, orthopaedics, general surgery and gynaecology (20).

Furthermore, there is evidence that the application of HA aids in the healing of acute and chronic wounds and burns (21, 22), it also augments mucociliary clearance and has been shown to reduce the frequency of acute exacerbations of chronic bronchitis (23). Recent studies have supported the role of HA in improving endoscopic and cytological parameters of ARS and CRS (24).

The aim of our work is to systematically review the published literature regarding all the potential therapeutic effects of HA, alone or in combination, in treatment of rhinosinusitis in adults and children and evaluate the effectiveness of HA in children affected by cystic fibrosis.

MATERIALS AND METHODS

Research and study selection

Relevant published studies were searched in Pubmed, Google Scholar and Ovid using either the following keywords or, in case of Pubmed database, Medical Subject Headings: ("sodium hyaluronate" and "rhinosinusitis"), ("sodium hyaluronate" and "acute rhinosinusitis"), ("sodium hyaluronate" and "chronic rhinosinusitis"),

(“sodium hyaluronate” and “upper airways”), (“sodium hyaluronate” and “sinusitis”), (“sodium hyaluronate” and “cystic fibrosis”) with no limit for the year of publication. Only studies in English, published in peer-reviewed journals, reporting data on the role of the topical administration of HA in the treatment of rhinosinusitis were included.

Literature reviews, technical notes, letters to editors, and instructional course were excluded. Two authors (CM and VP) independently assessed the full-text version of each publication, by selecting that based on its content and excluding papers without the specific content. The reference lists of the selected articles were fully and accurately reviewed to identify articles not included during the first electronic search.

Considering all the journals, articles strictly coherent with the topic were firstly identified and studies on animal models after a primary selection were excluded. Studies that were based on the use of HA resorbable nasal packing or dressing and HA after functional endoscopic sinus surgery were not included either. Furthermore, any scientific papers regarding invasive administration of HA

were not considered for the aims of our research.

We excluded 4 studies on the use of spray containing HA after functional endoscopic sinus surgery, 9 studies on the use of HA resorbable nasal packing in middle meatus after functional endoscopic sinus surgery and 1 study on invasive administration of HA. At the end of our selection process, 5 relevant publications were included as shown in Fig. 1 and Table III.

RESULTS

Five clinical studies, which investigated the role of HA, have been included in the present review, as below reported.

Two of them investigated the potential role of HA in reducing symptoms and preventing exacerbations of CRS in adult population, two of them in paediatric patients affected by upper respiratory tract infections, one of them in cystic fibrosis patients with bacterial rhinopharyngitis.

HA in adult CRS

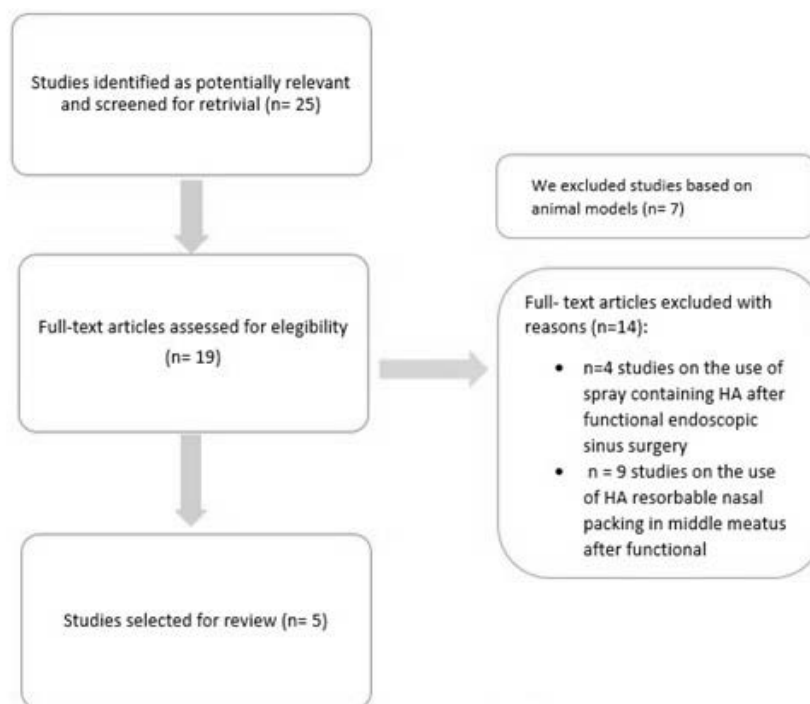


Fig. 1. Flow chart of study selection.

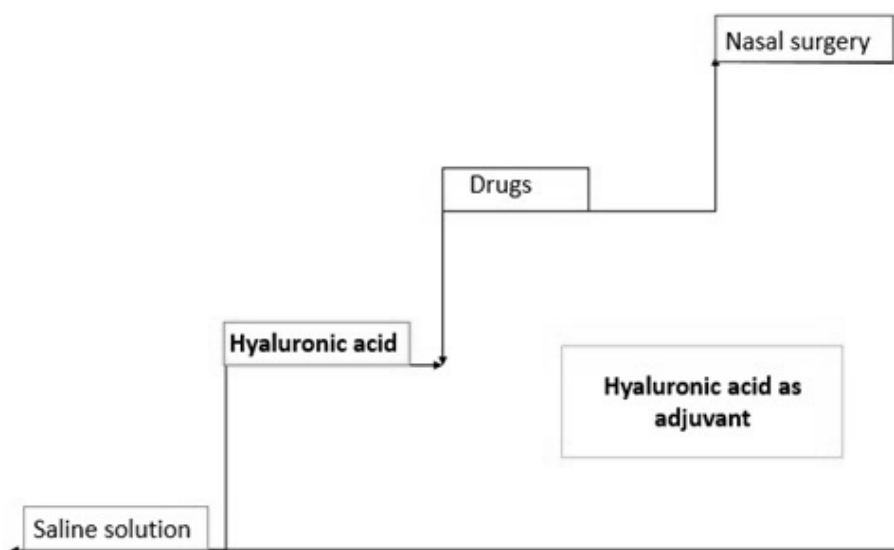


Fig. 2. Use of HA.

Casale et al. (24) evaluated the role of nebulized HA given twice a day for 10 days per month over 3 months through Rinowash and only saline solution (5 cc) twice a day in the last 20 days of the month, to minimize symptoms and prevent exacerbations of CRS compared with simple nasal washes with saline solution.

They proved that HA significantly improved quality of life in patients with sino-nasal symptoms in the CRS questionnaire score. The analysis of endoscopic evaluation showed that the combination of HA and Saline Solution (NS) was more effective than NS alone in improving mucosal edema of the middle turbinate and nasal secretions.

Another study written by Cassandro et al, (25) on CRS with nasal polyposis compared the effectiveness of nebulized NS compared to intranasal corticosteroid spray (ICS) or nebulized HA or both ICS and nebulized HA.

Results at 1 month, 3 months and 3 months after treatment showed that the addition of nebulized HA to ICS spray produced a rapid and significant improvement in nasal symptom, endoscopic scores, such as in radiological, rhinomanometry and saccharine clearance test in comparison to control group.

Di Cicco et al. (26), evaluated the changes in symptoms of nasal obstruction (mucopurulent rhinorrhea, hyposmia/anosmia, snoring, headache/facial pain), endoscopic assessment (status of nasal mucosa, mucopurulent secretion of osteomeatal complex, hypertrophy of the left and right turbinate), positive *Pseudomonas aeruginosa* and positive *Staphylococcus aureus* on sputum or nasal swab in children affected by cystic fibrosis (CF).

The analysis of endoscopic evaluation showed that the combination of HA and Tobramycin was more effective than HA alone in improving the status of the nasal mucosa and in reducing nasal mucopurulent secretions and in improving ENT symptoms (hyposmia/anosmia and headache/facial pain). The treatment was well tolerated without relevant side effects. More patients in the HA and tobramycin group had negative nasal swab culture for *S. aureus* at the end of treatment, while all the patients with positive nasal swab culture for *P. aeruginosa* showed a reduction of bacterial load.

HA in children CRS

Macchi et al. (27) were aimed at evaluating the effects of nasal washes with 9 mg nebulised sodium hyaluronate given for 15 days per month over 3

months in pediatric patients with recurrent upper respiratory tract infections (URTI). Ciliary motility was the primary study endpoint and was assessed by the contrast-phase microscopy analysis. Evaluation was based on a 0-3 point rating scale (0=absent, 1= $\leq$ 5 min, 2= $>$ 5 and $<$ 10 min, 3= $\geq$ 10 min). A semi quantitative scale was also used for the assessment of adenoid hypertrophy, presence of neutrophils, eosinophils, mast Cells and bacteria. They also evaluated the presence of rhinitis, post-nasal drip, basal dyspnea, mycetes and biofilm. The study demonstrated that intermittent HA treatment with 9 mg of sodium hyaluronate with nasal washes was useful in pediatric patients suffering from recurrent upper respiratory tract infections. In particular, the HA group had a significant improvement in ciliary motility, cytological, microbiological, endoscopic and clinical outcomes compared to saline group. The results also showed a significant adenoid size reduction and a lower presence of bacteria and neutrophils, both assessed through direct evaluation with optical microscopy.

Varricchio et al (28) showed that intranasal HA might be able to improve topical antibiotic efficacy in children with bacterial acute rhinopharyngitis. For each child, clinical examination, including assessing of complaints perception, rhino-pharyngoscopic examination, and nasal cytology were performed. They evaluated the effects of 125 mg of thiamfenicol plus 4 mL HA 0.2% plus xylitol 5% twice daily, administered by the nasal device Rinowash compared to control group treated with 125 mg of thiamfenicol diluted in 4 mL of saline isotonic solution, twice daily. The study showed that the combination of thiamfenicol plus HA induced a significantly reduction of VAS, neutrophil count, bacteria count, symptoms and infection parameters.

DISCUSSION

Many different treatment options for both ARS and CRS exist. Topical therapy has become an important tool in the otolaryngologist's armamentarium for rhinosinusitis, offering the potential benefit of high local concentration of drug at the specific site with low blood levels and hence, low potential for

systemic side effects and it is considered, with systemic therapies, an essential part of the medical treatment of ARS and CRS.

Several different topical therapies, alone or in association, could be proposed with a different level of effectiveness (Fig. 2).

HA is a versatile, biocompatible, biodegradable, and mucoadhesive biopolysaccharide (29). It has unique chemical-physical properties and is characterized by numerous biological functions that led to its use in different medical, pharmaceutical, and cosmetic applications (30). HA is a naturally occurring biopolymer, especially concentrated in soft connective tissue extracellular matrix, skin dermis, vitreous of the eye, hyaline cartilage, synovial joint fluid, disc nucleus and umbilical cord. It is also present on the airway surface, being an important component of normal airway secretions and plays an important role in the homeostasis in the upper airway (1).

HA acts in the nasal mucosa, regulating the vasomotor tone and significantly contributes to mucosal host defence by stimulating ciliary clearance of foreign bodies and retains enzymes that are important for homeostasis. Its high concentration in the upper and lower airways positively influences bio-mechanical forces, hydric balance, cellular functions, activity of growth factors and cytokine behaviour (31), thus suggesting that HA may play a therapeutic role in many respiratory diseases (32).

Thanks to its properties it has already been widely proven that the use of HA alone is useful in the treatment of allergic rhinitis, non-allergic rhinitis and in the postoperative phase of patients undergoing FESS (33-35).

Literature data that emerged from our analysis allow us to affirm that topical HA is a valuable therapeutic aid which can be used alone or in combination with other drugs for the treatment of rhinosinusitis in adult and paediatric population, especially in the treatment of CRS.

The study carried out by Casale et al. (24) showed that HA can be useful as adjuvant treatment in association with Saline Solution to minimize symptoms in patients suffering from CRS and to reduce the risk of exacerbations.

Table I. *Criteria for diagnosis of rhinosinusitis. At least 2 major criteria or 1 major + 2 minor criteria.*

Major symptoms	Minor Symptoms
• Purulent anterior nasal discharge	• Headache
• Purulent or discolored posterior nasal discharge	• Ear pain, pressure or fullness
• Nasal congestion or obstruction	• Halitosis
• Facial congestion or fullness	• Dental Pain
• Facial pain or pressure	• Cough
• Hyposmia or anosmia	• Fatigue
• Fever	

Table II. *Classification of rhinosinusitis.*

CLASSIFICATION	DURATION
ACUTE (ARS)	7 days to ≤ 4 weeks
SUBACUTE	4-12 weeks
RECURRENT ACUTE	≥ 4 episodes of ARS per years
CHRONIC (CRS)	≥ 12 weeks

In patients affected by CRS with nasal polyposis, the association of HA with topical corticosteroids has proved a valuable treatment for improving HR-QoL, nasal symptom score and nasal endoscopy scores (such as secretions and mucosal oedema); considerable improvement was also observed in radiologic, rhinomanometry and saccharine clearance scores. These data were confirmed by Cantone et al. in 2016, the study showed that the supplementation of HA to standard corticosteroid seems to be strongest in patients with allergic rhinitis associated with CRSwNP (36).

The therapeutic effect of HA was also validated in children with recurrent upper respiratory tract infections and as shown by Varricchio (28), the association with thiamphenicol is effective and safe in treating children with bacterial acute rhinopharyngitis.

The importance of HA was also evaluated in the treatment of severe pediatric diseases such as first cystic fibrosis. Children with this disease require daily intake of hypertonic solution in order to improve quality of life, lung function and reduce pulmonary exacerbations but some adverse events such as cough, narrowing of the airways and unpleasant salty taste are described, limiting its application.

Moreover, topical HA plus hypertonic saline solution significantly improved tolerability and pleasantness compared to hypertonic saline alone, and reduced adverse effects. In addition to these important results, it was also demonstrated that children affected by cystic fibrosis with bacterial rhinosinusitis treated with HA and tobramycin had improvement of ENT symptoms, nasal mucopurulent secretion, bacterial load and the status of the nasal mucosa.

Table III. *Classification of rhinosinusitis.*

Authors	Study design	Patients distribution	Mean age (years)	Disease	Therapy	Efficacy parameters	Results
Casale M, et al.	Prospective, open label, randomized, controlled, parallel group	39 Patients: HA group (21); Control group (18)	HA group (44); Control group (38)	Patients affected by CRS	HA group received HA (3ml of HA were dissolved in 2 ml of isotonic solution) twice a day for 10 days per months over 3 months by Rinowash. The control group received standard saline solution (5 cc) by Rinowash twice a day for 3 months	CRS questionnaire about main CRS symptoms shown. Endoscopic nasal examination performed by a flexible nasal fibroscope. The findings were scored with respect to middle turbinate edema and nasal secretions.	HA significantly improved quality of life in patients with sinonasal symptoms in the CRS questionnaire score, unlike control group that showed post-treatment CRS questionnaires similar to the pre-treatment questionnaire. Both the reduction of mucosal edema of the middle turbinate and the reduction of nasal secretions were statistically significant in the HA group.
Cassandro E, et al.	Prospective, open label, randomized, controlled, parallel group	80 Patients: 4 treatment groups of 20 patients	group 1 (38); group 2 (38); group 3 (39); group 4 (39)	Patients affected by CRS and nasal polyps undergoing FESS	group1 nebulized saline aerosol therapy bid; group2 intranasal corticosteroid spray mometasone furoate nasal spray 200 µg dbid; group3 nebulized HA: aerosol therapy with 3ml HA 9mg and 2 saline bid; group4 intranasal corticosteroid spray + nebulized HA. The treatment period was 3 months.	Nasal symptom score, endoscopic score, radiologic score, rhinomanometry and saccharine clearance tests before, during and after the treatment period.	Significant improvements in nasal symptom score, endoscopic score, radiologic score, rhinomanometry and saccharine clearance test were observed in the nHA, ICS and in ICS+nHA group after 1 month of treatment, these effect were also seen after 3 months after treatment. The treatment ICS + nHA appeared to be the most effective therapy, reaching a statistically improvements in the control of nasal symptoms.
Di Cicco M et al.	Prospective, double blind, randomized, controlled, parallel group	27 Patients: HA group (14); Control group (13)	HA+ tobramycin group (16); HA group (15)	Patients affected by cystic fibrosis and nasal infection (pseudomonas aeruginosa and/or staphylococcus aureus)	HA group: The test nasal spray formulation consisted of a 10-mL aqueous solution containing 0.2% HA and 3% tobramycin sulphate. Control group: nasal spray formulation was identical in presentation but contained only 0.2% HA. Each patient nebulized 100 µL of the assigned product into each nostril 3 times a day for 14 Days.	Endoscopic evaluation (eutrophic, hyperemic, dystrophic nasal mucosa, mucopurulent secretion, hypertrophy of the left and right turbinate). Symptoms of nasal obstruction (mucopurulent rhinorrhea, hyposmia, /anosmia, snoring, headache, /facial pain). Positive pseudomonas aeruginosa and positive staphylococcus aureus (sputum, nasal swab).	Combination of HA and tobramycin reduced hyposmia/anosmia, headache/facial pain, and nasal mucopurulent secretion, and improved the status of the nasal mucosa. More patients in the HA and tobramycin group had negative nasal swab culture for S. aureus at the end of treatment, while all the patients with positive nasal swab culture for P. aeruginosa showed a reduction of bacterial load.
Macchi A. et al.	Prospective, open label, randomized, controlled, parallel group	75 Patients: HA group (38); Control group (37)	HA group (6); Control group (7)	Recurrent upper respiratory tract infections	Selected patients started aerosol therapy with nasal washes. Intermittent treatment was administered twice daily for 15 consecutive days per month, for three consecutive months. Patients in HA group received 9 mg vial of HA plus 3 ml of saline solution, while patients in control group were only treated with 6 ml of saline solution.	Dichotomic variables: (1) rhinitis (2) post nasal drip (3) nasal dyspnea (4)mycetes (5) biofilm. Quantitative variables: (1) ciliary motility (2)adenoid hypertrophy (3)presence of bacteria (4)neutrophils (5)eosinophils (6) Mast cells	Treatment with HA was associated with Significant improvements compared to saline solution alone for rhinitis, nasal dyspnea and biofilm. The results showed significant differences which favoured HA group for ciliary motility, adenoid hypertrophy, presence of bacteria and neutrophils.
Varricchio A. et al	Randomized, single-blind, two arm parallel-group	51 Patients: Group A (24); Group B (27)	Group A (6 ± 2.4 years), Group B (5.8 ± 1.9)	Children with bacterial Acute Rhinopharyngitis	Group A : patients treated with 125 mg of thiamphenicol diluted in 4 ml of saline isotonic solution twice daily. Group B : patients treated with 125mg of thiamphenicol plus 4 ml of sodium hyaluronate 0,2 % plus xylitol 5% twice daily administered by Rinowash	VAS, spotting nasopharyngeal, nasal cytology (neutrophils, bacteria)	Thiamphenicol plus HA can significantly induce a greater effect on VAS, neutrophil count and bacteria count than thiamphenicol alone.



Fig. 3. *Spray-sol nebulizer.*

Currently the use of topical HA represents a relevant therapeutic advance in upper respiratory disease, avoiding systemic side effects while increasing local drug activity. However main factors influencing nasal and sinus distribution such as nebulized particle diameters, substance irrigation volume, viscosity of substance, the head position and the angle between nozzle and vestibule, nasal and sinus anatomy. At present, the spray is the main device used for the delivery of solutions based on HA.

The main limit of the spray is that it allows an atomization of large diameter particles (an average of 50 μm), thus allowing to introduce a small amount of substance into the nasal cavities, only easily reaching the most anterior and lower portion of the cavities, especially in the inferior turbinate head. It therefore does not ensure an adequate and homogeneous distribution in the entire nasal mucosa.

To increase the amount of substance at the middle meatal area has a key role in the pathophysiology of rhinosinusitis a nebulization by aerosol is required that allows particles of smaller diameter (between 10 and 20 μm). These properties are satisfied by compressed air nebulizer thanks to a nebulization of the particles between 10 and 20 μm but it is more expensive and unwieldy. A new, specific device for rhino-sinus cavities has been developed called Spray-sol (Brio Srl, Rome, Italy) (Fig. 3), in an attempt to have a device that is practical and manoeuvrable as much as a common spray and able to easily reach the middle turbinate, complex osteomeatal and paranasal sinuses.

The features of Spray-sol include: the ability to nebulize high viscosity substances such as an aerosol; nebulize particles of an average diameter of 10-20 μm ; very low administration times (10 s to 5 cc of substance); the cost and the possibility of its use for the duration of the treatment; it is portable

and does not need electricity as it is attached to a luer-lock syringe. We believe that HA nebulized with a device such as spray-sol or rhinowash may be an adjuvant therapeutic tool in patients affected by rhinosinusitis (Fig. 3).

Thanks to its properties, HA protects the airway epithelium against injury induced by bacterial products during infection, by acting as a lubricant at the airway epithelium surface, thus suggesting that HA may play a therapeutic role in a variety of respiratory diseases.

Data deriving from the present review of 5 clinical studies showed that HA could have a potential role in patients affected by ARS and CRS to minimize symptoms and prevent reactivation, with a significant improvement on their quality of life. The strong connection between particle diameter and site of the high concentration of nebulized particles in the upper aero-digestive tract suggests that it should be mandatory to carefully choose the nebulizer device in order to get better therapeutic results. However, further studies on larger populations are needed to confirm these encouraging results.

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POST-OPERATIVE NEBULIZED SODIUM HYALURONATE VERSUS SPRAY AFTER FUNCTIONAL ENDOSCOPIC SINUS SURGERY FOR CHRONIC RHINOSINUSITIS

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We prospectively evaluated the efficacy of nebulized Hyaluronic Acid (HA) as an adjuvant treatment to hasten the improvement of nasal respiration and to minimize patients' discomfort in the postoperative functional endoscopic sinus surgery (FESS) for chronic rhino-sinusitis (CRS). We enrolled 33 CRS adult patients who underwent endoscopic functional sinus surgery. They were randomly assigned into two groups: Spray-Sol group (18 patients) with HA nebulized with a new nasal device named Spray-Sol and Spray group (15 patients) with a HA nebulized with a common spray. Both groups were treated twice daily for 4 weeks. CRS questionnaire, Visual analogic scale (VAS) and nasal endoscopy were used to assess the outcomes of the treatments during the 1st month of follow up. The mean VAS score of the Spray-Sol group at 2 weeks was significantly lower than the Spray group (5.2 ± 2.1 vs 10.5 ± 3.7 ; $p < 0.05$). The VAS score remained significantly lower in the Spray-Sol group also at the 4 weeks (2.9 ± 0.8 vs 6.1 ± 3.4 ; $p < 0.05$). The CRS score was significantly better at week 2 and 4 in both groups in comparison with baseline values, with better results in the Spray-Sol group. Since the first visit the Spray-Sol group also showed significantly lower crusts, edema and secretions than the Spray group ($p < 0.05$). The compliance to treatment was similar in both groups. The results of this prospective study suggest a role nebulized of HA through new device (Spray-sol) as a supportive treatment for faster improvement of nasal respiration, also minimizing patient discomfort, promoting nasal mucosa healing in postoperative FESS for CRS.

Chronic rhinosinusitis (CRS) is one of the most common chronic diseases in the United States with significant morbidity and health care costs. It is estimated that CRS annually results in 73 million restricted activity days and \$2.4 billion in direct medical costs (1). Epidemiologic studies point out that CRS affects 10-15% of the US population with peaks between 30 and 60 years of age (1).

CRS is defined as inflammation of the nose and the paranasal sinuses characterized by two or more symptoms, one of which should be either nasal blockage/obstruction/congestion or nasal discharge

± facial pain/pressure ± reduction or loss of smell for ≥ 12 weeks (2). This purely symptom-based diagnosis must be supported with confirmatory evidence of mucosal inflammation, such as that seen on anterior rhinoscopy or radiographic imaging (3).

The pathogenesis of CRS remains controversial. Several factors, altering the host-environment interaction such as bacteria, fungi, viruses, allergens or environmental toxins may trigger the inflammatory process (4).

The ostiomeatal complex (OMC) plays a key role in the pathogenesis of rhinosinusitis, it is a

Key words: hyaluronic Acid, Spray-Sol, chronic rhinosinusitis, post-operative, FESS, discomfort, nebulization

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critical area for the drainage and ventilation of the paranasal sinuses. Stenosis created by inflammation and anatomic variations in this region causes an ideal ground for paranasal sinus infections by preventing the drainage and ventilation of the sinuses. Today it represents the main target of medical and surgical CRS therapy (5).

The currently available treatments aim to obtain disease clinical control that is the elimination or mitigation of symptoms, primarily focused on reducing mucosal inflammation, removing bacterial infection/colonization, and improving sinonasal function (6). The first line treatment is medical therapy, first of all nasal lavage, corticosteroid sprays, systemic steroids, and antimicrobials (7).

Recently, many studies have marked the role of topical therapies, such as sinonasal saline irrigations and topical nasal steroids, as an integral component in the management of CRS (8, 9). Topical therapy offers many advantages such as direct delivery of the medication to the targeted region, the ability to deliver high concentrations to specific site and avoidance of systemic effects (10). The disadvantages of topical medical therapy include challenges with application technique, variable sinus penetration and local adverse effects (e.g. epistaxis, throat irritation, nasal dryness, burning, and stinging) (11).

The choice of therapy and delivery method depends on multiple factors including the anatomy of the nasal cavity, the severity and type of disease, as well as patient preference and compliance (12). Despite a high number of CRS medical treatments, their efficacy is still debated and CRS is still one of most disabling chronic disease (12).

When medical management fails to adequately control symptoms, surgical intervention should be considered. Functional endoscopic sinus surgery (FESS) is a minimally invasive technique, in which sinus ventilation and drainage of the sinus cavities are restored (13) and it is considered the surgical gold standard treatment.

The functional results after FESS are good, with most studies reporting an 80-90% success rate with a significant improvement in quality of life (QOL) according to the most published articles (14). However, positive post-surgical effects are

noticeable not before 3-4 weeks because of a discomfort produced by nasal secretions, extensive crust and mucosal edema (15). For these reasons, clinicians are constantly engaged in research of agents able to promote cleansing of the nasal cavities and postoperative regeneration of the nasal mucosa and new device for topical CRS therapy (16).

Several studies evaluated the role of topical drugs used for improving symptoms, preventing complications, and aiding sequelae after FESS, especially in the first weeks after surgery (17, 18). In this regard, Hyaluronic Acid (HA) has recently been recognized as adjuvant treatment in the patients undergoing FESS (19). HA is a fundamental component of the human connective tissue and chemically definable as a glycosaminoglycan (20-27). It is present on the airway surface and is an important component of normal airway secretions. HA plays a pivotal role in the homeostasis of respiratory apparatus, contributes to mucosal host defense by stimulating ciliary clearance and is an important regulator of inflammatory response (28). In particular, topical application of HA significantly improves the healing process of the nasal mucosa, preventing extensive crust formation, and eventually leading to the recovery of smell parameters and cooling sensation (16, 29).

Although evidence of the effectiveness of HA on postoperative care following nasal surgery and FESS is currently available, there remains a scarce evidence regarding the best device to administrate HA in sinus and nasal cavities after functional surgical procedures (19, 30).

Patients undergoing FESS typically use nasal spray to administrate drugs in sinus and nasal cavities. It is known that nasal spray allows a nebulization of large diameter particles (average about 50 μ m), introduces a small quantity of the substance into the nasal cavities and easily reaches the most anterior portion and bottom of the nasal cavity, in particular the inferior turbinate (31).

In order to increase the amount of substance in nasal cavities, especially in the main surgical FESS fields such as the upper section of the nasal cavity, paranasal sinuses and at the ostio-meatal complex, a nebulization by aerosol of the particles between



Fig. 1. *Spray-sol device.*

10 and 20 mm and the availability to administer a greater amount of substance in the nasal fossae is advisable.

The most common devices currently used include: Lavonase (especially for the floor and the inferior turbinate), Squeeze bottle (it isn't able to reach the upper portion of the nasal cavities) and Rinowash (allows a good distribution of the drug in the ostiomeatal complex and in the middle turbinate thanks to a nebulization of the particles between 10 and 20 mm but it is more expensive and unwieldy) (32).

Recently, a new specific device has been developed for rhinosinus cavities called Spray-sol (BI4630R/REPA/rst patent and PCT/IB2014/065121) Brio Srl, Rome, Italy (Fig. 1), in an attempt to have a device that is as practical and maneuverable as a common spray and able to easily reach the middle turbinate, complex osteo-meatal and paranasal sinuses (33).

The goal of the present study was to evaluate the effect of intranasal administration of 9 mg of high molecular weight HA nebulized through a new specific device for nasal cavities named spray-sol on the improvement of postoperative discomfort of CRS patients undergoing FESS.

MATERIALS AND METHODS

We prospectively enrolled 33 CRS adult patients who underwent an endoscopic functional sinus surgery (from

May 2015 to April 2016 at the department of Otolaryngology of Campus Bio-Medico University (2). Demographic data are shown in Table I.

All participants gave their informed consent to participate in the study, which was fully approved by the local Board of Medical Ethics. The diagnosis of CRS was based on the European Position Paper on Rhinosinusitis and Nasal Polyps (EPOS) diagnostic criteria and confirmed by nasal endoscopy and computed tomography (CT) scans with axial, coronal and sagittal images.

Nasal endoscopy with a 4-mm 0-degree rigid endoscope (Storz, Tuttlingen, Germany) was performed before surgery and after postoperative treatment, without decongestant or local anesthesia. Patients suffering from Cystic fibrosis, immotile cilia Syndrome, immunodeficiency Syndrome, fungal sinusitis, Sinonasal tumors or obstructive lesions, acetylsalicylic acid (ASA) sensitivity, choanal atresia and with a history of previous interventions were excluded.

After surgery, the nose was packed for three days and the day after tampon removal all subjects were randomized into two groups and given the following treatment:

- *Investigation arm (Spray-sol group) (18 pts: 15M and 3F):* intranasal administration of high molecular weight HA (9 mg) 3 mL of HA is dissolved in 2 mL of isotonic solution, twice a day in the first postoperative four weeks, nebulized through Spray-Sol;
- *Control group (Spray group) (15 pts: 13M and 2F):* intranasal administration of high molecular weight HA (9 mg) 3 mL of HA dissolved in 2 mL of isotonic solution, twice a day in the first postoperative four weeks, nebulized through a common Spray.

Table I. *Demographic data of the two groups.*

The Demographic data of two group		
	Spray-Sol (11)	Spray (11)
Mean age (years)	45	43
Male\Female	7/4	10/1

To assess QoL, groups were asked to answer two questionnaires:

- *CRS questionnaire* was used to evaluate major and minor symptoms of chronic rhinosinusitis. It is simple and quick (can be completed just in 5 min). It is based on 16 parameters assessed and classified with a score ranging from 0 to 3 (Fig. 2).
- *Visual Analogue Scale (VAS) questionnaire*, which includes questions about headache, nose congestion, runny nose, secretion from the nose into the throat, feeling of fullness in the face, face pain. The VAS scale range was from 0 (most comfortable) to 10 (severe symptoms, dissatisfying situations).

QoL tools, as well as nasal endoscopy, was administered at baseline before surgery (T0), 15 days (T1) and 30 days (T2) after surgery.

Nasal fibroendoscopic findings were classified by means of a 3- point Scale (0=none, 1=mild, 2=moderate, and 3=severe) with respect to nasal crusts, swelling and secretion in nasal fossae middle meatus and osteo-meatal complex. The nasal fibroendoscopic findings were performed with a rigid endoscope.

Statistical analysis

Statistical analysis was performed using the Statistical

Package for Social Sciences Software (SPSS 10.0 for Windows, SPSS Inc., Chicago, IL, USA). Data are shown as mean and standard deviation.

Parametric (Student's *t*-test) and non-parametric (Wilcoxon test for paired data and Mann-Whitney *U* test for non-paired data) tests were used to compare different values. Our criterion for statistical significance was set at *p* values of less than 0.05 (2-tailed).

RESULTS

From thirty-three patients included in the study, thirty-two patients completed the study; 1 patient for onset of conjunctivitis, not connected to the surgery and/or treatment, dropped out of the study at T1. No side effects were recorded in both groups.

At baseline, no statistically significant differences were observed in the clinical, endoscopic, and QoL tools, nor in any of the examined parameters, confirming the homogeneity of the two groups.

VAS score

The mean VAS score of Spray-Sol group at T1 was significantly lower than Spray group (Spray-Sol group 5.2 ± 2.1 vs Spray group 10.5 ± 3.7 ; $p < 0.05$). These significantly better results in Spray-Sol group were confirmed also at T2 (Spray-Sol group 2.9 ± 0.8 vs Spray group 6.1 ± 3.4 ; $p < 0.05$).

CRS score

The CRS score was significantly better at T1 and T2 in both groups in comparison with T0. The mean CRS score values were lower (better) in the Spray-Sol group at T1 and T2, even if the difference was not significant (Spray-Sol group 2.9 ± 0.8 vs Spray group 6.1 ± 3.4 ; $p < 0.05$).

Endoscopic score

At first follow up visit the Spray-Sol group showed significantly less crusts, edema and secretions than the Spray group ($p < 0.05$). We also noticed that atraumatic cleaning of nasal cavities by suction in Spray-Sol group compared to Spray group is associated with less pain and bleeding from crusts detachments, especially for upper portions of nasal cavities and ostio-meatal complex.

Major /Minor Symptoms

The following is a list of nasal and sinus symptoms. Place a mark ("X") after symptom to indicate your discomfort.

0 = no discomfort
1 = mild discomfort
2 = moderate discomfort
3 = severe discomfort

	0	1	2	3
Facial pain or pressure				
Facial congestion or fullness				
Nasal blockage or congestion				
Nasal drainage/rhinorrhea				
Nasal dryness				
Nasal stinging				
Sneezing				
Stinging				
Change in taste or smell				
Headache				
Fever				
Bad breath				
Fatigue				
Tooth pain				
Cough				
Ear pain or pressure				

Fig. 2. CRS Questionnaire.

Other parameters

Both groups showed a good patients' satisfaction and the compliance of Spray-Sol group was higher than Spray group (98% versus 78%) (Table II).

DISCUSSION

CRS is one of the most common health care challenges for which the increasing demand for medical care significantly contributes to rising healthcare costs (34).

CRS patients not responding to medical therapies resort to FESS surgery, the gold surgical therapy for CRS (16). Since the majority of patients following FESS will require ongoing medical therapy to control inflammatory disease, the creation of an open sinus cavity has become a critical component to improve topical drug delivery and provide long-term clinical success (6).

However, because the postoperative first weeks after surgery are often painful and very uncomfortable for the patient, early and careful care following FESS is crucial to reducing post-operative discomfort and complications and improving long-term outcomes (35).

Little data are available regarding the precise effects of sinus surgery on mucosal integrity and wound repair at this level is poorly understood (36).

Wound healing is a highly organized process, involving inflammation, cell proliferation, matrix deposition or remodeling and is regulated by a wide variety of growth factors (37).

During the first weeks after surgery, the fibrin crusts formation overlying the epithelium and the reepithelization starts 7 days after wounding and is usually completed within 8-10 weeks (12), so that the patient feels a fastidious nasal congestion, especially in the first post-operative weeks.

Table II. Summary of the results.

	SPRAY-SOL	SPRAY	P
T0 (baseline evaluation)			
CRS	21.4±9.0	22.9±8.0	NS
VAS	10.3±4.0	13.4±3.5	NS
ENDOSCOPIC SCORE	4.0±1.8	4.8±2.1	NS
T1 (15 days Post-ESS)			
CRS	10.6±6.5	10.2±7.0	NS
VAS	2.7±2.4	5.7±3.0	P<0.05
ENDOSCOPIC SCORE	2.4±1.6	6.8±1.5	P<0.05
T2 (30 days Post-ESS)			
CRS	6.6±2.3	8.1±5.5	NS
VAS	2.6±2.5	4.1±3.0	P<0.05
ENDOSCOPIC SCORE	0.7±0.5	5.7±2.6	P<0.05

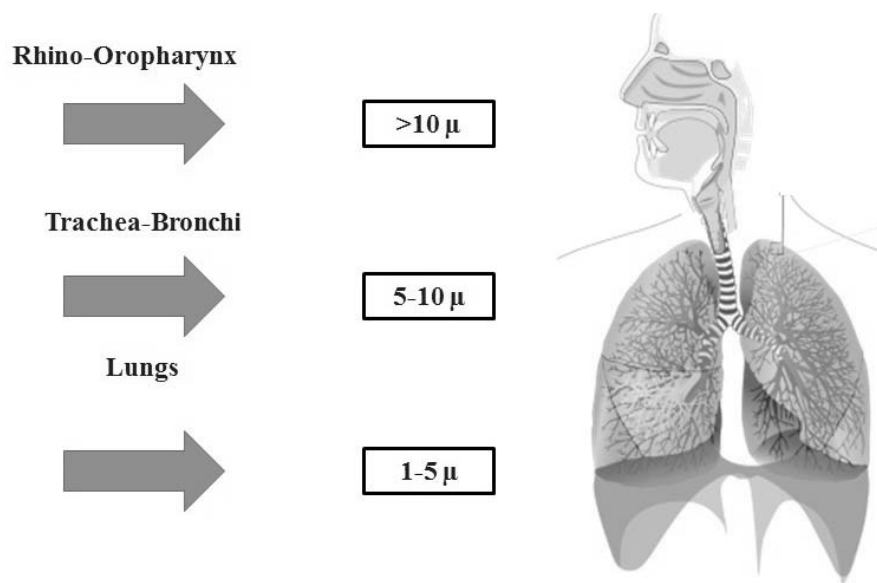


Fig. 3. *Distribution of nebulized particles.*

Nebulized and low-pressure and low-volume HA formulations include Rinowash and spray, respectively. These are positive pressure HA treatments that are used globally, despite insufficient evidence demonstrating safety or efficacy

This is likely in part because topical HA sprays are considered safe, they do not require a prescription, and they are heavily marketed. To date, there are no studies comparing high-volume, low-pressure devices with low-volume devices in the postoperative CRS patient.

The authors hypothesized that there is an advantage of nebulized over HA spray. The mechanical effect of nebulized irrigation debrides and cleans a larger surface area of sinonasal mucosa.

To optimize patient care and manage finite health care resources, strategies that optimize clinical outcomes and minimize subsequent revision surgery must be recognized and implemented (35). Although many studies have investigated the use of topical agents to improve postoperative intranasal symptoms and to aid healing following FESS, there is still an unmet medical need for an effective therapy. Postoperative care is not standardized and a formal evidence-based review of common clinical practices has never been performed for FESS (35).

Recently, HA treatment following FESS and nasal surgery has been shown to improve both mucociliary clearance and nasal mucosa regeneration due to a faster recovery of the impaired ciliated cells in CRS patients (38). Furthermore, additional evidence has reported the effectiveness of 9 mg nebulized high molecular weight HA in the treatment of sinonasal clinical, endoscopic, and psychological parameters in patients undergoing FESS for sinonasal remodeling (29).

Particle size during nebulization plays an important role in airway deposition; in particular, particle size of 1-5 micron are best for reaching the lung, whereas 5-10 micron particles deposit mostly in the trachea and bronchi, >10 micron particles deposit mostly in the nose (Fig. 3).

Many devices for nebulization particles in nasal and sinus cavities have been proposed in the recent literature for patients affected by CRS. The spray device remains the most utilized device, it is very comfortable but it shows some limitations. The nasal spray allows a nebulization of large diameter particles (average about 50 mm), introduces a small quantity of the substance into the nasal cavities and easily reaches the most anterior portion and bottom of the nasal cavity (inferior turbinate and

nasal floor), but very few substance arrives to main surgical FESS fields, in particular at the ostio-meatal complex, considering that the mucociliary clearance is severely impaired in the post-operative FESS (39).

To increase the amount of substance in the upper section of the nasal cavity and in paranasal sinuses a nebulization by aerosol is required to reach the rhino-sinus cavities, which allows the administration of a greater amount of substance in the nasal fossae. Thanks to the small diameter of the particles (around 20 μ m) and the availability to modulate the amount of substance, spray-sol can quickly achieve the ostiomeatal complex, the middle turbinate, the paranasal sinuses, the middle meatus and the deepest portions of the nasal cavities.

The new propose device Spray-sol, unlike the others devices used, shows low administration times (10 s to 5 cc of substance), low cost, single-patient, portable, does not require electricity and is very easy to use (20).

The results of this prospective study suggest a role of HA nebulized through a specific nasal device named spray-sol as a supportive treatment to minimize patients' discomfort and to quickly improve nasal respiration in the post-operative FESS with an optimal compliance. Further larger scale studies are needed to confirm the encouraging results obtained.

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THE USE OF HYALURONIC ACID IN THE TREATMENT OF ANKLE OSTEOARTHRITIS: A REVIEW OF THE EVIDENCE

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Ankle osteoarthritis (OA) is a progressive degenerative joint disease that causes ankle pain and functional limitation especially during walking. It tends to involve younger people with high functional request and has often a post-traumatic origin. Symptoms control through conservative treatment is essential to procrastinate as long as possible the need for surgery. Although few data are present in literature about the use of local viscosupplementation in ankle OA, their potential use for ankle OA has been suggested. We systematically reviewed literature to evaluate the best evidence about short and long term effectiveness of intra-articular HA injections in the treatment of ankle OA. After having screened titles and abstracts from PubMed, Ovid, Cochrane Reviews, Google Scholar, we identified 14 full text articles and collected the outcome rates of intra-articular cycles of HA injections in patients with symptomatic ankle OA. Only 4 randomized control trials were included. Ankle Osteoarthritis Scales (AOS), American Orthopedic Foot, Ankle Society (AOFAS) clinical rating score, visual analog scales (VAS), Western Ontario and McMaster Universities (WOMAC) OA Index of Pain, Stiffness, and Physical Function Score were most frequently used to evaluate outcomes. Although randomized trials showed scores improvement also in placebo-treated patients, current evidence suggests that viscosupplementation for treatment of ankle OA is a safe and effective method. More randomized controlled trials with a large number of patients that compare not only the different types, dosages and frequency of HA injections, but also the effectiveness of HA versus corticosteroids infiltrations and HA versus other types of conservative treatment are still needed.

Ankle osteoarthritis (OA) is a progressive degenerative joint disease that leads to a progressive erosion of articular cartilage until the exposure of subcondral bone. From a clinical point of view, this degenerative process causes progressive ankle pain and functional limitation especially during walking that affect patient quality of life (1). Ankle OA prevalence ranges between 5% (2) and 13% (3) of total cases of OA and between 1 and 4% of the whole population (4). Its origin is frequently post-traumatic (70%), most commonly secondary to

fracture or ligament chronic instability and, only in a smaller percentage of cases, idiopathic (7%) or a consequence of a systemic rheumatologic disease (12%) (1). Unlike hip and knee OA, this disease tends to involve younger people with high functional request that approximately in 10 to 20 years reach the end stage of progression (5). Symptoms control, an important therapeutic challenge for surgeons (6), is essential to procrastinate as long as possible the need for surgery, which not always guarantees a consistent relief at long-term follow-up and could

Key words: osteoarthritis, ankle, hyaluronic acid, injections

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lead to possible complications (2,7). Analgesics and nonsteroidal anti-inflammatory drugs (NSAIDs), intra-articular corticosteroid or hyaluronic acid (HA) injections, physiotherapy, activity modification, weight loss and orthotics can be used for symptoms control, according to disease severity and activity level of the patient (8). While the oral intake of drugs may lead to possible systemic toxic effects, intra-articular injections are effective and safe minimizing drug toxicity risks. Intra-articular NSAIDs injections have a detrimental effect on articular cartilage because of their interaction with cartilage metabolism that leads to disruption of extracellular matrix (9-11).

Although few data are present in literature about the use of viscosupplementation in ankle OA, its potential use for ankle joint has been suggested (12). In particular, injections with hyaluronic acid (HA) have aroused great scientific interest in the last decades, becoming a popular non-operative treatment for knee OA and a promising solution for other joints, such as hip, shoulder and trapeziometacarpal (13-17). HA is a high-molecular-weight polysaccharide synthesized by chondrocytes and synoviocytes. It is a critical constituent of synovial fluid and extracellular matrix of articular cartilage contributing to the viscoelasticity of

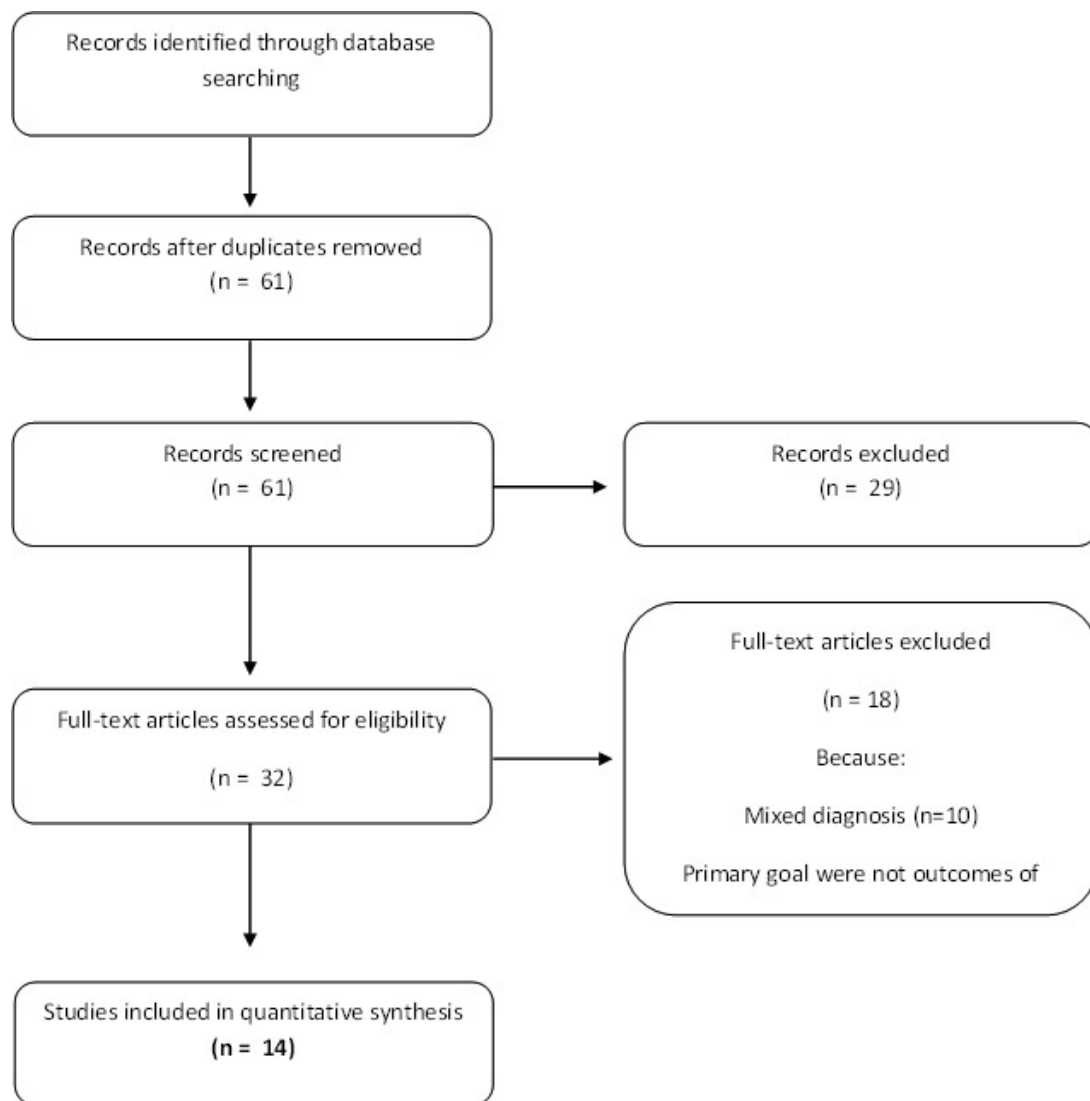


Fig. 1. *Studies inclusion process.*

synovial fluid and the structural integrity of cartilage matrix, carrying out numerous functions such as inducing proteoglycan synthesis and aggregation and modulating inflammatory response (18-27). In OA, as the depolymerization of hyaluronan causes a loss of these functions, the exogenous introduction of this molecule has been shown to recover lubricating and antioxidant properties and a better viscoelasticity of the synovial fluid (28) with improvement in joint pain (29).

The purpose of this review is to evaluate the best evidence about short and long term effectiveness of intra-articular injections with HA in the treatment of ankle OA.

MATERIALS AND METHODS

We performed a search using the keyword “ankle osteoarthritis” in combination with “hyaluronic acid”, “sodium hyaluronate,” and “viscosupplementation” with no limit regarding the year of publication. The following databases were accessed until February 24th, 2017: PubMed (<http://www.ncbi.nlm.nih.gov/sites/entrez/>); Ovid (<http://www.ovid.com>); Cochrane Reviews (<http://www.cochrane.org/reviews/>), Google Scholar. Only publications in English were considered in the current review. In addition, the search was extended by screening the reference list of all the articles. The investigators (RP and EA) read the abstract of each

Table I. Demographic data.

Author	Year	Level of Evidence	Study group	Number of ankles	M/F	Mean age (years)	Mean ankle OA grade	Follow up duration (months)
Carpenter et al. ³⁵	2008	IV	AAA group	12	6/6	55	n.r.	13
			AA + H group	14	6/8	59		
Cohen et al. ²²	2008	I	HYL group	15	14/1	56.2	2.8 (K-L)	6
			Control group	13	11/2	43.4		
DeGroot et al. ²³	2012	I	Active group	39	24/15	54.1	2.46 (K-L)	3
			Placebo group	25	12/13	61.9	2.72 (K-L)	
Han et al. ²⁶	2014	IV	/	40	21/19	60.6	2.1 (Takakura)	12
Lucas y Hernandez et al. ²⁷	2013	IV	/	26 (18 patients)	13/5	60.0	1/2 (M-W)	45.5
Karatoun et al. ²⁵	2008	I	HA group	15	9/6	52.1	3 (K-L)	12
			PE group	15	12/3	58.1		
Luciani et al. ²⁸	2008	IV	/	21	13/8	45	2 (K-L)	18
Mei-Dan et al. ²⁹	2010	IV	/	16	10/6	43	2/4 (K-L)	8
Murphy et al. ³⁰	2016	III	/	50	25/25	49	n.r.	12
Salk et al. ²⁴	2005	I	HA group	9	4/5	57.8	2-4 (K-L)	6
			PB - saline group	8	5/3	60.0		
Sun et al. ³¹	2006	IV	/	75	41/34	50.2	1.6 (K-L)	6
Sun et al. ³²	2011	IV	/	46	20/26	51.7	2.3 (K-L)	6
Witteveen et al. ³³	2008	IV	/	55	33/22	41	2 (van Dijk et al.)	6 (1 inj) - 9 (2 inj)
Witteveen et al. ³⁴	2010	IV	1 ml group	7	4/3	31	2 (van Dijk et al.)	6.7
			2 ml group	7	3/4	47		
			3 ml group	6	5/1	51		
			3 x 1 ml group	6	6/0	40		

AAA: ankle arthroscopy alone; **AA+H:** ankle arthroscopy plus hyaluronan; **n.r.:** not reported; **HYL:** Hyalgan; **K-L:** Kellgren-Lawrence classification; **M-W:** Morrey and Wiedeman classification; **HA:** Hyaluronic acid; **PE:** Progressive Ankle Exercises; **PB-saline:** phosphate-buffered [PB]-saline; **inj:** injection.

publication identified; if an abstract was not available, the article was excluded. We included studies which reported outcomes after HA injections in patients with ankle OA.

Only studies about rehabilitative and surgical procedures were excluded. Studies on animals, *in vitro* studies, case reports, literature reviews, technical notes, letters to editors and articles not specifically reporting outcomes were also excluded. To qualify, an article had to be published in peer-reviewed journals. These selection criteria allowed excluding papers based on the text of the abstract. We obtained full-text versions if the abstract did not permit to include or exclude the study. If there was doubt about inclusion of an article, the senior authors made a collegial decision. Considering all the journals, we first identified 32 articles coherent with the topic at hand. At the end of the study selection process, after primary selection excluding duplicated studies

and papers published on different journals, 14 relevant publications were included. Two authors (RP and EA) assessed and extracted data independently from each article. Relevant data were summarized into tables to provide a systematic overview of the included papers. The studies inclusion process is shown in Fig. 1.

RESULTS

After screening titles and abstracts from different databases, we identified 14 full text articles published from 2005 to 2016, and collected the outcome rates of intra-articular cycles of HA injections in patients with symptomatic ankle OA.

Study type

We included 14 studies, which focused on the use and effectiveness of HA in reducing ankle

Table II. HA, type of treatment and post-injection complications.

Study name	Year	Grade of OA	Side	Main intervention	Placebo/ Control
Bahadir et al.	2009	Eaton-Littler II and III	23 R, 17 L	Sodium hyaluronate (Ostenil , 5 mg/0.5 mL): 3 weekly injections	Triamcinolone acetone (Kenacort-A, 20 mg/0.5 mL): 1 injection
Di Sante et al.	2011	Eaton and Gleackel I to IV	n.a.	1mL solution sodium hyaluronate: 1 injection weekly for three weeks, US guided.	-
Figen et al.	2009	Eaton-Glickel I to IV	n.a.	HA- Hylan G-F 20, 1 ml: 1 injection	Control (contralateral hand) - saline, 1 ml: 1 injection
Frizziero et al.	2014	KL grade 2-3	n.a.	HA: Hyalgan 500-730 KDa: 1 injection 0.8 mL once weekly for 3 weeks	-
Fuchs et al.	2006	Kellgren II	n.a.	Ostenil mini, 1 ml: 3 weekly injections	Triamcinolone (Volon A10), 1 ml: 1 injection
Heyworth et al.	2008	n.a.	51 dominant	Hylan G-F 20, 1 ml: 2 weekly injections	I control group: Betamethasone acetate 1 ml: First week, 1 mL saline; second wk, 1 mL II control group: saline 1 ml: 2 weekly injections
Ingengoli et al.	2011	KL grade 3-4	n.a.	Hyalubrix 3.2kDa (Fidia pharmaceuticals), 1 injection 0.5 mL once weekly	-
Karalezli et al.	2007	Eaton grade 3-4	n.a.	Sodium hyaluronate (Ostenil , 5 mg/0.5 mL): 3 weekly injections	-
Mandl et al.	2009	KL grade 2-4	n.a.	HA: Synvisc (Hylan G-F 20), one injection 1mL weekly for 3 weeks	-
Monfort et al.	2014	KL grade 1-3	n.a.	Sodium Hyaluronate 5 mg/0.5 ml: 3 weekly injections	Betamethasone acetate, 3 mg/0.5ml: 3 weekly injections
Roux et al.	2007	Kellgren II to IV	n.a.	Sinovial, 1 ml: 1 injection	I control group: Sinovial, 1 ml: 2 weekly injections II control group: Sinovial, 1 ml: 3 weekly injections
Salini et al.	2009	KL grade 2-3	n.a.	Sodium hyaluronate 0.8-1.2 KDa, 1 injection 1mL 0.8%	-
Stahl et al.	2005	Eaton-Littler II	n.a.	Sodium Hyaluronate (Orthovisc) 1 ml/15mg: 1 injection	Methylprednisolone acetate (Depomedrol), 1 mL/40 mg: 1 injection
				2 ml group	1 / 4
				3 ml group	1 / 1
				3 x 1 ml group	3 7 1

OA symptoms. Four were randomized control studies. Three authors reported on groups of patients undergoing cycles or single injection of HA compared to patients undergoing intra-articular injection of saline placebo solution (30-32). In one study, the control group performed a progressive ankle rehabilitative program (33). Ten were case series of patients undergoing a different number of intra-articular injections with different types of HA and different temporal distance between injections (34-43). Of these, only 1 study (43) was conducted on patients undergoing surgery and aimed to compare the long-term success of arthroscopy alone to surgical treatment followed by 3 weekly HA infiltrations. All data are collected and summarized in Table I.

Study size and follow up

The data of 512 patients with ankle OA were analyzed in this review [from seventeen patient of Salk et al. (32) to sixty-four of DeGroot et al. (31)]. The mean age was 51.46 years, ranging from 40.2 (41) to 60.6 (34). The mean follow-up time in all studies ranged from 3 (31) to 45.5 months (35). Ankle OA was radiographically diagnosed in all patients and classified according with the Kellgren-Lawrence score in most of the studies. Some authors used Van Dijk score (41, 42), Takakura classification (34) and Morrey and Weiedeman classification (35). All other demographic data are summarized in Table I.

Type of treatment

All performed injections were intra-articular but different types of HA with different molecular weights were used in the enrolled studies. Different types of HA have been used in the studies, including Hylan G-F 20 (35, 36, 41, 43), Hyalgan® (30, 40) and Supartz® (31, 39). All other data are reported in Table II.

Reported outcomes

To evaluate the results, the authors used validated functional scores. Ankle Osteoarthritis Scales (AOS) (30-32, 36, 37, 39, 40) and American Orthopedic Foot, Ankle Society (AOFAS) (31, 33, 35, 37-40) clinical rating score were used in 7 studies. Seven authors have used the visual analog scales (VAS) (30, 31, 34, 36, 41-43). Western Ontario and McMaster

Universities (WOMAC) OA Index of Pain, Stiffness, and Physical Function Score were used by Cohen et al. (30), and Salk et al. (32). All the other scores and all outcomes data are reported in Table III.

Complications

The studies included in this review showed a low complication rate and were local and spontaneously resolved. Arthralgia and erythema were more frequently found at the injection site (32, 36, 39-42, 44). A groin lymph node enlargement (31), itching (40) and swelling 33, 34, 36 was also reported. Osteochondrosis dissecans (41) 4 months after study treatment, which the authors did not consider to be related to the treatment, was the only severe event described (Table II).

DISCUSSION

Ankle OA is a degenerative disease that frequently affects young adults, causing pain and stiffness. In order to keep surgery as last treatment option, conservative therapies have been object of great interest over the past few years. Nonsurgical alternatives for symptomatic OA remain NSAIDs, analgesics, weight loss, physical and occupational therapy and corticosteroid injections. Among possible treatments, intraarticular injections are an option with a high safety margin and minimal systemic risks.

Corticosteroid injections are an alternative treatment option frequently used in the clinical practice but not risk free, even if local and mild or transient (45). In literature, few data are available about efficacy and duration of corticosteroids treatment in big joints, such as knee, hip and small joints, such as the carpometacarpal joint of the thumb (46, 47). While, on one hand, it has been stated that corticosteroids do not wear joint cartilage and improve symptoms; on the other hand, few randomized studies have been conducted to obtain certain efficacy data (48). Ward et al (49) investigated the use of corticosteroids on multiple foot and ankle sites. However, no specific guidance on the effectiveness of this treatment in the ankle has been provided.

Table III. HA, type of treatment and post-injection complications.

Author	Year	Outcomes							
Carpenter et al. ³⁵	2008	AAA group	pre-intervention	Pain score	Median reduction in pain				
			post-intervention	8 (7.5, 9.5)	5.5 (5, 6)				
		AA + H group	pre-intervention	9 (8, 9)	7.5 (6, 9)				
			post-intervention	1 (0, 2)					
Cohen et al. ²²	2008	HYL group	Baseline	AOS total (mean ± SD)	WOMAC total (mean ± SD)				
			6 Months	64.1 ± 12.8	55.9 ± 15.1				
		Control group	Baseline	30.9 ± 8.0	32.4±8.6				
			6 Months	52.5 ± 14.6	45.9 ± 17.5				
DeGroot et al. ²³	2012	Active group	Baseline	AOFAS score	VAS score	AOS pain score			
			Change from Baseline to 12 Weeks	69.4 (13.9, 2.2)	33.4 (22.4, 3.7)	41.4 (18.8, 3.0)			
		Placebo group	Baseline	4.97 ± 10.3	-4.1 ± 26.5	-5.3 ± 21.6			
			Change from Baseline to 12 Weeks	63.5 (17.0, 3.4)	35.0 (23.1, 4.7)	46.4 (22.3, 4.8)			
Han et al. ²⁶	2014	1 week after third injection	Baseline	VAS score (mean ± SD)	"satisfactory outcomes" in VAS				
			6 months	8.1 ± 1.5	/				
			12 months	3.2 ± 2.2	/				
			12 months	4.33 ± 2.9	positive= 21 negative= 19				
Lucas y Hernandez et al. ²⁷	2013		Baseline	5.3 ± 2.7	positive= 16 negative= 24				
			4 months	AOFA score (mean ± SD)					
			12 months	61.8 ± 15					
			12 months	74.4 ± 14.5					
Karatosun et al. ²⁵	2008	HA group	Baseline	73.7 ± 16.6					
			12 months	AOFA score (mean ± SD)					
		PE group	Baseline	61.6 ± 16.8					
			12 months	90.1±9.7					
Luciani et al. ²⁸	2008		Baseline	72.1±16.6					
			6 months	87.5±17.5					
			12 months	AOS-A (mean ± SD)	AOS - B (mean ± SD)	VAS score (mean ± SD)			
			18 months	44.5±19.9	48.9±20.8	6.6±2.4			
Mei-Dan et al. ²⁹	2010		6 months	39.1±18.6	33.3±16.3	4.6±2.5			
			12 months	35.7±17.5	32.4±18.2	4.6±2.3			
			18 months	34.4±20.8	32.6±19.8	4.6±2.6			
			18 months						
Murphy et al. ³⁰	2016		Baseline	VAS score pain	VAS score stiffness	VAS score functioning	score for subjective function and disability	Ankle ROM	Ankle-Hindfoot Scale
			week 32	5.3	6.8	5.6	44.21	Average improvement of 15% (20%)	Average improvement of 7 points
				3.1	3.8	3.3	47.78		
Salk et al. ²⁴	2005	HA group	baseline	Quality of life (mean ± SD)	Sports (mean ± SD)	Activities of daily living (mean ± SD)	Pain (mean ± SD)	Burden of symptoms (mean ± SD)	
			6 - 12 months	28 ± 12	54± 7	62 ± 6	48 ± 7	54 ± 8	
		PB-saline group	Baseline	64 ± 5	70± 5	86 ± 11	84 ± 9	82 ± 7	
			6 months	AOS score	Ankle girth (cm) (mean ± SD)	Ankle ROM (mean ± SD)	EQ-5D	SF-12	
Sun et al. ³¹	2006	HA group	Baseline	58	26.17 ± 2.54	31.2 ± 7.52			
			6 months	25	25.70 ± 2.02	34.4 ± 8.46			
		PB-saline group	Baseline	58	26.4 ± 2.56	29.10 ± 7.85	no significant differences between groups	significant difference in mean scores for vitality domain (47.22 vs 25.00)	
			6 months	37	26.53 ± 2.52	31.1 ± 7.88			
Sun et al. ³¹	2006		Baseline	AOS score (mean ± SD)	AOFAS score (mean ± SD)	Ankle sagittal ROM (mean ± SD)	Satisfaction rate (%)		
			3 months	5.1 ± 1.9	64 ± 17	36.9 ± 14.6			
			6 months	2.6 ± 1.7	78 ± 15	38.1 ± 14.3	90.7		
			6 months	2.4 ± 1.9	78 ± 14	38.7 ± 13.6	86.7		
Sun et al. ³²	2011		Baseline	AOS score (mean ± SD)	AOFAS score (mean ± SD)	Clinical balance test scores (mean ± SD)	Satisfaction rate (%)		
			3 months	5.5 ± 2.1	60.5 ± 19.1	SLS:17.3 ± 21.5; FRT:23.1 ± 6.3; TUG:9.6 ± 3.3;BBS:51.1 ± 5.2	/		
						SLS: 25.6 ± 25.0;FRT:27.2 ± 7.6; TUG:8.0 ± 2.9; BBS:53.2 ± 3.6			
							93		

			6 months	3.2 ± 1.9	76.7 ± 19.5	SLS: 25.6 ± 26.0; FRT: 26.3 ± 6.7; TUG: 7.9 ± 2.6;BBS:52.9 ± 3.5	93
				Study Ankle OA Pain VAS (mm)	Ankle OA scale (mm)	Patient global assessment - VAS	SF-36 scale
Witteveen et al. ³³	2008	Baseline	68.0	65.8(pain) - 63.6 (disability)	64.9	physical component: 36.2 Mental component: 49.9	
		3 months	33.8	35.7 (pain) - 36.1 (disability)	34.6	physical component: 43.9 Mental component: 54.3	
				Pain during walking activities (VAS, mm) median			
Witteveen et al. ³⁴	1 ml group	Baseline	43				
		week 27	49				
	2 ml group	Baseline	81				
		week 27	74				
	3 ml group	Baseline	48				
		week 27	41				
	3 x 1 ml group	Baseline	61				
		week 27	31				

SLS = single-leg stance test, *FRT* = functional reach test, *TUG* = timed up-and-go test (higher scores represent poorer performance), and *BBS* = Berg Balance Scale.

HA, locally delivered, has anti-inflammatory effects (50) and the capability of restoring synovial fluid viscoelasticity and normalizing endogenous HA synthesis. All these effects result in alleviation of pain and improvement in joint ROM (51). For this reason, HA injections are widely recognized as non-operative treatment for knee OA (52, 53) and a promising solution for other joints, such as hip, shoulder and trapeziometacarpal (13-17).

Three out of the 4 randomized studies included in this review compared patients undergoing intraarticular injections of HA to saline placebo, while 1 study compared them to progressive ankle physiotherapy.

Cohen et al. (30) studied the effects of five weekly fluoroscopically guided intraarticular injections with specific sodium HA (Hyalgan - HYL) versus phosphate-buffered saline (control). The two groups, HYL group (15 patients) and control group (13 patients), significantly differed for age and baseline AOS scores and WOMAC pain scores, which were higher in the HYL group. At 3 months follow-up, there was a statistically significant difference between the two groups in the AOS score, in favour of the HYL group (-17.4 versus -5) that showed a 36% improvement from baseline. These outcomes did not remain significant at 6 months follow-up, though both groups showed persistent benefit from treatment. Moreover, the HYL group showed

an earlier (2 weeks) improvement in pain scores compared to that achieved in the AOS disability scores. For the WOMAC scores, only at 3 months follow-up, the scores of the two study groups differed significantly, in particular for pain. The overall improvement of WOMAC scores were greater for the HYL group (35%) at 3 months follow-up than control group, which had a peak improvement at 2 weeks follow-up (13%).

De Groot III et al. (31) compared the effects of a single injection with low molecular weight HA in a group of 39 patients (active group) with a single normal saline solution injection in a group of 25 patients (control group). All infiltrations were fluoroscopically guided. Active treatment group showed statistical improvement from baseline values in the AOFAS scores at 6 and 12 weeks follow-up, in both cases equal to 4.97 points. Moreover, the same indices also improved after 12 weeks in the control group (5.43 points) and the inter-group differences were never statistically significant. The AOS and VAS scores were significantly reduced in the control group but not in the active group at 12 weeks follow-up, although the difference between the two groups was not always statistically significant.

In a study with 17 patients, Salk et al. (32) observed the possible clinical and functional improvement following weekly intra-articular administration for 5 weeks of HA (9 patients) or phosphate-buffered

(PB) -saline (8 patients). The demographic data of the two groups were similar. Both groups showed a significant improvement in AOS pain and disability subscores at 6 months follow-up, but the inter-group difference was not statistically significant. The improving trend was always in favour of the active group. In particular, at 6-month follow-up, a greater number of subjects treated with HA showed a 50% improvement in VAS score. No statistical significance was found in the comparison of WOMAC score and ankle ROM, although the latter remained better in the HA group.

In literature, only one work compared the effects of a 3-weekly infiltrations cycle with HA (Adant) with progressive six-week ankle exercises (PE) therapy. Karatosun et al. (33) included 43 ankles (30 patients), dividing them into HA group (19 ankles) and PE group (24 ankles). Both average AOFAS score and pain during activity measured by VAS scale were significantly improved in the two groups at one-year follow-up, respectively from 61.6 to 90.1 and 5.4 to 1.4 for the HA group and from 72.1 to 87.5 and from 4.7 to 2.4 for the control group. The inter-group differences were not significant. The PE group also showed improved gait control at the end of the study.

Only one study, although not randomized, evaluated the effectiveness of HA post-operative injections (43). The authors evaluated the results in terms of pain perceived by the patient, measured in a 10-point categorical scale, in a group of 12 (46%) patients who underwent ankle arthroscopy only (AAA group), compared to a control group of 14 (54%) patients who underwent the same type of surgery, combined with a successive cycle of 3-weekly ankle infiltrations with hylan G-F 20 (Synvisc), starting from the first postoperative week (AA + H group). The average reduction in pain score at the postoperative average of 13-months follow-up compared to baseline was about 5.5 in the AAA group and 7.5 in the AA + H group. This difference was statistically significant. In particular, in the pre- and post-operative period the average pain score was respectively 8 and 3 for the AAA group, and by 9 and 1 in the AA + H group. The post-operative inter-group assessment differed in a statistically

significant way for the two types of treatment.

Several studies, however, analyse the effects of HA infiltration without comparing the results with a control group.

Han et al (34) conducted a retrospective review of 40 consecutive patients undergoing 3 weekly HA infiltrations. The baseline value of VAS decreased of about 50%, respectively in 62.5% and 52.5% of patients at 3 months and 6 months follow up. Specifically, in the respective follow-up period, 22 out of 25 subjects and 19 out of 21 subjects were suffering from early stage arthritis according to Takakura classification, with a statistically significant association between an early radiographic osteoarthritis and a greater benefit of the treatment. In addition, shorter symptom duration (≤ 12 months) was associated with satisfactory results with improvement in VAS value. However, after 12 months, 60% of patients reported negative outcomes.

Similar results were obtained in the study by Lucas et al. (35), which showed a satisfactory symptomatology improvement after 73% of infiltrations performed. They reported that 5 patients underwent surgery about 27 months after the last cycle of infiltrations. Luciani et al (36) showed that improvement of AOS disability and VAS scores was already significant in the first 6 months after the third infiltration (VAS: from 6.6 ± 2.4 to 4.6 ± 2.5 and AOS disability: from 48.9 ± 20.8 to 33.3 ± 16.3), persisting also in successive evaluations (12 and 18 months). Overall, final results were good for 71% of patients according to AOS score (improvement ≥ 10 points) and 48% according to VAS scales (improvement ≥ 10 points). Murphy et al. (38) performed a series of three low-MW HA injections administered at 2-week intervals in 50 patients. The improvement of the score was statistically significant for each domain of Foot and Ankle Outcomes Score (FAOS); specifically pain domain score (from 48 ± 7 to 84 ± 9), activities of the daily living domain score (from 62 ± 6 to 86 ± 11), sports and recreational activities domain score (from 54 ± 7 to 70 ± 5) and quality of life domain score (from 28 ± 12 to 64 ± 5).

Sun et al. performed two consecutive studies respectively evaluating the efficacy of a 5- and

3-HA infiltration cycles in patients with unilateral ankle pain. The first study (39) included 75 patients undergoing a 5-week infiltration cycle with significant statistical results in terms of pain and disability reduction already one week after the end of the cycle. AOS total score decreased from baseline 5.1 ± 1.9 to 3.2 ± 2.1 after 1 week and 2.4 ± 1.9 after 6 months. However, ankle sagittal ROM did not improve significantly during treatment (baseline 36.9 ± 14.6 ; 6 months 38.7 ± 13.6). Only 20% of patients had a reduction of benefit over the next 4 months. In this study, they also found a significant reduction in the use of Acetaminophen at 6 months from the end of the cycle. Similar results were obtained in the second study conducted, that involved 46 patients with unilateral ankle OA, in which the authors showed a significant reduction in the mean AOS score and AOFAS Ankle-Hindfoot Score at 1, 3 and 6 months after the third injection (40).

Mei-Dan et al. (37) evaluated the effects of 5 weekly infiltrations with HA in 16 patients with OA higher than stage II according to Kellgren and Lawrence. Scores of the VAS questionnaire ranged from 1 to 10 and were statistically significant at 32-week follow-up compared to baseline in terms of pain (from 5.29 to 3.05), stiffness (6.83 to 3.80), function and disability (5.61 to 3.33). In addition, ankle range of motion improved of 15° in 20% of subjects and the Ankle-Hindfoot Scale score of about 7 points at the final follow-up. These results remained stable 7 months after the last infiltration.

Witteveen et al, performing one or two infiltrations in a group of 55 patients, obtained a decrease in VAS score of 68.0 mm (baseline) at 33.8 mm at 3 months, which was maintained at 6 months (34.2 mm, $p < 0.001$) (41). In a subsequent study, the same authors randomized patients in four study groups: 1 single HA infiltration of 1, 2 or 3 ml dose or a cycle of 3 1-ml infiltrations. They showed that the 3 x 1 ml dose group showed a statistically significant decrease at week 7 for 'pain during walking' and 'pain at rest', which remained significant even at week 15. Each group did not show a significant reduction in VAS scores (42). Moreover, no study reported a high rate of complications, which, if observed, were mostly local and transient.

In this review, we found that only four randomized studies were conducted to compare the efficacy of HA intra-articular injections with placebo or other conservative therapies. No study compares the use of HA and corticosteroids. Moreover, randomized trials are very heterogeneous, in terms of selection of patients, type of HA used, number and location of infiltrations and type of score used for pre- and post-operative assessment.

These differences do not allow an objective evaluation of the results and therefore, do not allow to effectively understanding if HA injections are the best conservative treatment for patients with ankle OA. Overall, we can conclude that current evidence suggests that viscosupplementation for treatment of ankle OA is a safe and effective method even though no data suggest the best group of patients that can benefit from this treatment.

CONCLUSION

Despite the lack of reliable data in the literature, we can conclude that HA injections in patients with ankle OA improve symptoms and quality of life of the treated patients. However, more randomized controlled trials with a large number of patients that compare not only the different types, dosages and frequency of HA injections, but also the effectiveness of HA versus corticosteroids infiltrations and HA versus other types of conservative treatment, are still needed.

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HYBRID HYALURONIC ACID VERSUS HIGH MOLECULAR WEIGHT HYALURONIC ACID FOR THE TREATMENT OF OSTEOARTHRITIS IN OBESE PATIENTS

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Osteoarthritis (OA) of the knee is one of the most relevant and debilitating health problems. Obesity represents one of the major risk factor for early development of OA. In the obese population, knee replacement should be delayed and eventually avoided and prefer conservative treatments including intrarticular hyaluronic acid (HA) viscosupplementation. In the present clinical randomized trial, we present a comparison between two groups of 24 obese patients which were randomized to be treated with two intrarticular injections of hybrid (low and high molecular weight) hyaluronic acid (Group A) or two injections of high molecular weight hyaluronic acid (Group B). Patients were followed-up through to 6 months and assessed through IKDC and KOOS scores, pain was evaluated with VAS. All patients reported a significant improvement when compared to baseline value in all outcome measures. At 3-month follow-up, IKDC had significantly improved in patients of Group A, compared to Group B (53.1 ± 1.9 vs 51.4 ± 2.4 , $p=0.0079$) and the same for KOOS (52.1 ± 2.0 vs 50.1 ± 2.9 , $p=0.010$). Furthermore, the difference in KOOS was persistently significant at 6-month follow-up (54.7 ± 2.3 vs 51.7 ± 4.9 , $p=0.014$). The VAS reduced significantly more in Group A at 3 months (3.7 ± 0.5 vs 5.2 ± 0.7 , $p<0.001$). In an obese population, where basal inflammatory pattern increases symptoms of OA and conservative treatment is recommended, HA viscosupplementation improved function and pain of the knee. The treatment with hybrid HA showed better outcomes than high molecular weight HA in obese patients. The combination of the anti-inflammatory action of low molecular weight HA on chondrocytes and the biomechanical role of high molecular weight HA might explain the different results.

Osteoarthritis (OA) is the most common joint disease and one of the most common causes of disability. Indeed, OA represents a painful, debilitating disease that drastically affects quality of life and functional capacity of patients. It is a metabolically active and dynamic process, characterized by the degeneration of cartilage and all joint tissues (1).

Obesity, a rising epidemic and one of the major public health problems, is considered an important

independent risk factor in the development of OA (2). It represents a global burden and people affected are constantly increasing worldwide. In a previous report, the World Health Organization (WHO) stated that 500 million of the world's population were obese (3). Both the higher prevalence of obesity and the increasing aging population contribute to make OA a critical problem. The numbers of obese people developing early OA and undergoing total knee replacement (TKR) has consistently been rising (4).

Key words: hyaluronic acid, obesity, osteoarthritis, knee

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However, there have been major concerns among surgeons and patients about clinical outcomes and complications of TKR in obese patients, especially due to the morbidities associated such as type 2 diabetes mellitus, hypertension and coronary artery disease, in addition to OA (5) which have detrimental effects on TKR outcomes.

Conservative approaches are core strategies for the management of OA in obese people and these include appropriate disease-related education, lifestyle modification, physical therapist-prescribed exercise programs to address physical impairments and weight loss. Among conservative strategies, intrarticular hyaluronic acid (HA) viscosupplementation is currently widely used in clinical practice, with good results reported in several studies (6, 7). HA is universally accepted as conservative treatment for OA due to its ability to increase the viscoelasticity of synovial fluid toward normal levels, decrease pain, and improve the natural protective functions of synovial fluid in the joint. The high-molecular weight HA (HMWHA) (Sinovial Forte, IBSA) utilized for the present experimentation is composed of a hyaluronan polymer of 800-1.200 kDa (7) and it has been extensively used for clinical experimentation, for the treatment of OA (8-15). The Hybrid Hyaluronic Acid (HHA) (Sinovial HL; IBSA) represents a recent formulation that conjugates a low molecular weight HA (LMWHA, 80-100 kDa) and an HMWHA (1.100-1.400 kDa) obtained through NAHYCO™ technology (16). It has been shown to stimulate extracellular matrix *in vitro* (7), due to the presence of LMWHA and it has proven to significantly reduce the inflammatory process on human primary chondrocytes cultures (17).

In this randomized controlled trial, we evaluated the effects of viscosupplementation with either HMWHA or HHA for the treatment of OA in obese patients.

MATERIALS AND METHODS

This study was performed at the Department of Orthopedic and Trauma Surgery of Campus Bio-Medico University Hospital. The study was conducted in accordance to Helsinki declaration principles and Good

Clinical Practice normative.

Inclusions criteria were Body Mass Index (BMI) >30, radiographic evidence of degenerative changes to the knee (stage 3 according to Kellgren-Lawrence radiographic system), symptomatic unilateral pain condition, unresponsive to non-injective conservative therapies (rehabilitation, cryotherapy, rest, NSAIDs). Exclusion criteria were previous surgery on the knee, excessive axial deformity (>5°), knee instability and osteochondral lesion. Hematologic and general health status exclusion criteria were also applied. Since November 2015, 48 obese patients with clinical and radiographic evidence of degenerative changes to the knee were enrolled in the study. Patients were randomized into 2 groups: Group A - 24 patients received 2 injections of HHA (Sinovial HL, 3.2%, 64mg/2ml, 32 mg High-MW 1.100-1.400 kDa+32 mg Low-MW 80-100 kDa; IBSA) and Group B - 24 patients received 2 intra-articular injections of conventional HA (Sinovial Forte, 1.6% 32mg/2ml, MW: 800-1.200 kDa; IBSA)

Clinical assessment

International Knee Documentation Committee (IKDC), Knee injury and Osteoarthritis Outcome Score (KOOS) and Visual Analogue Scale (VAS) were administered to all patients before starting the treatment (baseline), at 3 and 6 months from the end of management. A different and independent investigator, unaware of the previous measurement to ensure a blinded status, recorded each measurement.

Injection procedure

An orthopaedic surgeon in the outpatient clinic injected the product. Knee injection was performed through a lateral approach. The upper pole of dorsal margin of the patella and the femoral condyle were considered as landmarks to identify the point of least resistance. After the sterile dressing of the skin with a 22-G parallel needle to the surface of the bed and tilted toward the articular surface of the patella, operator penetrates through the skin and releases the product after lateral subluxation of patella. Immediately after the injection, the affected knee underwent passive flexion and extension for 3 times, followed by 10 min of resting in supine position.

Statistical analysis

Statistical analyses were blinded. The analyses were

performed using SPSS version 16.0.1 (SPSS Inc., Chicago, Illinois). Primary end-point was the postoperative difference in the IKDC and KOOS scores and VAS scale compared to baseline. Paired *t*-test was used to perform this analysis and unpaired *t*-test to compare the postoperative results between the 2 groups. A *P* value of 0.05 was set as a threshold for statistical significance. Data were presented with average value and standard deviation (SD).

RESULTS

Demographic and basal characteristics

For the present experimentation, 48 obese patients have been enrolled (BMI 32.6 ± 3.4 Kg/m²). In the first group (Group A) there were 24 subjects (10 males and 14 females), with a mean age of 61.5 y (range 53-70). In the second group (Group B) there were 24 subjects (11 males and 13 females), with a mean age of 60 y (range 51-69). Baseline evaluation did not find any significant difference between the groups in terms of age, sex and clinical outcomes (IKDC, KOOS and VAS). No patient was lost at follow-up.

Clinical and pain outcomes

In terms of clinical outcomes (IKDC and KOOS), a significant improvement was observed in both groups at each follow-up, when compared to baseline ($p < 0.001$). Similarly, VAS improved over time significantly at each follow-up ($p < 0.001$). Intergroup analysis showed superiority of the treatment with HHA compared to HMWHA at 3 months in terms of IKDC (53.1 ± 1.9 vs 51.4 ± 2.4 , $p = 0.0079$) and KOOS (52.1 ± 2.0 vs 50.1 ± 2.9 , $p = 0.010$) and the difference in KOOS was also significant at 6-month follow-up (54.7 ± 2.3 vs 51.7 ± 4.9 , $p = 0.014$). The VAS resulted reduced significantly more in the Group A at 3 months (3.7 ± 0.5 vs 5.2 ± 0.7 , $p < 0.001$) but this difference was not observed at last follow-up (3.0 ± 0.8 vs 3.1 ± 0.7 , $p = 0.839$). Details of the clinical outcomes are shown in tables I, II and III.

Complications

In Group A, one patient developed aseptic acute arthritis of the knee, while another showed swelling at the site of injection, without any sequelae. In Group B, one patient developed synovitis of the knee and

one patient had persistent pain at the site of injection.

DISCUSSION

The authors carried out a prospective controlled trial to investigate the clinical outcomes of HA viscosupplementation with either HHA or HMWHA in an obese population affected by knee OA. According to the results obtained in the present study, both treatments showed to be effective in relieving symptoms. In particular, knee function evaluated through IKDC and KOOS scores significantly improved from baseline in both groups. Furthermore, VAS reduced at each follow-up stage compared to baseline in both groups, thus showing that viscosupplementation can be a beneficial approach in obese patients. However, HHA showed higher effectiveness in terms of pain reduction and function compared to HMWHA. This was evident especially at mid-term follow-up (3 months), with a statistically significant improvement in clinical scores of patients treated with HHA compared to those treated with HMWHA. HHA treatment had better scores also at 6-month follow-up, although this difference is not significant.

Viscosupplementation is considered the main conservative intervention for management of early OA and several HA preparations are nowadays available on market. Its fundamental role is to provide mechanical support to the joint, in order to avoid excessive shear stress on the cartilage. However, FDA approved only a few pharmaceutical preparation of HA, such as Hyalgan®, Supartz®, Orthovisc®, Synvisc® (Hylan G-F 20) and Euflexxa® (18). In the present study, we chose a standard HA, which is widely utilized in clinical practice in Europe. It is composed of a high molecular weight polymer of 800-1.200 kDa. Rheological studies demonstrated that this composite has comparable viscoelastic properties to HHA, since the HMWHA component is similar (7). On the other hand, HHA associates the HMWHA and the LMWHA in its formulation, combined together thanks to NAHYCOTM patented technology; both molecules are not cross-linked and have a respective molecular weight of 1.200 ± 2 kDa and 100 ± 5 kDa. Our group (6) demonstrated that

Table I. *IKDC values over time.*

Follow-up	Group	Average	SD	Intergroup analysis P value
Baseline	HHA	38.8	4.8	0.541
	HMWHA	39.8	5.8	
3 months	HHA	53.1	1.9	0.0079
	HMWHA	51.4	2.4	
6 months	HHA	60.4	2.1	0.075
	HMWHA	57.4	4.8	

Table II. *KOOS values over time.*

Follow-up	Group	Average	SD	Intergroup analysis P value
Baseline	HHA	43.4	4.7	0.907
	HMWHA	43.6	5.5	
3 months	HHA	52.1	2.0	0.010
	HMWHA	50.1	2.9	
6 months	HHA	54.7	2.3	0.014
	HMWHA	51.7	4.9	

Table III. *VAS values over time.*

Follow-up	Group	Average	SD	Intergroup analysis P value
Baseline	HHA	7.6	0.3	0.371
	HMWHA	7.4	0.5	
3 months	HHA	3.7	0.5	<0.0001
	HMWHA	5.2	0,7	
6 months	HHA	3.0	0.8	0.839
	HMWHA	3.1	0.7	

HHA is effective and superior to platelet rich plasma (PRP) in improving clinical outcomes in end career athletes with OA at 1-year follow-up (6). Moreover, *in vitro* studies showed that HHA significantly reduced inflammation biomarkers in a model and human chondrocytes respect to both HMWHA or LMWHA separately considered at transcriptional and protein level (16). In the present study, we showed that significant superiority for all the clinical outcomes assessed was achieved in the group treated with HHA at 3 months follow-up in obese patients affected by knee OA. These outcomes suggest that HHA is a suitable treatment to improve functional outcomes and relieve pain in obese patients.

Better outcomes may be due to the presence of both LMWHA and HMWHA in the same injection. Indeed, next to adequate viscoelastic supplementation (mechanical effect) provided by the HMWHA present in both composites, HHA formulation is efficient in reducing inflammation inside the joint (biological effect) (17) through the LMWHA. *In vitro* studies showed that markers including TNF- α and IL-6 were reduced in primary human chondrocytes cultures (17). Furthermore, it has been reported that LMWHA inhibits prostaglandin E2, nitric oxide and IL-1 having also an antioxidant role, protecting chondrocytes from free oxygen radicals damage (1). Therefore, the therapeutic effectiveness of HHA is based on a strong balance between biomechanical properties of viscosupplementation and biological features that contrast the pathogenic mechanisms of osteoarthritic degeneration of cartilage, chondrocyte damage and inflammatory response.

It is well demonstrated that obese patients represent a critical population, particularly susceptible to articular cartilage damage, increased risk and early development of OA (19, 20) due to the higher mechanical weight but also to the constitutive inflammatory pattern which characterizes obesity (21). Inflammation-induced proteinase and collagenases activity significantly affects matrix contents in structural proteins of the extracellular matrix of joint cartilage (17), thus there is a need of supplementation of such proteins. Chua et al. (22), showed that HA content in synovial fluid of obese patients affected by OA was negatively correlated

with medial joint space width and positively correlated with Kellgren-Lawrence radiographic progression of OA (22).

Obesity is, indeed, a predisposing factor for the development of early OA (23) and furthermore it is a factor associated with poor clinical and functional outcomes after surgical management with TKR, yielding also to an increased rate of post-operative complications (23), because of predisposition to cardiovascular events and development of peri-operative infections. Thus, several systemic and local treatments for chondral proteins supplementation have been developed, in order to delay surgical intervention as long as possible and eventually avoid it at all in this critical population. However, there are a few studies concerning specifically obese patients in the literature. A recent study by Nelson et al. investigated the role of oral supplementation of HA and other GAG (Oralvisc®) in obese patients affected by OA, showing that this therapy was safe and effective in terms of pain soothing, systemic inflammation reduction and turnover of the synovial fluid (20). Concerning local injection of HMWHA (1.500-3.600 kDa), it was shown by Eymard et al., that main predictors of failure of injective therapy are the obesity itself and radiologic grade of OA (24).

To our knowledge, this is the first study evaluating HHA in an obese population and is one of the few recent studies that evaluate the treatment of obese population affected by OA. Furthermore, the randomized design avoids allocation bias and confounding factors related to patients' anthropometric and demographic characteristics, which were homogeneous between groups at baseline. However, the paucity of the cohort is the main limitation of the present study, which needs further and larger investigation to find definite scientific significance.

CONCLUSION

In the present clinical randomized trial, we demonstrated that HHA is more effective in soothing pain and improves function in obese patients affected by knee OA compared to HMWHA, especially at

mid-term follow-up. However, further analysis and a longer follow-up are still necessary to assess the best treatment in this challenging population.

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THE USE OF HYALURONIC ACID AS AN ADJUVANT IN THE MANAGEMENT OF GINGIVITIS

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Recently, a specifically designed device was proposed that is able to nebulize particles with a diameter of approximately 16 micrometres to be used mainly in the management of diseases of the upper airway respiratory tract. The purpose of this pilot study is to evaluate the potential efficacy of nebulized hyaluronic acid in the management of gingivitis. The results of the statistical analysis demonstrate that there was no difference between the pocket depth as measured in the treated sites at time 0 (pre-treatment) and time 1 (15 days post-treatment). However, the difference between bleeding on probing as measured at time 0 and time 1 indicated an improvement on both sides, with a slightly greater improvement on the side treated with HA.

Hyaluronic acid (HA) is a natural polysaccharide consisting of a linear chain of glucuronic acid and N-acetylglucosamine molecules (1-8). It is synthesized by the serosa glands of the submucosa and it is an important component of the extracellular matrix of connective tissue, synovial fluid, embryonic mesenchymal tissue, the vitreous humour, the skin and many other organs and tissues in the human body.

According to its molecular weight, treatment with hyaluronic acid results in different responses in the body.

Of the many beneficial effects of hyaluronic acid, perhaps the most significant function, is the role it plays in reducing and managing inflammation. This is particularly true with regards to low-molecular-weight hyaluronic acid (<300 kD) due to its role in reducing peripheral vascularization; while in the case of high-molecular-weight hyaluronic acid (1000 kD), there is increasing evidence to suggest that, thanks to its inherent immunosuppressive effect on a cellular

level, it also helps to regulate excessive exacerbation of inflammation (9). Furthermore, hyaluronic acid is bacteriostatic and anti-inflammatory.

Many inflammatory diseases in dentistry, such as gingivitis and periodontitis, require ongoing and constant management over time.

HA, interacting with type I and II toll-like receptors, regulates the body's innate immunity against bacteria and viruses by preventing penetration, promoting angiogenesis and by modulating the proliferation, migration and differentiation of the cells responsible for tissue repair; thus playing a key role in wound healing (9-25).

The emollient and restructuring action it exerts on the mucous membranes is also very important, which is caused by its reparative (it stimulates angiogenesis) and soothing properties (hyaluronic acid is used in wound care to improve the processes of wound healing) which are effective in treating the symptoms of local inflammation and irritation (9-25).

Key words: hyaluronic acid, gingivitis

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HA is widely used in aesthetic medicine to improve aesthetic surgery results (26-35). There are several methods of administration depending on the molecular weight of the hyaluronic acid involved and according to how and where it is to be used.

Recently, in the management of diseases of the upper airway respiratory tract, nebulized hyaluronic acid particles with a diameter of approximately 16 micrometres have been used, and in this pilot study the purpose is to evaluate the potential efficacy of nebulized hyaluronic acid in the management of gingivitis.

MATERIAL AND METHODS

The study involved the selection of 5 patients who presented gingivitis and showed a maximum probing depth under 4 mm, bleeding on probing and/or spontaneous bleeding. Informed consent was obtained from all individual participants included in the study.

The oral hygiene of the patients was assessed at time 0 and 15 days (time 1) using the Simplified Oral Hygiene Index, which included analysis of the Tartar Index (TI) by applying the simplified Green-Vermilion Index, the Plaque Index (PI) by applying the simplified Green-Vermilion Index, evaluating Pocket Depth (PD) expressed in millimetres, and Bleeding on Probing (BOP) (yes/no). Patients underwent a treatment which included closed SRP (scaling and root planing) on all four quadrants, in addition to the administration of HA, nebulized via Spray-sol (IBSA FARMACEUTICI ITALIA Srl, Lodi, Italy), to one side only (either the right or the left) immediately after treatment. Patients were then provided with a sufficient quantity of nebulized HA to make two self-administrations per day for 15 days on that same side. At the end of the first and the second week the parameters listed above were re-measured and statistically compared to the initial results.

RESULTS

While this clinical trial involving only 5 patients, it is not sufficient to indicate a statistically relevant result, nevertheless, it can give a good indication of the potential efficacy of HA in the treatment of gingivitis.

The results of the statistical analysis demonstrate that there was no difference between the pocket depth as measured in the treated sites at time 0 (pre-treatment) and time 1 (15 days post-treatment). However, the difference between bleeding on probing as measured at time 0 and time 1 indicated an improvement on both sides, with a slightly greater improvement on the side treated with HA. In fact, on the side that was not treated with HA the statistical analysis indicated that bleeding on probing worsened by 40%.

The analysis of the tartar index (TI) at time 0 and time 1 indicated a one hundred per cent improvement, yet there was no statistically significant difference between TI recorded post-treatment for the side that was treated with HA and the side that was not, while the analysis of the plaque index (PI) showed a 100% improvement on both sides, with a slightly greater improvement seen on the side treated with HA.

DISCUSSION

Admittedly, the results obtained in regards to plaque and tartar management cannot be said to be directly affected by the introduction of HA during the treatment of gingivitis. Nevertheless, in the case of acute pathologies, such as gingivitis, the results provisionally demonstrate the most significant effect of using HA as an adjuvant treatment is primarily related to its ability to reduce bleeding post-treatment. It may be speculated that HA's ability to promote angiogenesis, which is important for wound healing, most likely contributes to an overall improvement in the oral hygiene of the patient.

While long-term post-operative treatment with antibiotics is usually not required in the case of acute gingivitis, for severe bacterial colonization, HA's bacteriostatic and anti-inflammatory properties (9), as well as its ability to prevent the penetration of foreign agents at the site of treatment, helps fight infection during wound healing without the same risks associated with chronic use of antibiotics and the potential development of resistant bacterial strains.

CONCLUSION

In our opinion, the result of this clinical trial are very promising with regards to the benefits of using HA as an adjuvant in the standard treatment of gingivitis in order to reduce post-operative bleeding, contributing to an overall improvement in oral health of the patient. Further studies are required with a larger sample size in order to confirm the results obtained.

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THE USE OF HYALURONIC ACID AS AN ADJUVANT IN THE MANAGEMENT OF MUCOSITIS

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In recent years, with an increase in the number of implants, there has been a related increase in cases of pathologies related to infections around the implant site and on the implant surface i.e. mucositis and peri-implantitis. The purpose of this pilot study is to evaluate the potential efficacy of nebulized hyaluronic acid in the management of mucositis. The results of the statistical analysis demonstrate that there was no difference between the pocket depth as measured in the treated sites at time 0 (pre-treatment) and time 1 (15 days weeks post-treatment). However, the difference between bleeding on probing as measured at time 0 and time 1 indicated an improvement on both sides, with a slightly greater improvement on the side treated with HA.

Hyaluronic acid, a natural polysaccharide consisting of a linear chain of glucuronic acid and N-acetylglucosamine molecules, is synthesized by the serosa glands of the submucosa and it is an important component of the extracellular matrix of connective tissue, synovial fluid, embryonic mesenchymal tissue, the vitreous humour, the skin and many other organs and tissues in the human body.

Treatment with hyaluronic acid can bring about different responses in the body according to the molecular weight used. In recent years, numerous studies have highlighted some of the properties of hyaluronic acid. In particular, low-molecular-weight hyaluronic acid (<300 kD) promotes the stimulation of cell proliferation and has an anti-inflammatory action, while in the case of high-molecular-weight hyaluronic acid (1000 kD), there is increasing evidence to suggest that, due to its inherent

immunosuppressive effect, it helps to regulate excessive exacerbation of inflammation (1-9). The most significant functions and properties of HA are due to some of its specific characteristics: it has a hygroscopic nature and viscoelastic properties; it is non-antigenic yet it is antioxidant, anti-oedematous and biocompatible. Moreover, it is bacteriostatic and anti-inflammatory.

These properties prevent the selection and formation of resistant bacterial strains even when used over an extended period (10), which is one of the most significant side effects that occur in the chronic use of antibiotics, making it especially useful in the management of chronic diseases, particularly in respiratory disorders.

HA is widely used in aesthetic medicine to improve aesthetic surgery results (11-20). It is well known in dentistry that there are numerous chronic conditions that require ongoing and constant management over

Key words: hyaluronic acid, mucositis

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time, the most noteworthy being periodontal disease, gingivitis and periodontitis. To this list, in recent years, due to an increase in the number of implants, there are increasing pathologies related to infections around the implant site and on the implant surface, i.e. mucositis and peri-implantitis.

HA, interacting with type I and II toll-like receptors, regulates the body's innate immunity against bacteria and viruses by preventing penetration, promoting angiogenesis and by modulating the proliferation, migration and differentiation of the cells responsible for tissue repair and thereby plays a key role in wound healing (1, 21-36).

The emollient and restructuring action it exerts on the mucous membranes is also very important, caused by its reparative (stimulates angiogenesis) and soothing properties (hyaluronic acid is used in wound care to improve the processes of wound healing) which are effective in treating the symptoms of local inflammation and irritation (1, 21-36).

There are several methods of administration depending on the molecular weight of the hyaluronic acid involved and according to how and where it is to be used.

Recently, a specifically designed device has been proposed that is able to nebulize particles with a diameter of approximately 16 micrometres to be used especially in the management of the upper airway respiratory tract.

The purpose of this pilot study is to evaluate the potential efficacy of nebulized hyaluronic acid in the management of mucositis.

MATERIAL AND METHODS

The study involved the selection of 5 patients who have at least one implant in different hemi-arches, who have worn a prosthesis for at least 6 months, and who presented with mucositis which showed a maximum probing depth under 4 mm, bleeding on probing and/or spontaneous bleeding. Informed consent was obtained from all individual participants included in the study.

The oral hygiene of the patients was assessed at time 0 and 15 days (time 1) using the Simplified Oral Hygiene Index, which included analysis of the Tartar Index (TI) by applying the Simplified Green-Vermilion Index, the

Plaque Index (PI) by applying the Simplified Green-Vermilion Index, as well as evaluating Pocket Depth (PD) expressed in millimetres, and Bleeding on Probing (BOP) (yes/no). The type of implant connection was noted for each case.

Patients underwent treatment which included closed SRP (scaling and root planing) on all four quadrants, in addition to the administration of HA, nebulized via Spray-sol (Ibsa Farmaceutici Italia Srl, Lodi, Italy), to one side only (either the right or the left) immediately after treatment. Patients were then provided with a quantity sufficient to make two self-administrations of nebulized HA per day for 15 days on the same side.

At the end of the first and the second week, the parameters listed above were re-measured and statistically compared to the initial results.

RESULTS

While this pilot study included only 5 patients and therefore the results are not sufficient to indicate a statistically relevant result, it can, nevertheless, give a good indication of the potential efficacy of HA in the treatment of mucositis.

The results of the statistical analysis demonstrate that there was no difference between the pocket depth as measured in the treated sites at time 0 (pre-treatment) and time 1 (15 days post-treatment). However, the difference between bleeding on probing as measured at time 0 and time 1 indicated an improvement on both sides, with a slightly greater improvement on the side treated with HA. In fact, on the side that was not treated with HA, the statistical analysis indicated that bleeding on probing worsened by 60%.

The analysis of tartar index (TI) at time 0 and time 1 indicated a 100% improvement, yet there was no statistically significant difference between TI recorded post-treatment for the side that was treated with HA and the side which was not. The analysis of the plaque index (PI) similarly showed a one hundred per cent improvement on both sides, with no difference recorded between the sides.

DISCUSSION

As can be seen from the results above, the most significant effect of using HA as an adjuvant to the

standard treatment of gingivitis is primarily related to its ability to reduce bleeding post-treatment. Admittedly, the results obtained as regards plaque and tartar management cannot be said to be directly affected by the introduction of HA during the treatment of mucositis. However, it may be speculated that the better management of bleeding, brought about due to HA's ability to promote angiogenesis, which is important for wound healing, most likely contributes to an overall improvement in the oral hygiene of the patient. Any treatment of this nature resulting in an open wound in the soft tissue that requires healing can lead to the colonization of bacteria.

Generally, antibiotics are prescribed to manage such infections. However, as mentioned in the introduction, the chronic use of antibiotics can cause the selection and formation of resistant bacterial strains. Recent studies have suggested that HA has bacteriostatic and anti-inflammatory properties and can prevent the penetration of foreign agents at the site of treatment, which both helps fight infection during wound healing and does not carry the same risks as the chronic use of antibiotics as mentioned above.

CONCLUSION

The result of this clinical trial show that HA is beneficial in the management of post-operative bleeding when used as an adjuvant in the standard treatment of mucositis. Further studies are required with a larger sample size in order to confirm the results obtained.

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THE USE OF HYALURONIC ACID AS AN ADJUVANT IN THE MANAGEMENT OF PERIODONTITIS

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The emollient and restructuring action exerted on the mucous membranes by hyaluronic acid is of particular significance. This is thanks to its reparative (it stimulates angiogenesis) and soothing properties (hyaluronic acid is used in wound care to improve the processes of wound healing), which are effective in treating the symptoms of local inflammation and irritation. The purpose of this clinical trial is to evaluate the potential efficacy of nebulized hyaluronic acid in the management of chronic periodontitis in adults. The results of the statistical analysis demonstrate that there was a slight improvement in the measurement of pocket depth in the side treated with HA at time 0 (pre-treatment) and time 1 (15 days post-treatment). Furthermore, the difference between bleeding on probing as measured at time 0 and time 1 indicated an improvement on both sides, with a slightly greater improvement on the side treated with HA.

Hyaluronic acid is an important component of the extracellular matrix of connective tissue, synovial fluid, embryonic mesenchymal tissue, the vitreous humour, the skin and many other organs and tissues in the human body. It is a polysaccharide consisting of a linear chain of glucuronic acid and N-acetylglucosamine molecules, which is synthesized by the serosa glands of the submucosa.

Low-molecular-weight hyaluronic acid (<300 kD) promotes the stimulation of cell proliferation and has an anti-inflammatory action, while increasing evidence suggests that high-molecular-weight hyaluronic acid (1000 kD), due to its inherent immunosuppressive effect, also helps to regulate exacerbation of symptoms caused by excessive inflammation (1-9). HA is widely used in aesthetic medicine to improve aesthetic surgery results (10-19).

Firstly, it is non-antigenic, yet it is antioxidant, anti-oedematous and biocompatible. Moreover, it is bacteriostatic and anti-inflammatory, and thus it prevents the selection and formation of resistant bacterial strains, even when used over an extended period (20), which is one of the most significant side effects that occur in the chronic use of antibiotics, making it especially useful in the management of chronic diseases, particularly in respiratory disorders.

In dentistry, one of the most important chronic pathology that requires constant management is periodontal disease.

HA plays a key role in wound healing, interacting with type I and II toll-like receptors, it regulates the body's innate immunity against bacteria and viruses by preventing penetration, promoting angiogenesis and by modulating the proliferation, migration and

Key words: hyaluronic acid, periodontitis

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differentiation of the cells responsible for tissue repair (1, 21-36). HA can be administered using several methods, depending on the molecular weight of the hyaluronic acid involved and according to how and where it is to be used.

It has recently been made possible, using a device (Spray-sol, Ibsa Farmaceutici Italia Srl, Lodi, Italy), designed specifically for the nasal administration of HA in the treatment of rhinosinusitis, to nebulize particles with a diameter of approximately 16 micrometres to better increase the management of diseases of the upper airway respiratory tract.

The purpose of this pilot study is to investigate how nebulized hyaluronic acid can be used as a supportive treatment in the management of chronic periodontitis in adults.

MATERIAL AND METHODS

The study involved the selection of 5 patients who presented chronic adult periodontitis and showed a maximum probing depth between 4 and 7 mm, bleeding on probing and/or spontaneous bleeding. Informed consent was obtained from all individual participants included in the study.

The oral hygiene of the patients was assessed at time 0 and at 15 days (time 1) using the Simplified Oral Hygiene Index, which included the Tartar Index (TI) by applying the simplified Green-Vermilion index, the Plaque Index (PI) by applying the simplified Green-Vermilion Index, as well as evaluating Pocket Depth (PD) expressed in millimetres and Bleeding on Probing (BOP) (yes/no).

Patients underwent treatment which included closed SRP (scaling and root planing) on all four quadrants, in addition to the administration of HA, nebulized via Spray-sol (Ibsa Farmaceutici Italia Srl, Lodi, Italy), to one side only (either the right or the left) immediately after treatment. Patients were then provided with a quantity sufficient to make two self-administrations of nebulized HA per day for 15 days on that same side.

At the end of the first and the second week, the parameters listed above were re-measured and statistically compared to the initial results.

RESULTS

This clinical gives a good indication of the

potential efficacy of nebulized hyaluronic acid as a supportive treatment in the management of chronic periodontitis in adults, despite the fact that only 5 patients were observed, and the results are therefore not sufficient to be statistically relevant.

There was a slight improvement in the measurement of pocket depth in the side treated with HA at time 0 (pre-treatment) and time 1 (15 days post-treatment). Furthermore, the difference between bleeding on probing as measured at time 0 and time 1 indicated an improvement on both sides, with a slightly greater improvement on the side treated with HA.

The analysis of the tartar index (TI) at time 0 and time 1 indicated a ninety per cent improvement, yet there was no statistically significant difference between TI recorded post-treatment for the side that was treated with HA and the side which was not. While, the analysis of the plaque index (PI), showed a one hundred per cent improvement on both sides, but with a slightly greater improvement seen on the side treated with HA.

DISCUSSION

As seen from the results above, the most significant effect of using HA to supplement the standard treatment of periodontitis is primarily related to its ability to reduce bleeding post-treatment. While plaque and tartar may be contributing aetiological agents in adult periodontitis, the results obtained cannot be said to demonstrate that the introduction of HA during the treatment of periodontitis brought about significant improvements in these areas. However, it may be speculated that the better management of bleeding, brought about due to HA's ability to promote angiogenesis, which is important for wound healing, most likely contributes to an overall improvement in the oral hygiene of the patient. The pocket created by periodontitis is chronic, and while standard treatment can result in a reduction in pocket depth, the remaining open wound, during post-operative healing, is highly susceptible to recolonization by bacteria. Moreover, due to its nature, periodontitis is likely to recur, leading to further treatment, and further bacterial recolonization.

Naturally, antibiotics are prescribed to manage such infections, yet the chronic use of antibiotics can cause the selection and formation of resistant bacterial strains, which is of particular significance in the case of chronic and recurrent periodontitis. As an adjuvant treatment, HA has the potential to combat this issue thanks to its bacteriostatic and anti-inflammatory properties and its ability to prevent the penetration of foreign agents at treatment site as suggested by recent studies (1), by helping to fight infection during wound healing and significantly reduce the risks associated with the chronic use of antibiotics, as mentioned above.

CONCLUSION

The result of this clinical trial demonstrate that HA is beneficial in the management of post-operative bleeding when used as an adjuvant in the standard treatment of periodontitis, and may be speculated to have an concordant benefit by reducing the necessity to prescribe long-term post-operative antibiotics in order to manage bacterial colonization during wound healing. Further studies are required with a larger sample size in order to confirm the results obtained.

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THE USE OF HYALURONIC ACID AS AN ADJUVANT IN THE MANAGEMENT OF PERI-IMPLANTITIS

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It is well known in dentistry that there are numerous chronic conditions that require ongoing and constant management over time, the most noteworthy being periodontal disease, gingivitis and periodontitis. Yet, in recent years, with the increase in the number of implants being placed, mucositis and peri-implantitis have become more and more prevalent pathologies. The results of the statistical analysis demonstrate that there was a slight difference between the pocket depth as measured in the treated sites at time 0 (pre-treatment) and time 1 (15 days post-treatment), although the difference was so small as to render it statistically irrelevant. Bleeding on probing as measured at time 0 and time 1 indicated an improvement on both sides, but with no greater improvement noted on the side treated with HA.

In recent years, with an increase in the number of implants being placed, pathologies related to infections around the implant site and on the implant surface, such as mucositis and peri-implantitis, have become increasingly prevalent. In severe cases, peri-implantitis results in bone loss and the consequent failure of the implant, which requires extensive surgery and bone regeneration procedures to rectify. Many implants today are designed with a rough surface, considered ideal to facilitate osteointegration, but the same surface treatment is the ideal breeding ground for bacteria. If the connection between the implant and the abutment is not sufficiently stable, the penetration of bacteria within the connection is likely to take place. In order to achieve significant, long-term results both to prevent total implant failure and to stimulate reosteointegration, targeted detoxification of the implant surface is required to eliminate biofilm colonization (1).

Hyaluronic acid is a natural polysaccharide consisting of a linear chain of glucuronic acid and N-acetylglucosamine molecules, and it is an important component of the extracellular matrix of many tissues in the human body.

Numerous studies carried out in the last decade have demonstrated the various beneficial properties of hyaluronic acid. Depending on its molecular weight, treatment with hyaluronic acid results in different responses in the body. On the one hand, low-molecular-weight hyaluronic acid (<300 kD) promotes the stimulation of cell proliferation and has an anti-inflammatory action. On the other, increasing evidence suggests that high-molecular-weight hyaluronic acid (1000 kD), due to its inherent immunosuppressive effect, also plays a role in the regulation of excessive inflammation (2-10). The most significant functions and properties of HA are as follows: it has a hygroscopic nature and

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viscoelastic properties; it is non-antigenic, yet it is antioxidant, anti-oedematous and biocompatible; it is bacteriostatic and anti-inflammatory. HA is widely used in aesthetic medicine to improve aesthetic surgery results (11-20).

During the post-operative phase of the treatment of peri-implantitis, it is often necessary to prescribe antibiotics to minimize and manage bacterial recolonization and to improve wound healing in the soft tissues affected around the implant site. The literature indicates that the use of HA as an adjuvant treatment can help to prevent the selection and formation of resistant bacterial strains, even when used over an extended period (21), which is one of the most significant side effects that occur in the chronic use of antibiotics. This makes it especially useful in the management of chronic diseases, which may be particularly significant in the case of peri-implantitis. Furthermore, HA has been shown to regulate the body's innate immunity against bacteria and viruses by preventing penetration, through its interaction with type I and II toll-like receptors, promoting angiogenesis and by modulating the proliferation, migration and differentiation of the cells responsible for tissue repair; thus playing a key role in wound healing (2, 22-37).

Recently, a device has been designed especially for the nasal administration of HA to treat rhinosinusitis that is able to nebulize particles with a diameter of approximately 16 micrometres. The purpose of this pilot study is to evaluate the potential efficacy of nebulized hyaluronic acid in the management of peri-implantitis.

MATERIAL AND METHODS

The study involved the selection of 5 patients who had at least one implant in different hemi-arches, who have worn a prosthesis for at least 6 months, and who presented with peri-implantitis which showed a probing depth between 4 and 7 mm, bleeding on probing and/or spontaneous bleeding. Informed consent was obtained from all individual participants included in the study.

The oral hygiene of the patients was assessed at time 0 and after 15 days (time 1) using the Simplified Oral Hygiene Index, which included analysis of the Tartar

Index (TI) by applying the Simplified Green-Vermilion Index, the Plaque Index (PI) by applying the simplified Green-Vermilion index, as well as evaluating Pocket Depth (PD) expressed in millimetres, and Bleeding on Probing (BOP) (yes/no). The type of implant connection was noted for each case.

Patients underwent treatment which included closed SRP (scaling and root planing) on all four quadrants, in addition to the administration of HA, nebulized via Spray-sol (IBSA FARMACEUTICI ITALIA Srl, Lodi, Italy), to one side only (either the right or the left) immediately after treatment. Patients were then provided with a quantity sufficient to make two self-administrations of nebulized HA per day for 15 days on that same side. At the end of the first and the second week, the parameters listed above were re-measured and statistically compared to the initial results.

RESULTS

While this pilot study included only 5 patients and therefore the results are not sufficient to indicate a statistically relevant result, it can, nevertheless, give a good indication of the potential efficacy of HA in the treatment of peri-implantitis.

The results of the statistical analysis demonstrate that there was a slight difference between the pocket depth as measured in the treated sites at time 0 (pre-treatment) and time 1 (15 days post-treatment), although the difference was so small as to render it statistically irrelevant. Bleeding on probing as measured at time 0 and time 1 indicated an improvement on both sides, but with no greater improvement noted on the side treated with HA.

The analysis of the tartar index (TI) at time 0 and time 1 indicated a one hundred per cent improvement, yet there was no statistically significant difference between TI recorded post-treatment for the side that was treated with HA and the side that was not. The analysis of the plaque index (PI) similarly showed a one hundred per cent improvement on both sides, but with no difference between the two sides.

DISCUSSION

As seen from the results above, HA as an

adjuvant to the standard treatment of peri-implantitis did not seem to bring about a statistically significant improvement in post-operative bleeding or in pocket depth measurements. Similarly, the introduction of HA during treatment did not appear to have resulted in an improvement as regards plaque and tartar management. It can be speculated that the contamination of implant surfaces significantly favours the development of peri-implantitis and while HA has been proven to be effective in the management of infection, due to its bacteriostatic and anti-inflammatory properties and to promote angiogenesis during wound healing, these beneficial effects act primarily on the soft tissues, and logically, in the case of implant failure due to bone loss, it cannot be expected to help with this issue. The results obtained in this study support the literature as regards the necessity to carry out targeted cleaning and detoxification treatments of the implant surface in order for reosseointegration to be successfully achieved (1).

CONCLUSION

We can conclude that HA's ability to control and improve post-operative bleeding and to promote the healing of soft tissue does not appear to be directly relevant in cases relating to the management of peri-implantitis. However, its ability to reduce and control inflammation may aid to minimize the inflammatory response induced by biofilm colonization on the implant surface, both during decontamination treatment and post-operative healing. This case-study made use of a device that nebulizes HA that is not designed for oral administration, therefore the poor results in terms of general improvements to the oral health of the patients as well as the parameters specifically targeted may have been affected by the fact that, in the case of peri-implantitis, it is fundamental that both the surgeon and patient, during the post-operative phase, are capable of site-specific oral administration. Further studies will be useful in order to evaluate the effectiveness of HA as an adjunct to treatments designed to decontaminate implant surfaces both in open to the closed surgical procedures.

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THE USE OF HYALURONIC ACID AS A SUPPORT OF TWO DIFFERENT MICRONIZED BIOMATERIALS IN CRESTAL SINUS LIFT PROCEDURES. A REPORT ON TWO CASE STUDIES WITH VOLUME COMPARISON

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In the context of the trans crestal maxillary sinus lift, a wide variety of biomaterials have been used to fill the sub-antral space over the years. The materials that have a pasty consistency and are smooth and free from lumps are the most suitable to come into contact with the Schneiderian membrane which, if torn, cannot perform its graft containment function. In this study, a micronized heterologous bone in a collagen matrix of two different percentages was used in order to fill the maxillary sinus. Before using biomaterial as filler, a spray form of hyaluronic acid was used to disinfect the surgical site before and after the surgery, along with more consistent and pasty form of gel of hyaluronic acid being used in order to facilitate the detachment of the membrane. The surgical procedures were designed and carried out using computer-planned surgery. The filling volume obtained was measured with a comparative software programme and using an ellipsoid formula. This technique allows the surgery to be performed in a way that is both minimally traumatic and invasive, fully careful of the membrane and represents a viable alternative to those surgical techniques for crestal sinus lift currently in use.

When there is increased pneumatization of the maxillary sinus, various surgical lifting techniques of the maxillary sinus floor lift are required. These techniques involve filling part of the maxillary sinus with biocompatible materials that after some months, due to reabsorption and remodelling processes, are transformed into bone, resulting in adequate bone volume for implant-prosthesis.

When large cavity fillings and anatomical structures are required, it is generally necessary to resort to vestibular approaches to the maxillary sinus. Actually, the crestal approach is increasingly employed where small quantities of biomaterial are required to fill the sinus cavity. In these cases, it is generally possible

to insert the implants immediately. These ‘mini lifts’ are therefore advisable when the initial osseous thickness is between 5 and 6 mm (1).

Over the years, numerous maxillary sinus lift techniques using a crestal approach have been described in the literature (2). A transcristal hydraulic lift technique has been used in this study. A specially made injector, named ML, linked to a new a specially designed polyoxymethylene micrometric piston weighing only 15 grams (ML easy) was used. It allows for the elevation of the membrane and the filling of the sinus cavity simultaneously (3, 4).

One of the most frequent reasons for failure during a maxillary sinus-floor lift operation is connected

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to the possibility of a rupture of the Schneiderian membrane that, if lacerated, cannot perform the function of graft containment. For this reason, it is important to choose smooth biomaterials in order to avoid damaging the membrane (2-4). In order to reduce the incidence of complications, it is necessary to cut the hard tissue with extreme accuracy, and as little trauma as possible, while saving the soft tissue. The precision of pre-operation measurements obtained through endoral X-rays, dental-scans and cone-beam CT allows us to approach and cut the sinus cortical floor with delicacy.

With the advent of computer-planned surgery, it is possible to design and carry out minimally traumatic and invasive procedures that allow for the filling of the maxillary sinus via a crestal approach, which is precisely calibrated, thus reducing the risk of failure (5-10).

In this study, using a fluído-dynamic technique of transcresal maxillary sinus lift performed with the aid of computer-planned surgery. Two different micronized heterologous bone in a collagen matrix biomaterials in two different clinical cases are used, both of which have a granulometry between 300 and 600 microns but the first one has 20% of collagen (Putty by Osteobiol-Tecnoss, Italy) and the second 40% of collagen (Gel 40 by Osteobiol-Tecnoss, Italy).

The cortical of the maxillary sinus is reduced by using calibrated burs (ROEN Torino, Italia), to obtain a hole that enables both access to the maxillary sinus and subsequently, the lifting of the Schneiderian membrane. In order to facilitate the detachment of the membrane, a gel form of hyaluronic acid was also used, a viscous material, with reepitalization properties. Ballini et al. (11) suggested that autologous bone combined with an esterified low-molecular HA preparation seems to have good capabilities in accelerating new bone formation in infrabony defects (11-17). HA is widely used in aesthetic medicine to improve surgery results (18-35).

Each stage of the operation was monitored and all the devices used passed through a custom-made template, which acts as a surgical guide. The sinuses were filled using two different fluid biomaterial

distributed through the dispenser ML, which could fit specifically for this technique. Due to the reduction in trauma and the fact that the process is much less invasive, this technique could be a valid alternative to the techniques known and used to date.

Work time is reduced in the cortical thinning operation and percussive trauma is avoided, while it is important to fill the sinus slowly and with care. In the cases described, the filling process took 3 min to fill the sinus with both biomaterials using an ML injector and a specially designed polyoxymethylene micrometric piston weighing only 15 grams (ML easy). There was no stops during the injection of Gel 40 while it was necessary to clean the injector two times using Putty.

The volume of the sinus to be filled was calculated using a specific software program that, in addition to an accurate volume measurement, allows for an accurate comparison between the projected and clinical outcome of the implant position.

The aim of this study is to compare the volumetric measurement and the behaviour of two different micronized heterologous bone in a collagen matrix biomaterials as a sinus filler, using a fluído-dynamic technique for transcresal maxillary sinus lift, performed with the aid of computer-planned surgery that allows for a precise reduction in the cortical thickness without entering the sinus and supported by hyaluronic acid.

MATERIALS AND METHODS

The operation field was prepared using Hyaluronic acid, which was delivered in a nebulized form using a patented device (Yabro spray sol, IBSA Italy). The cortical of the maxillary sinus was reduced through the use of calibrated burs (ROEN Torino, Italia) that allows for an opening to be created to give access to the maxillary sinus and subsequently, the lifting of the Schneiderian membrane can be achieved. In case number 1, the detachment and lifting was achieved using HA gel with a molecular weight of (Sinovial HL, IBSA Italy) micronized heterologous bone in a 20% collagen matrix (Putty by Osteobiol-Tecnoss, Italy) and the second one micronized heterologous bone in an 40% (Gel 40 by Osteobiol-Tecnoss, Italy) and whose granulometry was

between 300 and 600 microns .

Each stage in the operative process was planned through a computer-guided software (BIONOVA, LA SPEZIA, Italy). Fluid biomaterials, distributed through a dispenser, which had been created specifically for this technique, were used to fill the sinus.

Case study

Two cases with similar clinical situations were treated and have been described in detail below to illustrate the method used. Informed consent was obtained from all individual participants included in the study.

Both patients were not-smokers, with no systemic

disease, nor on regular prescribed medication. Both patients were periodontally treated. The first patient was a healthy, 48-year-old male whose second upper left molar was fractured and had been extracted 8 months previous. A regeneration procedure was carried out 6 months previous using a palatal cortical heterologous lamina of the thickness of 1 mm and micronized heterologous bone in an 20% collagen matrix whose granulometry was between 300 and 600 microns (Putty by Osteobiol-Tecnoss, Italy) (Fig 1, 2, 3). The crest showed four-millimetre residual bone height with a horizontal thickness of nine millimetres (Fig 4). The second was a 65-year-old male whose first and second upper right premolars had been extracted 5 years



Fig. 1. Second upper left molar fractured and extracted 8 months before in patient 1.

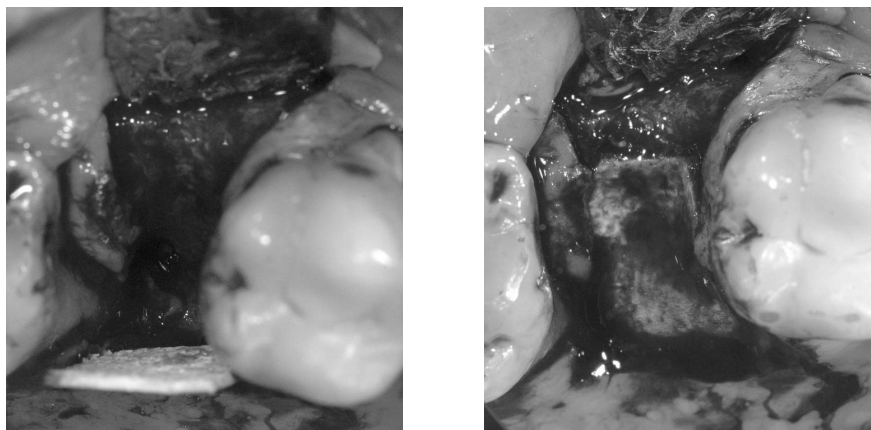


Fig. 2, 3. Regeneration of palatine side six months before in patient 1.



Fig. 4. Pre-operative TC Scan patient 1.

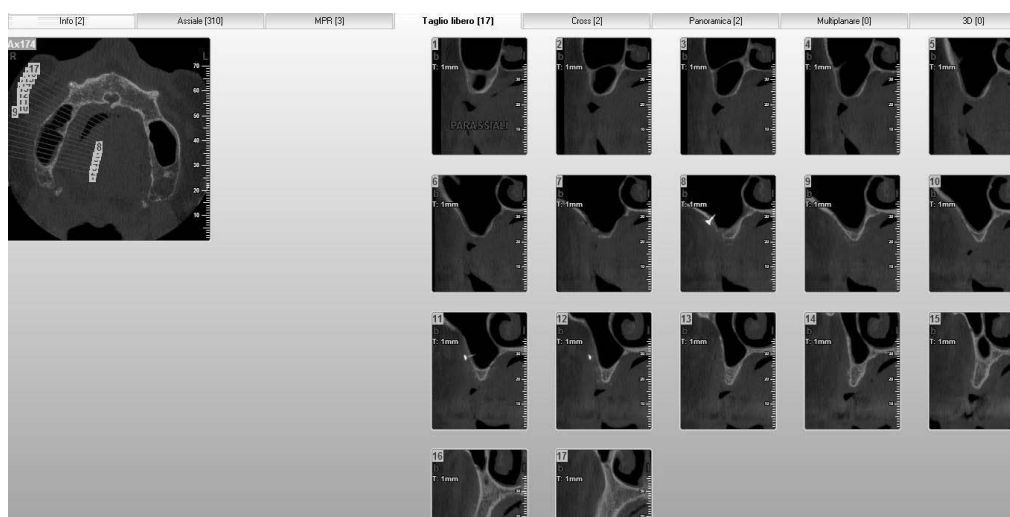


Fig. 5. Pre-operative TC Scan patient 2.

previous and showed approx. 5 millimetre residual bone height with a horizontal thickness of 5 millimetres in the position of second premolar (Fig 5).

A micronized heterologous bone in a 40% collagen matrix whose granulometry was between 300 and 600 microns (Gel 40 by Osteobiol-Tecnoss, Italy) was used. Neither patients showed signs of inflammation of the sinus membrane. The same dental surgeon performed the operations and the preoperative planning was carried out in the following phases:

- 1) A cone beam CT (GENDEX GXCB-500) scan.
- 2) The Dicom files obtained were processed with

specialised software (ModelGuide, BIONOVA, LA SPEZIA, Italy). The residual ridge measurement was found to be 4 mm in patient 1, and 5 mm in patient 2, with a horizontal thickness of 9 mm and 5 mm, respectively. The positioning of a 10-mm implant with a diameter of 3.5 mm (Instradent, Straumann Group; Switzerland. Drive CM) was planned to obtain a sinus floor elevation of 6 mm in patient 1 (Fig. 7, 8), and a 10-millimetre implant with a diameter of 3.5 mm (Instradent, Straumann Group; Switzerland. Alvim CM) to obtain a sinus floor elevation of five millimetres in patient 2 in order to completely cover



Fig. 6. *Injection of HA gel to first detachment of membrane.*

the implant with biomaterial (Fig. 9).

- 3) A Intradent surgery kit was used and two instruments for this technique were used: a calibrated burs not cutting in the apex of 3.5 mm diameter (ROEN Torino, Italia) and an ML dispenser (ROEN Torino, Italia) (Fig. 7).
- 4) An operculectomy was carried out with the use of a rotating circular tissue punch mounted on a hand-piece in patient 1 and a flap in patient 2, after which a pilot drill was used to cut the cortical bone and assess the consistency of the bone. In both patients, nebulized HA was used to prepare the operative field (Yabro spray sol, IBSA Italy). Subsequently, two burs, a pilot of 2 mm and a second one of 3.5 mm were used to enlarge the hole and to reach the sinus

floor (Fig. 10, 11).

- 5) Through a calibrated bur, not cutting in the apex of 3.5 mm diameter (ROEN Torino, Italia) the complete erosion of the cortical bone of the maxillary sinus was achieved; after which it was possible to insert a calibrated smooth, convex apex ML dispenser (ROEN Torino, Italia) to enter 6 mm inside the sinus of patient 1 and 5 mm in patient 2.

This tool allowed for both the detachment and lifting of the Schneiderian membrane and the simultaneous insertion of a fluid biomaterial. In both patients, there was pre-detachment of the membrane using HA gel (IBSA, Italia. Sinovial HL 1 m) (Fig. 6) and a mixture of micronized heterologous bone, both with a granulometry between 300 and 600 microns, in an 20% collagen matrix in patient n 1 (Putty by Osteobiol-Tecnoss, Italy) while in patient 2, a 40% collagen matrix (Gel 40 by Osteobiol-Tecnoss, Italy) were used. These materials were chosen because the small size of the granules better facilitates the injection process (Fig. 7).

- 6) The accuracy of the measurements was monitored using an endoral X-ray (Fig. 8, 9).
- 7) After the first filling, the ML injector was rotated by 90° to obtain a radial filling of the sinus, which formed a dome shape. The process of filling was performed slowly, with great care, in order to avoid rupturing the membrane, and took about 3 min. A specially designed polyoxymethylene micrometric piston weighing only 15 g (ML easy) was used (Fig. 12). After the ML dispenser had been removed, an



Fig. 7, 8. *ML filling the sinus and implant in patient 1.*

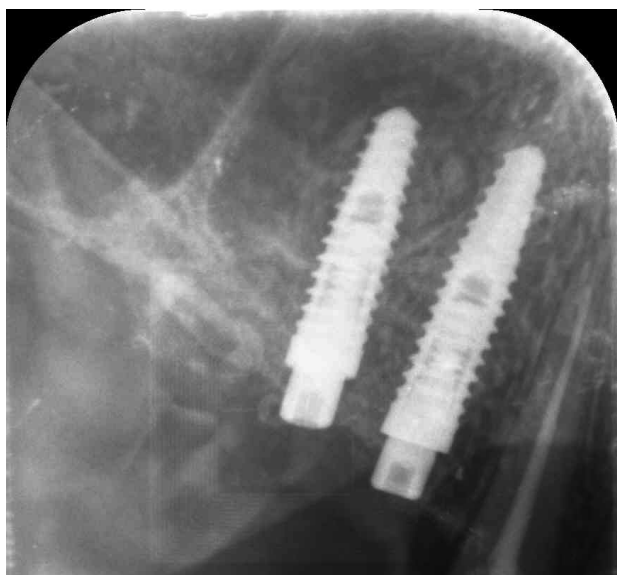


Fig. 9. *Implant in patient 2.*

implant with a diameter of 3.5 mm and height of 10 mm was inserted using an aggressive thread, which gave higher primary stability, thus reducing the risk of the implant accidentally sliding into the sinus (Instradent, Straumann Group, Switzerland. Drive CM and Alvim CM). Finally, the healing screw was positioned. In both patients, nebulized HA was used on the tissue after suturing (Yabro spray sol, IBSA Italy).

- 8) The precision of the final implant position was monitored through an endoral X-ray (Fig. 8, 9).
- 9) A measurement of the final filled volume was carried using the ellipsoid formula and taking into account the measurements of the three anteroposterior

diameters. In the first case the volume was 0.758 cubical centimetres and in second case was 0.664 cubical centimetres.

RESULTS

In the cases described above, a ≥ 6 mm augmentation of osseous volume was obtained. This result is comparable to those obtained in lifts where conventional techniques were applied as we can see in the X-rays and TC scan after 4 months at the moment of final prostheses (Fig. 13, 14, 15, 16).

DISCUSSION

In both cases, the distribution of biomaterial in the sinus was very regular and the quantity of biomaterial used was enough to create a dome, which formed a cover over the implant, as well as being capable of supporting the elevation of the membrane

After becoming stable and remodelled, the biomaterial transforms into bone acting as a filler and as support to the implants located in the sinus. There was no stops during the injection of Gel 40 while it was necessary cleaning the injector two times using Putty.

In recent years, there has been an increased tendency to use instruments and techniques to reduce the incidence of the type of complication, such as membrane laceration, as mentioned above. Biomaterials that have a pasty consistency, and are

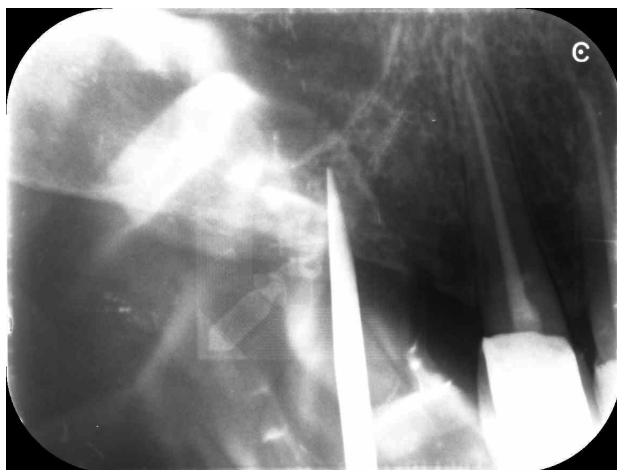


Fig. 10, 11. *Pilot drill in patient 2.*

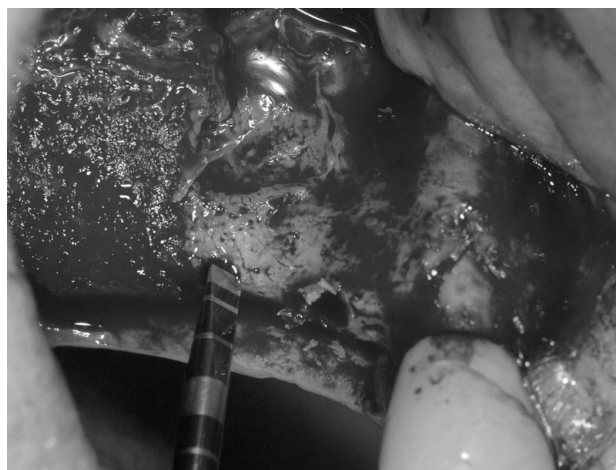




Fig. 12. *Specially designed polyoxymethylene micrometric piston weighing only 15 grams (ML easy).*

smooth and free from lumps are the most suitable to come into contact with the Schneiderian membrane, which is true for the 2 different biomaterials used in these 2 different clinical cases. Both of them have a granulometry between 300 and 600 microns but the first one has 20% of collagen (Putty by Osteobiol-Tecnoss, Italy) while the second one 40% of collagen (Gel 40 by Osteobiol-Tecnoss, Italy). Before using the filler it was very important to use a HA to reduce the contamination before surgery with a nebulized formula and to start the detachment of the membrane with a more viscous formula.

Topical HA can be useful as a coadjuvant treatment in gingivitis, chronic periodontitis, as well as during the post-operative period, for implant and sinus lift procedures, for faster healing and to reduce the patients' discomfort during the postoperative period.

In particular, the aim of this study is to compare the final filling volume of the sinus with the fluidodynamic technique of trans-crestal maxillary sinus lift performed with the aid of computer-planned surgery, which allows for a precise reduction in the cortical thickness without entering the sinus, while concurrently saving the soft tissues.

Such characteristics not only meet the requirements of maxillary sinus surgery, but also are much less traumatic than more traditional techniques. Even the risk of membrane rupture is reduced due to the high-precision system used and thanks to the smooth consistency of the biomaterials without lumps.

The improvement in diagnostic tests and new biomaterials available, allow the clinician to use computer-planned surgery in the case of crestal sinus lift operations in order to increase accuracy and reduce trauma.

In addition, there is the elimination of percussion, with a direct decrease in patient discomfort. Furthermore, the technique reduces the number of radiographic intraoperative checks necessary during the procedure, with consequent reduction in post-operative recovery time.

The measurement of the filled volume is compatible with the amount of material in the biomaterial syringe and the amount retained in the ML and in his connector. The use of a very light and short micrometer piston allows a correct handling of the biomaterial syringe avoiding the risk of enlarge the hole in the residual crest.

CONCLUSIONS

Even with the limited number of case studies carried out to date, is it possible to foresee that the results obtained with the technique described above are encouraging. The 2 biomaterials used have a similar pasty consistency but the second one (Gel 40 by Osteobiol-Tecnoss, Italy) seems to be easy to introduce in the sinus and seems to have a similar clinical behaviour in the first surgical phase, however, the results must be monitored during the remodelling time. It is possible that the second one could reabsorb too quickly so that a further support of small quantity of granules is advisable.

Both the reduced percussive trauma and the low invasiveness mean such a technique should be considered a valid and concrete alternative to those known and used thus far.

Further studies are necessary in order to investigate the higher or lower efficacy in comparison

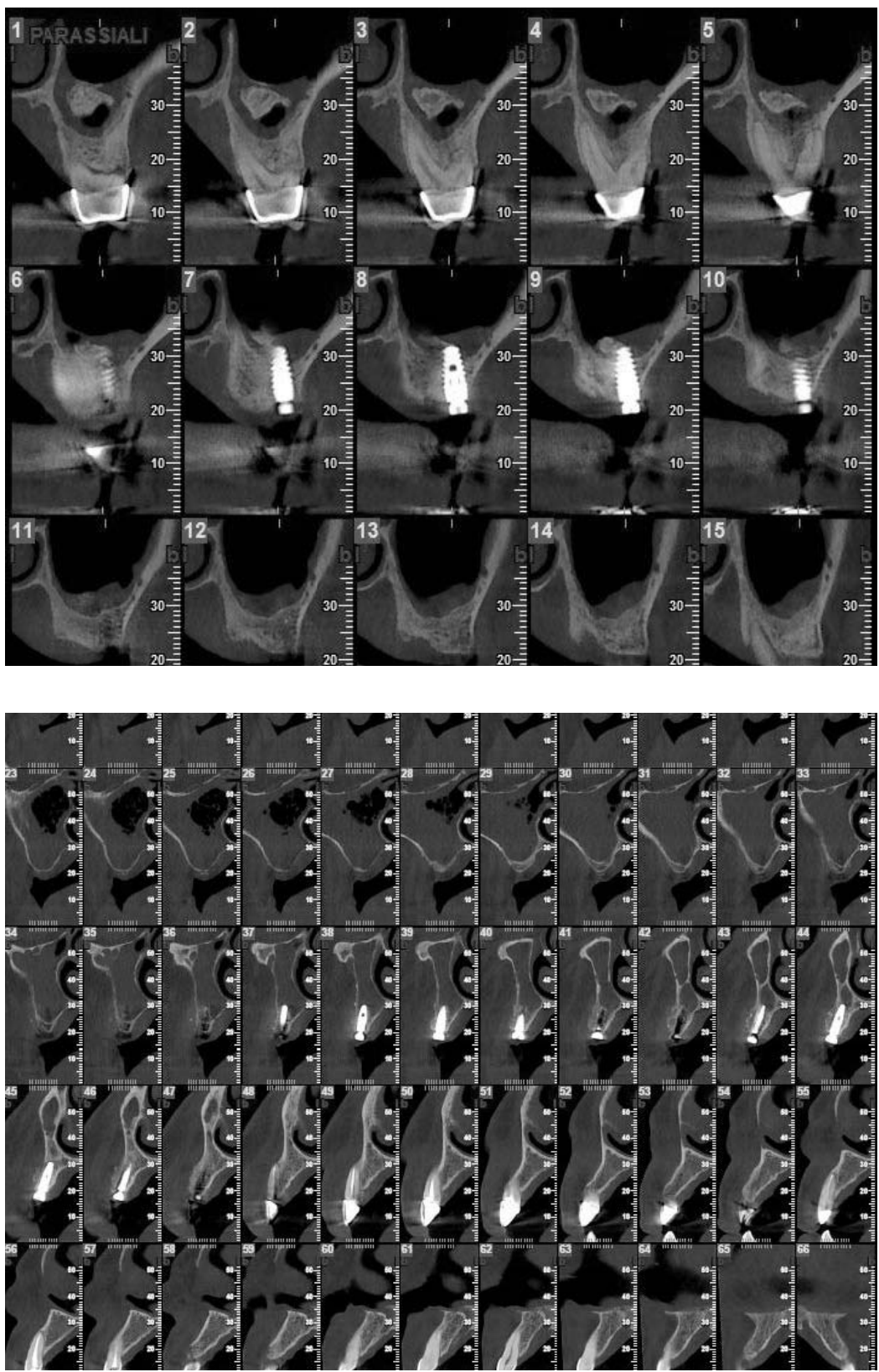


Fig. 15, 16. TC scan after four months at the moment of final prostheses.

with statistically significant success and to check the filling volume of the sinus over time. Histologic studies are also needed in order to confirm the quality of bone formed.

ACKNOWLEDGEMENTS

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EFFECT OF BIOSTIMULATION ON ORAL FIBROBLAST: A PILOT STUDY

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Bio-stimulation is a technique in aesthetic medicine in which different drugs such as nucleotides, antioxidants and glucosaminoglycans precursors are injected in the dermis to improving the anabolic function of dermal fibroblasts, i.e., protein synthesis, replication and production of extracellular matrix components. It can be achieved with multiple intra-dermal injections, using two protocols: 1) Polydeoxyribonucleotide (PDRN) plus glucosamine sulphate (Gluc); 2) N-acetylcysteine (NAC) and amino acids (Aa) (named Bio- NAC procedure). Since the role of drugs used in biostimulation on human dermal fibroblasts is not completely understood, the aim of this study is to evaluate the effect of these substances in primary cell cultures by using RT-PCR and a panel of specific genes (ELN, DSP, FN1, FBN1, ITGA1, ITGA2, ITGA5, ITGB1, COL1A1, COL3A1) to detect their effect on cell metabolism and extracellular matrix components. Both the treatments were responsible for Elastine and Desmoplakin genes activation. Only NAC plus Aa treatment enhance the expression of other genes related to tissue growth and elasticity like FBN1, ITGA1 and ITGB1. All the other genes investigated (FN1, ITGA5, ITGA2, COL1A1, COL3A1) were down-regulated by both treatments. Since the precise role of these proteins in tissue integrity and aging is not known, this study confirms the usefulness of biostimulation therapies in enhancing some of the genes responsible of cellular wellbeing. This study could be useful to consider the possibility of injective biostimulation in oral cavity, clinical applications in oral healing and in gingival atrophy as well.

Bio-stimulation (BS) is a technique in aesthetic medicine in which different drugs as nucleotides, antioxidants and glucosaminoglycans precursors are injected in the dermis (1) to improving the anabolic function of dermal fibroblasts, i.e., protein synthesis, replication and production of extracellular matrix components (ECM) (2).

It can be achieved with multiple intra-dermal injections, using two protocols: 1) Polydeoxyribonucleotide (PDRN) plus glucosamine

sulphate (Gluc); 2) N-acetylcysteine (NAC) and amino acids (Aa) (named Bio- NAC procedure) (1, 3, 4).

In the first protocol, polynucleotide fragments (as replication booster) and glucosamine sulfate (as hyaluronate precursor) are used. These drugs are commonly used for their anti-inflammatory function (1, 5).

PDRN is demonstrated to increase nucleic acid biosynthesis and enhance both cellular replication

Key words: biostimulation, aging, real time PCR, gene expression, oral healing

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and protein synthesis. PDRN is indicated in wound healing for the ability of the purine nucleosides and deoxyribonucleotides to enhance cell proliferation and intracellular cAMP, by increasing the extracellular concentration of adenosine. It has been suggested that adenosine regulates inflammation via interaction with one or more of its 4 known receptors (A1, A2A, A2B, and A3). Stimulation of adenosine A2 and A3 receptors has been shown to alter the cytokine network by decreasing inflammatory cytokine secretion by macrophages in vitro (6, 7).

Gluc is classified among the anti-inflammatory non-steroid drugs and used in knee arthrosis. It is one of the major precursors in glucosaminoglycans synthesis. The glucosamine and acetic acid together produce N-acetylglucosamine and its polymerization with glucuronic acid gives Hyaluronic Acid (HA) (5).

HA is widely used in aesthetic medicine to improve aesthetic surgery results (3-5, 8-22). The second BS protocol, named Bio- NAC, provides the administration of N-acetylcysteine (NAC) and amino acids (Aa) instead. NAC is the acetylated form of the amino acid cysteine, one of the three components of glutathione (gamma-glutamyl-cysteinyl-glycine), the major antioxidant of the body.

The SH groups of NAC are essential against reactive oxygen species (ROS) created during oxidative stress (ionizing radiation, environmental pollution and sun exposure) and responsible for aging pathway (23, 24). ROS are usually sequestered and removed by anti-oxidant enzymes. However, scavenging cannot be completely efficient because the antioxidant ability of aged cells is decreased or ROS production in unfavorable environmental conditions is increased (25, 26). Aging is a consequence of the cellular senescence of fibroblasts, decreased synthesis and increased degradation of dermal matrix components and oxidative stress. The Bio-NAC therapy is performed as a further step when clinical response to classic bio-stimulation is poor because the biosynthetic activity of aged fibroblasts is decreased.

Since the role of drugs used in BS on human dermal fibroblasts is not completely understood, the aim of this study is to evaluate the effect of these substances in primary cell cultures by using RT-

PCR and a panel of specific genes (ELN, DSP, FN1, FBN1, ITGA1, ITGA2, ITGA5, ITGB1, COL1A1, COL3A1) to detect their effect on cell metabolism and extracellular matrix components.

MATERIALS AND METHODS

Primary human gingival fibroblast cell (HGF) culture

Human connective tissue was obtained from gingival biopsies of three healthy patients (a 68-year-old man, a 20-year-old man and a 63-year-old woman). The gingival pieces were fragmented with a scalpel and transferred in culture dishes containing Dulbecco's modified Eagle medium (DMEM, Sigma-Aldrich, Inc., St. Louis, Mo) supplemented with 20% foetal calf serum (FBS) and antibiotics, i.e. penicillin 100U/ml and streptomycin 100µg/ml (Sigma Aldrich, Inc.).

Cells were incubated in a humidified atmosphere of 5% CO₂ at 37°C. The medium was changed the next day and twice a week. After 15 days, the pieces of gingival tissue were removed from the culture dishes. Cells were harvested after additional 24 h of incubation.

Cell culture

Human fibroblasts at the second passage were seeded on a layer of two different medical devices:

1- Polydeoxyribonucleotide (PDRN) plus glucosamine sulphate (Gluc), at the concentration of 50 µg/ml;

2- N-acetylcysteine (NAC) plus amino acids (Aa) at the concentration of 50 µg/ml;

A set of untreated cells were used as controls. The cells were maintained in a humidified atmosphere of 5% CO₂ at 37°C for 48 h. After the end of the exposure time, cells were trypsinized and lysed for RNA extraction.

RNA Isolation and Reverse Transcription

After treatment, the cells were trypsinized, harvested and lysed for RNA extraction. The total RNA was isolated using GenElute™ Mammalian Total RNA Miniprep kit (Sigma-Aldrich, Inc., St. Louis, Mo), following the manufacturer's instructions. Then, 2.5µg of total RNA was used for synthesize cDNA using M-MLV Reverse Transcriptase (Sigma-Aldrich, Inc., St. Louis, Mo)

in according to the manufacturer's instructions.

Real Time Polymerase Chain Reaction (Real Time PCR)

cDNA was amplified by real-time PCR. For all but 2 genes (COL1A1 and COL3A1), amplification was performed by using Power SYBR® Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) and the specific assay designed for the investigated genes. For COL1A1 and COL3A1, we performed amplification by using the TaqMan universal PCR master mix (Applied Biosystem, Foster City, CA, USA) instead.

SYBR® Green assays reactions were performed in a 20-μl volume using the ABI PRISM 7500 (Applied Biosystems, Foster City, CA, USA). Each reaction contained 10 μl 2X Power SYBR® Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA), 400 nM concentration of each primer, and cDNA.

TaqMan assays amplification, instead, were

performed in a 20 μl volume using the ABI PRISM 7500 (Applied Biosystems, Foster City, CA, USA). Each reaction contained 10 μl 2X Power SYBR® Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA), 400 nM concentration of each primer, 200nM of the specific probe and cDNA.

All experiments were performed including non-template controls to exclude reagents contamination. PCRs were performed with two biological replicates. Expression was quantified using real time RT-PCR. The gene expression levels were normalized to the expression of the housekeeping gene RPL13.

Gene expression was evaluated as fold changes relative to the expression of the untreated HGF. Quantification was done with the delta/ delta calculation method. Forward and reverse primers for the selected genes were designed using primer express software (Applied Biosystems, Foster City, CA, USA) and are listed in Table I and II.

Table I. Primers sequences for SYBR® Green assay.

Gene symbol	Gene name	Primer sequence (5'>3')
DSP	Desmoplakin	F- ATGACCTGAGGAGAGGACGAA R- AGGCTCTCTCTTCTGTACCAC
ELN	Elastin	F- CTAAATACGGTGCTGCTGGC R- CATGGGATGGGGTTACAAAG
FN1	Fibronectin 1	F – CCATAGCTGAGAAGTGTTTTG R- CAAGTACAATCTACCATCATCC
FBN1	Fibrillin-1	F- AAAGATCTTGATGAGAGTGTGC R- GTATGGTGTGGGTAAATCC
ITGA1	Integrin Subunit Alpha 1	F- CAGTTGGAATTGTACAGTATG R- TGTCTATTCCAAGAGCTGTC
ITGA2	Integrin Subunit Alpha 2	F- GGTGGGGTTAATTCAGTATG R- ATATTGGGATGCTCTGGGATG
ITGA5	Integrin Subunit Alpha 5	F- AAGCTTGGATTCTTCAAACG R- TCCTTTCAGTAGAATGAGGG
ITGB1	Integrin Subunit Beta 1	F- ATTCCTTTCTCAGAAGTC R- TTTTCTCCATTTCCCTG
RPL13	Ribosomal protein 13	F - ATTCACAAGAAGGGAGACAG R - GAAATTCTTCTTCTCCTCAGTG

Table II. Primers and probes sequences for TaqMan® Gene Expression Assay.

Gene symbol	Gene name	Primer and Probe sequence (5'>3')
COL1A1	Collagen type I alpha1	F- TAGGGTCTAGACATGTTACGCTTTGT R- GTGATTGGTGGGATGTCTTCGT P- CCTCTTAGCGGCCACCGCCCT
COL3A1	Collagen type III alpha1	F- CCCACTATTATTTTGGCACAACAG R- AACGGATCCTGAGTCACAGACA P- ATGTTCCCATCTTGGTCAGTCCTATGCG
RPL13	Ribosomal protein 13	F- AAAGCGGATGGTGGTTCCT R- GCCCCAGATAGGCAAACCTTC P- CTGCCCTCAAGGTCGTGCGTCTG

RESULTS

Fibroblasts' behaviour was evaluated by measuring the gene expression levels of several connective growth, wealth and elasticity related genes (ELN, DSP, FN1, FBN1, ITGA1, ITGA2, ITGA5, ITGB1, COL1A1, COL3A1) after exposition to 2 different medical device: NAC plus Aa and PDRN plus Gluc.

Both the treatments were responsible of Elastine and Desmoplakin genes activation. Only NAC plus Aa treatment enhance the expression of other genes related to tissues growth and elasticity like FBN1, ITGA1 and ITGB1. All the other genes investigated (FN1, ITGA5, ITGA2, COL1A1, COL3A1) were down-regulated by both treatments.

DISCUSSION

Aging is a complex biological process of multifactorial origin. Intrinsic cellular senescence and sun exposure are leading elements in the production of reactive oxygen species (ROS). In fact ROS production is also a physiological consequence of mitochondrial oxidative energy generation (26).

Cellular damage occurs when ROS production

exceeds the detoxifying ability of antioxidant enzymes, which is caused by both excessive production or decreased performance in scavenging of aged cells.

ROS react with a great variety of molecules like DNA, proteins, and membrane phospholipids. These reactive species can induce single- and double-strand breaks in nuclear DNA, but it is also well documented that mitochondrial DNA mutations and damage are involved, and in particular the base excision- repair pathway is less efficient in aging (27, 28).

In medical therapy, the direct use of anti-oxidants and polyphenols by diet is recommended. The dosage can be critical; in fact, excess of anti-oxidant supplementation (e.g., ascorbic acid) can produce a paradoxical pro-oxidant effect (29). Moreover, the metabolic fate of dietary ingredients do not assure their presence in the target tissue.

In chemical bio-stimulation, different drugs are injected to improve dermal components. Polynucleotides, glucosaminoglycans precursors and amino acids have the aim of enhancing the anabolic function of fibroblasts, i.e., protein synthesis and replication and simultaneously NAC is a precursor for anti-oxidative enzyme.

Since no study is available on BS therapy on oral

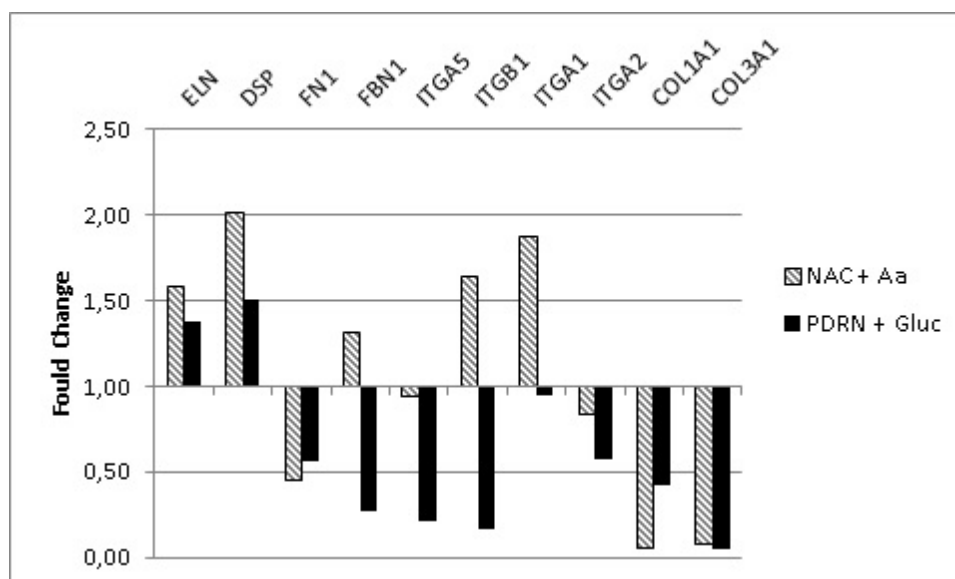


Fig. 1. Human Dermal Fibroblasts gene expression profile after 48 h of treatment with NAC plus Aa and PDRN plus Gluc.

cavity, we investigated the genetic effect of BS on gingival fibroblast. Both the treatment NAC plus Aa and PDRN plus Gluc enhance the expression of ELN and DSP.

DSP encodes desmoplakine, an essential component of desmosomes that anchor intermediate filaments to desmosomal plaques (30, 31). Desmosomes are intracellular junctions with a key role in cellular adhesions. A correct DSP synthesis and functioning is essential to assure epithelial integrity. In turn, this will prevent environmental damages (e.g. UV) and bacterial infections (32, 33). Its up-regulation suggests the involvement of this protein in cell-cell and cell-matrix adhesion. ELN encodes elastin, the major component of the elastic fibres (90%) of the connective tissue. This protein is one of the main ones responsible for conferring dermal elasticity and flexibility, not only to the skin but also to the blood vessels (34). Moreover, it has a known role in damaged skin reparation and regeneration (35).

FBN1, ITGB1 and ITGA1 were up-regulated by NAC plus Aa. FBN1, a member of fibrillin family, is involved in many processes related to the structural integrity of tissues, binding tropoelastin during elastic fiber formation and targeting growth factors such as TGF- β and bone morphogenic protein (BMP) to the ECM (36,37). FBN1 plays a key role in cell adhesive and migratory behavior (38).

ITGB1 gene encodes the beta 1 subunit of integrin receptors. This protein heterodimerizes with the alpha 1 subunit encoded by the ITGA1 gene to form a cell-surface receptor for collagen and laminin (39). Integrin- α 1-deficient fibroblasts showed reduced expression of type I collagen and connective tissue growth factor and failed to differentiate into myofibroblasts in wound reparation (40). This receptor is involved in cell adhesion, inflammation and fibrosis.

Since the precise role of these proteins in tissues integrity and aging is not known, this study confirms the usefulness of biostimulation therapies in enhancing some of the genes responsible of cellular wellbeing.

This study could be useful to consider the possibility of injective biostimulation in oral cavity

too, clinical applications in oral healing and in gingival atrophy.

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BIOREVITALIZATION: AN IN VITRO STUDY ON GINGIVAL FIBROBLAST

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Bio-revitalization is a therapy commonly used in aesthetic medicine to improve skin quality by directly integrating hyaluronic acid alone or added to other molecules (i.e. vitamins) through intradermal injections. These injections are not aimed to fill roughness but to achieve extracellular matrix optimization. The injective medical devices used in aesthetic medicine differ for hyaluronic acid content and for the presence of additional molecules that characterize the formulation of a particular company. The aim of the present study is to compare HA with different compounds in regard to their effects on cultured fibroblasts over time by using RT-PCR and a panel of genes (ELN, DSP, FN1, FBN1, ITGA1, ITGA2, ITGA5, ITGB1, COL1A1, COL3A1) involved in connective integrity. Bio-revitalization is able to activate genes involved in tissue integrity. The reported data add new insight in the comprehension of molecular mechanism related to BR. These preliminary data have to be developed through additional experiments. However, an injective therapy seems to be effective in gingival fibroblast stimulation.

Age, lifestyle, smoking, sun exposure, facial expressions and stress are some of the factors that can accelerate the normal aging process (1). Cosmetic imperfections are due to the disorganization of autologous collagen that leads to the alteration of the elasticity of the skin mantle. This phenomenon, in combination with the dehydration processes, determines the formation of wrinkles (2, 3). Other imperfections may be due to trauma, surgery, acne or chickenpox scars. In every case, depletion of the dermal matrix and of the adipose tissues, lead to an accentuated and ugly blemish defined as “groove” or “depression”.

For the correction of all these skin problems, the plastic and cosmetic surgery makes use of different products with different applications fields. Bio-revitalization is a therapy commonly used

in aesthetic medicine to improve skin quality by directly integrating hyaluronic acid (HA) alone or added to other molecules (i.e. vitamins) (4,5) through intradermal injections. These injections are not aimed to fill roughness but to get extracellular matrix (ECM) optimization.

HA is a high-molecular polymer of dimeric units of N-acetylglucosamine and glucuronic acid. Among its important biological functions, there are the modulation of cellular proliferation, migration and differentiation, regulation of the extracellular water content and protein homeostasis. HA is largely used in aesthetic medicine for its hydration capability. It is a polyanion that can self-associate and that can also bind to water molecules giving it a stiff, viscous quality similar to gelatin (6-8).

HA is omnipresent in the human body occurring in

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almost all biological fluids and tissues, although the highest amounts of HA are found in the extracellular matrix of soft connective tissues, in the synovial fluid, in the vitreous humour of the eye, and in the hyaline cartilage (9).

Two main receptors capable to connect HA to cell surface are described. The Cluster of Differentiation 44 (CD44), located on the plasma membrane and regulating cell adhesion and migration, and the Receptor for Hyaluronan-Mediated Motility (RHAMM), located in the cytoplasm and responsible for cell growing and migration.

For the characteristics of consistency, biocompatibility, and hydrophilicity, HA is an excellent moisturizer in cosmetic dermatology and skin-care products. Its unique viscoelasticity and limited immunogenicity permit its use in several biomedical applications including osteoarthritis treatment, eye surgery, wound regeneration. In addition, HA has currently been explored as a drug delivery agent (6-8). HA is widely used in aesthetic medicine to improve aesthetic surgery results (5,10-26).

The injective medical devices used in aesthetic medicine differ for HA content and for the presence of additional molecules that characterizes the formulation of a particular company. The aim of the present research is to compare different HA containing compounds as regard their effects on cultured fibroblasts over time by using RT-PCR and a panel of genes (ELN, DSP, FN1, FBN1, ITGA1, ITGA2, ITGA5, ITGB1, COL1A1, COL3A1) involved in connective integrity.

MATERIALS AND METHODS

Primary human gingival fibroblast cell (HGF) culture

Human connective tissue biopsies were obtained from 3 healthy patients (a 68-year-old man, a 63-year-old woman and a 20-year-old man) during oral surgery. The gingival pieces were fragmented with a scalpel and transferred in culture dishes containing Dulbecco's modified Eagle medium (DMEM, Sigma-Aldrich, Inc., St. Louis, Mo) supplemented with 20% foetal calf serum (FBS) and antibiotics, i.e. penicillin 100U/ml and streptomycin 100µg/ml (Sigma Aldrich, Inc.).

Cells were incubated in a humidified atmosphere of

5% CO₂ at 37°C. The medium was changed the next day and twice a week. After 15 days, the pieces of gingival tissue were removed from the culture dishes. Cells were harvested after additional 24 h of incubation.

Cell cultures

Human fibroblasts at the second passage were seeded on a layer of five medical devices different for HA content and for the presence of additional molecules:

1. Solution of HA 6.2 mg/ml with Aa and vitamins (Skinkò E Viscoderm Ibsa//Revitacare, Saint Ouen d'Aumone, France)
2. HA gel 10 mg/ml and polynucleotides (Newest Mastelli, Sanremo, Italy)
3. HA gel 10 mg/ml in saline solution (Hyalgan, FIDIA Farmaceutici S.p.A. - Abano Terme, Italy)
4. HA 13.5 mg, mannitol 9 mg, phosphate buffer pH 7,2 (Juvéderm® Hydrate™ Allergan, inc. Irvine, California).
5. HA gel 20 mg/ml in saline solution phosphate buffered (Restylane® Vital, GALDERMA, Uppsala, Sweden).

A set of untreated cells were used as control. The cells were maintained in a humidified atmosphere of 5% CO₂ at 37°C for 24 h. After the end of the exposure time, that was 24 h, 48 h, and 72 h, cells were trypsinized and lysed for RNA extraction.

RNA Isolation and Reverse Transcription

After treatment, the cells were trypsinized, harvested and lysed for RNA extraction. The total RNA was isolated using GenElute™ Mammalian Total RNA Miniprep kit (Sigma-Aldrich, Inc., St. Louis, Mo), following the manufacturer's instructions. Then, 2.5µg of total RNA was used for synthesise cDNA using M-MLV Reverse Transcriptase (Sigma-Aldrich, Inc., St. Louis, Mo) in according to the manufacturer's instructions.

Real Time Polymerase Chain Reaction (Real Time PCR)

cDNA was amplified by real-time PCR. For all but two genes, (COL1A1 and COL3A1) amplification was performed by using Power SYBR® Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) and the specific assay designed for the investigated genes. For COL1A1 and COL3A1, we performed amplification by using the TaqMan universal PCR master mix instead

(Applied Biosystem, Foster City, CA, USA).

SYBR® Green assays reactions were performed in a 20-µl volume using the ABI PRISM 7500 (Applied Biosystems, Foster City, CA, USA). Each reaction contained 10 µl 2X Power SYBR® Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA), 400 nM concentration of each primer, and cDNA.

TaqMan assays amplification, were performed in a 20 µl volume using the ABI PRISM 7500 instead (Applied Biosystems, Foster City, CA, USA). Each reaction contained 10 µl 2X Power SYBR® Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA), 400 nM concentration of each primer, 200nM of the specific probe and cDNA.

All experiments were performed to exclude reagents contamination, including non-template controls.

PCRs were performed with two biological replicates. Expression was quantified using real time RT-PCR. The gene expression levels were normalized to the expression of the housekeeping gene RPL13. Gene expression was evaluated as fold changes relative to the expression of the untreated HGF. Quantification was done with the delta/delta calculation method. Forward and reverse primers for the selected genes were designed using primer express software (Applied Biosystems, Foster City, CA, USA) and are listed in Table I and II.

RESULTS

Fig. 1. shows the effects of the five different medical devices on the gene expression profiling of treated fibroblast. All but 2 genes resulted up-

Table I. Primers sequences for SYBR® Green assay.

Gene symbol	Gene name	Primer sequence (5'>3')
DSP	Desmoplakin	F- ATGACCTGAGGAGAGGACGAA R- AGGCTCTCTCTTCCTGTACCAC
ELN	Elastin	F- CTAAATACGGTGCTGCTGGC R- CATGGGATGGGGTTACAAAG
FN1	Fibronectin 1	F – CCATAGCTGAGAAGTGTITG R- CAAGTACAATCTACCATCATCC
FBN1	Fibrillin-1	F- AAAGATCTTGATGAGAGTGTGC R- GTATGGTGTGGGTAAATCC
ITGA1	Integrin Subunit Alpha 1	F- CAGTTGGAATTGTACAGTATG R- TGTCTATTCCAAGAGCTGTC
ITGA2	Integrin Subunit Alpha 2	F- GGTGGGGTTAATTCAGTATG R- ATATTGGGATGTCTGGGATG
ITGA5	Integrin Subunit Alpha 5	F- AAGCTTGGATTCTTCAAACG R- TCCTTCAGTAGAATGAGGG
ITGB1	Integrin Subunit Beta 1	F- ATTCCCTTTCTCAGAAAGTC R- TTTTCTCCATTTTCCCTG
RPL13	Ribosomal protein 13	F - ATTCACAAGAAGGGAGACAG R - GAAATCTCTCTCTCCTCAGTG

Table II. Primers and probes sequences for TaqMan® Gene Expression Assay.

Gene symbol	Gene name	Primer and Probe sequence (5'>3')
COL1A1	Collagen type I alpha1	F- TAGGGTCTAGACATGTTACGCTTTGT R- GTGATTGGTGGGATGTCTTCGT P- CCTCTAGCGGCCACCGCCCT
COL3A1	Collagen type III alpha1	F- CCCACTATTATTTGGCACAACAG R- AACGGATCCTGAGTCACAGACA P- ATGTTCCCATCTTGGTCAGTCCTATGCG
RPL13	Ribosomal protein 13	F- AAAGCGGATGGTGGTTCCT R- GCCCAGATAGGCAAACCTTC P- CTGCCCTCAAGGTCGTGCGTCTG

regulated after 48 h of treatment with four of the five medical devices. In fact, COL1A1, COL3A1 were the only, although weakly, down-expressed genes.

Significantly deregulated genes are those with a fold-change value superior to 2 (up-regulated) and less than 0.5 (down-regulated). Hyalgan treatment induce the significative up-regulation of ELN, DSP, FN1, FBN1, ITGB1 AND ITGA1 (Fold change >2). Restylane Vital enhances the expression of FN1, ITGB1 and ITGA1. Newest stimulate the significative expression of FN1 gene. Juvederm induce the over-expression of ELN and DSP. This treatment is the only one to determine a down-regulation (not statistically significative) of ITGA5 and ITGB1 genes that are over-expressed as a result of the other treatments.

DISCUSSION

There can be different reasons to resort to aesthetic facial rejuvenation. Among these, ageing is the most common. Human skin, in fact, is constantly subjected to various kind of stress. Among these cellular metabolism, ionizing radiation, environmental pollution and exposure to UV radiation can lead to

the production of harmful species (free radicals). Accumulation of such substances and a defective production of structural proteins such as elastin, collagen and elastic fibers can inevitably lead to skin ageing (21, 23, 25-28).

To correct skin blemishes, different techniques can be employed.

Bio-revitalization is an anti-aging therapy, directed to skin improvement through intra-dermic injection of Hyaluronic acid (HA) at different concentrations. HA is a polymer of dimeric units of *N*-acetyl-glucosamine synthetized at the inner surface of plasma membrane, and then secreted in extracellular space (5, 29).

The purpose of this study is to investigate the effect of bio-revitalization therapy. To this aim, some fibroblastic expressed genes after exposition to five medical devices were evaluated. These products contain different concentrations of HA and all but one, are commonly used in aesthetic medicine. Only Hyalgan is used in viscosupplementation in knee arthrosis.

ELN encodes elastin, one of the major components of the dermal matrix. This protein is responsible in conferring dermal elasticity and flexibility due

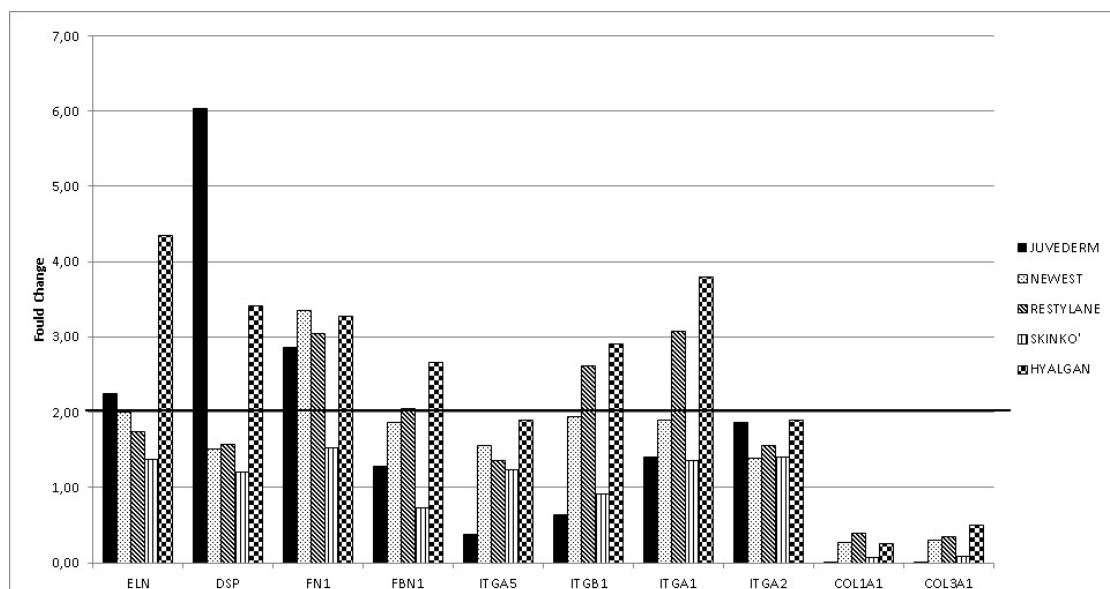


Fig. 1. Effects of the 5 different medical devices on fibroblast gene expression.

to its role in forming elastic fibres. Moreover it has a known role in damaged skin reparation and regeneration (30).

Desmoplakin (DSP) is a protein belonging to the plakin family of proteins that act as cytoskeletal linker and is the major component of desmosomes (31, 32). Desmosomes are intracellular junctions with a key role in cellular adhesions. A correct DSP synthesis and functioning is essential to assure tissue integrity.

DSP interacts with a variety of intermediate filaments including desmin in cardiac muscle, keratin in epithelial tissues, and vimentin in certain specialized tissues such as the arachnoidal tissue of the brain meninges and the dendritic reticulum of lymphoid follicles. A possible explanation for DSP increased expression in bio-revitalization is the assessment of a three-dimensional network for fibroblast and keratinocyte communications.

Fibrillin (FN1) is of great importance in cellular migration and adhesion. The main function of FN1 is to serve as a scaffold regulating cell proliferation and differentiation (33, 34). In healthy adult tissue, FN1 is expressed at low levels. Its expression is reactivated transiently as a result of tissue injuries, ranging from skin wounds to tissue inflammation (35).

Another up-regulated gene in treated cell was fibrillin 1 (FBN1). Members of fibrillin family, are involved in many processes related to the structural integrity of tissues, binding tropoelastin during elastic fiber formation and targeting growth factors such as TGF- β and bone morphogenic protein (BMP) to the ECM (36, 37). ITGB1 gene encodes the beta 1 subunit of integrin receptors. This protein heterodimerizes with the alpha 1 subunit encoded by the ITGA1 gene to form a cell-surface receptor for collagen and laminin (38).

Integrin- β 1-deficient fibroblasts showed reduced expression of type I collagen and connective tissue growth factor, and failed to differentiate into myofibroblasts the involved in wound reparation (39). Integrin Subunit Alpha 5 (ITGA5) is a receptor for fibronectin and fibrinogen. This gene has been suggested as important factors in the healing cascade (40, 41). Integrin family members participate in a variety of processes including cell adhesion, immune

response, metastasis of tumor cells as well as embryogenesis, hemostasis, and tissue repair (40). In conclusion, Bio-revitalization is able to activate genes involved in tissue integrity.

The reported data add new insight in the comprehension of molecular mechanism related to BR. These preliminary data have to be expanded with additional experiment. However, an injective therapy seems to be effective in gingival fibroblast stimulation.

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