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CONTENTS

SPECIAL ISSUE:
"**FOCUS ON PEDIATRIC CARDIOLOGY**"

This special issue provides a rich blend of news, reviews and clinical studies on many aspects of the understanding, management and prevention of cardiovascular disease in childhood. Primary care in pediatric cardiology, principally, must concern with prevention of heart disease and early detection of existing illness. In addition, pediatricians must be able to maintain the vigilance of "healthy" children in order to prevent cardiovascular risk in adulthood. With these goals, the themes presented in this "mosaic" of topics include

- Cardiovascular involvement in genetic syndromes, SIDS and infectious, endocrine, kidney, storage or autoimmune diseases.
- Pulmonary hypertension and patent ductus arteriosus in premature infants.
- Cardiovascular risk in obese or hypertensive children.
- Bicupid aortic valve and aortic dysfunction
- Covid 19 and heart disease in childhood

I thank the Researchers and Professors of Catanzaro, Palermo, Pavia, Brescia, Taormina and Messina University for their interesting and up-to-date scientific contributions, and I hope the information gathered in this issue will be useful to the reader.

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Hypertension in childhood

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Hypertension is a growing health problem in children, and it is an important parameter of cardiovascular risk for adults. It is classified as primary (influenced by obesity, sedentary lifestyles and poor-quality food) or secondary to underlying causes. The AAP 2017 guidelines recommend measuring blood pressure every year from the age of three and in children under the age of three only if they have known risk factors. The measurement of infantile hypertension is relatively complicated and instable and, for this reason, ambulatory blood pressure monitoring (ABPM) and multiple office BP measurement (mOBPM), especially in infants who are not collaborating are indicated. High blood pressure may have an adverse effect on the heart, the vessels, the kidney, and the central nervous system so it is important recognize it and act promptly. Hypertension is initially treated with lifestyle changes such as weight loss, a healthy diet, and regular exercise, but, if non-pharmacological interventions have failed, a pharmacological treatment with angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, calcium channel blockers, thiazide diuretics and/or beta blocker may be indicated.

Definition

Hypertension (HTN) is a relevant clinical problem in pediatrics because it is an alarming cardiovascular risk factor. In adolescents and adults, HTN is defined as blood levels > 140/90 mmHg according to European guidelines, while according to 2017 AHA guidelines it is defined as blood levels > 130/80 mmHg (1). In children, definitions that categorize BP values have been modified by the 2017 AAP guidelines, taking into account two age groups: 1) In children between 1 and 13 years of age - **normal BP** is defined when both Systolic BP (SBP) and diastolic BP (DBP) are < 90th percentile (matched by age, gender, and height); **elevated BP**: SBP and/or DBP > 90th percentile but < 95th percentile; - **stage 1 HTN**: SBP and/or DBP ≥ 95th percentile; - **stage 2 HTN**:

Key words: hypertension, children, cardiovascular disease

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SBP and/or DBP ≥ 95th percentile + 12 mmHg. 2) For children ≥ 13 years of age, - **normal BP**: BP < 120/80 mmHg; - **Elevated BP**: SBP 10-129 mmHg and DBP < 80 mmHG; - **stage 1 Hypertension**: BP between 130/80 to 139/89 mmHg; - **stage 2 Hypertension**: BP ≥ 140/90 mmHg (2, 3).

Epidemiology and risk factors

The prevalence of hypertension in pediatric age is 1.5-5% (4). The incidence of HTN is approximately 10.4% in well-fed children, but if it is associated with obesity or being overweight, or a diet rich in salts and fats and a sedentary lifestyle, this percentage can raise up to 68%. The Hispanic race and black children have an increased risk of developing hypertension, as well as children of hypertensive parents (5).

Maternal pre-pregnant BMI and maternal hypertension in pregnancy was positively correlated with infant weight gain during the perinatal period (6), in particular, with abdominal obesity and, therefore, with pediatric hypertension and cardiovascular risk (7, 8). The risk of childhood hypertension increases rapidly with puberty and could be associated with hormonal changes.

Etiology

Even in children and adolescents, hypertension can be classified as primary (essential) or secondary. Primary hypertension is the predominant diagnosis for children and adolescents older than 6 years of age who have a positive family history of HTN, are overweight or obese and do not have symptoms or signs suggestive of a secondary HTN. Some chronic conditions, such as coarctation of the aorta, Cushing syndrome, hyperthyroidism, excess mineralocorticoids (congenital adrenal hyperplasia, aldosterone-secreting tumors), pheochromocytoma, disorders of sleep (i.e. obstructive sleep apnea, night snoring, sleep fragmentation), chronic renal disease as renal vascular hypertension (RVH) and parenchymal renal disease, rheumatological disorders and drugs such as contraceptives, steroids, sympathomimetics / stimulants can be related to secondary childhood HTN.

A previouse study supported the hypothesis that inherited variations of the CART gene could influence the development of obesity and complication in Italian children (9).

Increased levels of aldosterone may be responsible for hypertension and for cardiovascular complications associated with obesity (10).

From measurements to diagnosis

Unlike the 2016 European Hypertension Society guidelines that recommended screening every two years from the age of three years, the American Academy of Pediatrics (AAP) 2017 guidelines recommend measuring blood pressure every year from the age of three. Children and adolescents with risk factors (i.e. those who are obese; who have kidney disease, aortic arch obstruction, diabetes mellitus, or who are taking a drug known to increase blood pressure) should have measured the BP at each health check.

High-mobility group protein B1 (HMGB1) takes part in numerous medical conditions, including pregnancy (11, 12). It plays an important role in the inflammatory process associated with childhood obesity. HMGB1 can be passively secreted from damaged or necrotic cells (13), it is one of the most important DAMP molecules, initiating and perpetuating immune responses both in non-infectious and/or infectious inflammatory processes (14, 15). HMGB1 serum levels may therefore represent a predictive marker of disease in pregnant women (16) and it may be an important diagnostic marker for obesity-related complications (17) like hypertension.

In children under the age of three, blood pressure should be measured if they have the same risk factors as older children or if they are premature, if they have a family history of congenital kidney disease, a history of organ or bone marrow transplant malignancy, elevated intracranial pressure or systemic diseases known to increase blood pressure (2).

The measurement of infantile hypertension is relatively complicated and blood pressure values are unstable and change according to the size of the cuff, the level of activity and anxiety, the intake of substances such as caffeine or cocoa, some drugs, the day or night hours and to the positioning of the child (18). The measurement is performed with the auscultatory method or an oscillometric (automatic) device. The bracelet must be applied on bare skin and

its width must be at least 40% of the circumference of the arm, while the length between 80 and 100% of the circumference of the arm. It is preferable to use the right arm for the measurement since on the left arm the measurement can provide erroneously low readings if coarctation of the aorta coexists. Regardless of the initial method used, an initial high blood pressure reading should be followed with at least two other auscultatory blood pressure measurements to avoid false positive cases.

To identify patients with *white coat* hypertension and those with *masked hypertension*, ambulatory blood pressure monitoring (ABPM) is indicated, in which BP is measured throughout the day and night at intervals varying between 20 and 60 minutes A minimum of 1 records for hour for a 24-hour period is considered necessary for a correct interpretation of the data and to correctly distinguish patients with normal pressure, or those with "white coat hypertension" or "masked hypertension",

Table I. *Etiologies of hypertension and suggestive findings in children and adolescents*.

Etiology	Hystory	Physical examination	Laboratory and other test
Primary hypertension			
	Adolescent age, diet high in fat and salt, family history of essential hypertension or early cardiovascular disease or diabetes, obesity and dyslipidaemias, limited physical activity	Acanthosis nigricans (insuline-resistance), diabetes and obesity	Hyperlipidemia Impaired glucose tolerance or type 2 diabetes
Secondary hypertension			
• ***Parenchimal renal disease*** (glomerulonephritis, polycystic kidney disease, congenital anomalies of the kidneys and urinary tract and CDK)	Enuresis, family history of renal disease, age <6 y, recurrent urinary tract infections	Abdominal mass, edema gross hematuria, growth retardation	Abnormal blood urea nitro¬gen, creatinine, anemia, urinalysis or urine culture, abnormal renal ultrasonography
• ***Renal artery stenosis*** (fibromuscular dysplasia and Takayasu's arteritis)	Prior umbilical artery catheterization	Epigastric and/or upper abdominal bruit	Ultrasonography, angiography if response to antihypertensive drugs is poor
• ***Coarctation of the aorta***	Hystory of neurofibromatosis, Williams syndrome, Alagille syndrome, or Takayasu arteritis	BP difference in the upper or lower limbs of 20 mm Hg or more, possible difference in BP between right and left arms, a systolic murmur with radiation in the back, diminished femoral pulses	Echocardiography
• ***Glucocorticoid excess*** (Cushing Syndrome, adrenocortical carcinoma)	Family hystory of endocrinopathies, cronic therapy with glucocorticoids	HTN, signs of Cushing syndrome, (acne, hirsutism, striae, moon facies, truncal obesity	Elevated cortisol levels
• ***Mineralocorticoid excess*** (congenital adrenal hyperplasia,	Family hystory of endocrinopathies	Ambiguous genitalia Muscle weakness	Elevated plasma aldosterone levels,

prehypertension, ambulatory hypertension, or severe ambulatory hypertension. Recently, it has been proposed a new method, named multiple Office Blood Pressure Measurements (mOBPM) that provide reliable measurements of BP and likely may be used instead of ABPM, particularly in infancy. This method is a simpler and faster tool than ABPM, especially in infants who are not collaborating (19).

After establishing a diagnosis of high BP or hypertension, it is necesary to identify a possible underlying etiology and any comorbidities. Nutritional history should assess for intake of foods that are associated with high BP such as high salt and caffeine, and low potassium. Physical activity, smoking status, and alcohol intake should also be explored. Patients should be screened for a family history of hypertension, other cardiovascular risk factors, diabetes, obesity, dyslipidaemias and familial renal or endocrine diseases. A sleep disturbance history should be investigated because an association between sleep disorders and hypertension is well documented in adults and it cannot be excluded in children, although on this topic the reports are limited. Screening for kidney, endocrinological diseases, rheumatological, cardiological and taking drugs that can increase blood pressure is indicated (Table I) endovascular techniques, and surgery. We aimed to describe the course of renovascular hypertension (RVH(20, 21).

Consequences

HTN is known to be one of the most relevant factors for the development of atherosclerosis and cardiovascular disease in adults. The most important aspect of the management of hypertensive children is the detection of early changes of "target-organs" and the assessment of the individual cardiovascular risk. Increased carotid intima-media thickness (cIMT), high pulse wave velocity, left ventricular hypertrophy (LVH) and microalbuminuria have been identified as "end organ-effects", expression of injury related to HTN.

Electrocardiography has high specificity but very low sensitivity for identifying children with LVH; for this reason, clinicians should not perform EKG in hypertensive children for evaluating LVH. Echocardiography, instead, is a good tool to assess heart damage secondary to HTN. In particular, it has been documented that height-adjusted left ventricular mass, assessed by echocardiography, is correlated to both body mass index and systolic blood pressure and this has been suggested to be a good indicator of the need for pharmacological terapy in pediatric patients with HTN.

In obese children waist circumference and/or body mass index and biohumoral and inflammatory parameters can help predict cardiac structural and functional alterations (22). New risk factors, such as homocysteine, lipoprotein (a), apolipoprotein B, adipokines, fibrinolysis and inflammation markers, associated with obesity, insulin resistance and hypertension significantly increase the risk of cardiovascular events in adulthood (23).

Also in obese children and adolescent, screenings for diabetes, steatohepatitis, and dyslipidemia as additional cardio-vascular risk factors are recommended through the evaluation of fasting glucose, hemoglobin A1c, alanine and aspartate aminotransferases, and a fasting lipid panel (24). Abdominal obesity with visceral obesity might be predicting factor for accumulation fatty liver (25).

Vascular lesions in the retina, frequently detected in adults, can also be found in children with severe HTN. Neurological manifestations occur more in hypertensive crisis than in chronic hypertension. Recently it has been reported reduction in cognitive function in children with sustained hypertension (26) untreated hypertension and 75 frequency-matched normotensive controls had baseline neurocognitive testing as part of a prospective multicenter study of cognition in primary hypertension. Subjects completed tests of general intelligence, attention, memory, executive function, and processing speed. Parents completed rating scales of executive function and the Sleep-Related Breathing Disorder scale of the Pediatric Sleep Questionnaire (PSQ-SRBD.

Therapy

Accurate identification of secondary hypertension is extremely important because many causes are reversible. The goals of the treatment include achieving a blood pressure level less than the 90th

percentile for age, height, and gender for patients younger than 13 years of age, or less than 130/80 mm Hg for those ≥ 13 years of age. All children with HTN should undertake, first of all, lifestyle changes, such as nutritional intervention dietary (DASH or Dietary Approaches to break down hypertension including poor intake of fat milk products, considerable intake of fruits, vegetables and low salt input) and physical exercise as, at least, 60 min of moderate to vigorous activity, three to five days per week with less than 2 hours of sedentary activity daily.

Despite of the limited availability of studies on pediatric symptomatic hypertension (eg, headache, cognitive changes), if hypertension remains at stage 2, symptomatic or with evidence of "organ damage" after six months of lifestyle changes, it is necessary to choose pharmacological intervention, while if there is normalization of blood pressure, patients can be followed up every 3-6 months.There is no consensus about the best initial antihypertensive drug in children, and there have been no clinical trials of hypertension treatment that evaluate long-term outcomes in children.

First line therapy for hypertension in children is represented by angiotensin-converting enzyme inhibitors (ACEi), angiotensin receptor blockers (ARB), calcium channel blockers, and thiazide diuretics that are effective, safe, and well tolerated, while beta-blockers are not recommended as initial treatment. Pharmacological therapy should be initiated with single medication at the low end of the dosing range, increasing the dosage every two to four weeks until the blood pressure goal is achieved (BP <90th percentile or <50th percentile if chronic kidney disease). If a single drug is not enough to control BP, a second agent, preferably a thiazide diuretic, can be added to therapy.

Drug choice should take into account the underlying pathophysiology and the presence of comorbidities: ACEi or ARB are the most appropriate first-line agents in the presence of diabetes mellitus and microalbuminuria, CKD and proteinuria and in obese pediatric patients with LVH. Beta-blockers or calcium channel blockers are used in children with hypertension after the surgical repair of aorta coarctation. Beta-blockers are contraindicated in asthmatic or diabetic children, while ACEi and ARB are contraindicated in female adolescents at high risk of pregnancy to avoid potential fetal risks. A close surveillance (every 4-6 weeks) is mandatory in the first months of treatment, while once the BP values are stabilized and the right drug is found at the appropriate dose, controls can be spaced over time.

The recommendations for the treatment of resistant hypertension (persistent hypertension despite the treatment with 3 or more antihypertensive agents of different classes) foresee the reconfirmation of the pressure values with adequate sized caps, the ABPM, the dietary control (27), the identification of secondary causes not diagnosed with hypertension, optimizing current therapy and, if necessary, adding other agents.

Regarding participation in competitive sports, the guidelines recommend that children with stage 2 hypertension be restrict from high-static sports, even in the absence of end organ damage, until their BP normalizes. Children with evidence of target organ damage require an adequate specialist assessment before starting participation in competitive sports (28).

Recent AAP guidelines have reviewed the classification of hypertension and stressed the importance of ABPM for the diagnosis and management of childhood hypertension. Children ≥6 years of age must be advised not to request systematically a thorough investigation into the secondary causes of hypertension if they have a positive family history, are overweight or obese and do not have symptoms or signs suggestive of a secondary HTN.

Adequate strategies are needed to prevent the development of hypertension in children and adolescents: these are mainly based on lifestyle modification, including appropriate diet, regular exercise and obesity treatment. A pharmacological treatment is indicated if non-pharmacological interventions have failed, if hypertension is symptomatic or stage 2 persists and organ damage, such as left ventricular hypertrophy, is present. The main choice of antihypertensives should include acei, arb, long acting calcium channel blocker or thiazide diuretic, while beta blockers should never be used as initial antihypertensives.

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Cardiovascular risk factors in childhood obesity

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Childhood obesity is the "disease of the century". This article reviews the early cardiovascular risk factors and the recommendations to prevent them in the overweight and obese children. A comprehensive search of published literature was carried out to identify all articles published on this topic in English and Italian from 1999 to 2020.

Childhood obesity is one of the major public health problems. Since the 1980s, the worldwide prevalence of childhood overweight and obesity has increased by 47% in both developed and developing countries (1).

The definition of this problem in pediatric age is difficult. Some national societies define children with "body mass index" (BMI) between 85% and 95% percentile as overweight: obese those above 95% percentile by age and gender. BMI is the ratio between the weight in kilograms and the square of the height in meters (Kg/m^2) (2). For a more sensitive valuation, it would be reasonable to include, also, waist circumference (WC) measurement and the waist/height ratio (WHtR) calculation in the routine assessment of overweight or obese children. Waist circumference predicts abdominal fat and the WHtR may be more practical than WC because it does not require conversion to a percentile. In conclusion they have been shown to predict cardio-metabolic risk better than BMI (3). In obese children cardiac parameters obtained from echocardiogram tests may be in the normal range. However, other parameters may be altered in the early phase, showing that infantile obesity can compromise myocardial functions, even in the absence of comorbidities (4).

Childhood obesity is associated with development of cardiovascular risk factors (CVRF) in children and adolescents and with adverse long-term outcomes. In addition, a high BMI in childhood is known to be associated with a high risk of obesity in adulthood. The negative effects of obesity on cardiovascular (CV) health are accelerated progression of atherosclerosis, high rates of ventricular remodelling and a high risk of associated diseases, including stroke, myocardial infarction, and heart failure in adults (5).

Cardiovascular pathophysiology: "adiposopathy or sick fat"

Adiposity is excessive adipose tissue and it can cause adipocyte and adipose tissue anatomic and functional abnormalities, known as "adiposopathy"

Key words: obesity, childhood, hypertension, atherosclerosis, cardiovascular risk and disease.

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or "sick fat". Causes of adiposopathy are positive caloric balance, sedentary lifestyle, genetic predisposition (6-9) and environmental causes. During positive caloric balance, any stage of the adipogenicity processes is impaired causing adipocyte hypertrophy and pathological responses of endocrine tissue and the immune system (10).

The clinical importance of adiposity is not only how fat is stored (adipocyte proliferation vs adipocyte hypertrophy), but also where fat is stored. Adipose tissue can be deposited into pericardial, subcutaneous abdominal, peri muscular, perivascular, orbital, and paraosseal tissue and it contributes to dysregulated local secretion of vasoactive and inflammatory factors that may contribute to atheroma instability and other cardiovascular pathophysiology. In addition, adipocyte hypertrophy may contribute to intracellular hypoxia with further cellular and organ dysfunction and it, also, increases circulating free fatty acids (FFA), causing pathologic disruption and lipotoxicity of non-adipose tissue organs, such as the liver, muscle, pancreas, and blood vessels. These pathophysiological manifestation of adiposopathy are shown in Fig. 1 (10).

In conclusion, adiposopathy may directly promotes atherosclerosis endothelial dysfunction and contributes to CVD through pericardial and perivascular effects on the myocardium and blood vessel and indirectly through promoting or worsening major CVD risk factors such as type 2 diabetes mellitus, high blood pressure, dyslipidaemias and metabolic syndrome. There is a simplified relationship between pathogenic adipose tissue, cardiovascular risk factors and cardiovascular disease (10). Also, weight gain, apart from obesity per se, can be considered an important and hitherto unrecognized additional risk factor for idiopathic intracranial hypertension especially in children (11).

Cardiovascular risk factors

The main risk factors for CV disease and clinical manifestation of adiposopathy in childhood obesity are: high blood pressure (including systolic and diastolic blood pressure), excessive visceral adipose tissue (including BMI, WC, WHtR, insulin resistance) and metabolic alterations (including total cholesterol, low density lipoprotein-LDL, high density lipoprotein-HDL, triglycerides and glucose) (3).

Adiposity is the greatest risk factor for essential hypertension. It is determined by underlying mechanisms like activation of the sympathetic nervous system and the renin-angiotensin-aldosterone system, which increases the absorption of sodium and reduces natriuresis. In childhood, it is mostly subclinical and unknown; at present, there are some tables for diagnosis with cut-off values of blood pressure and normality ranges specific for age, sex and height (12).

Adipose tissue is responsible for releasing proinflammatory cytokines with a consequent minimal inflammatory state. This systemic inflammatory state has been demonstrated by high serum High-mobility group protein B1 (HMGB1) levels in obese children (13). Moreover this "alarm", has been studied in obese children as a new marker of metabolic syndrome (14). In the obese child, it is also present an immune dysregulation characterized by impaired complement and B-cell, T-cell, and NK cell activity; phagocyte deficiency; and decreased levels of anti-inflammatory cytokines, such as IL-10. All these factors predispose the child to Recurrent respiratory infections (15).

Young people with obesity and overweight have also premature signs of insulin resistance and hyperinsulinemia. The increased visceral fat (relative to subcutaneous peripheral fat) is dysfunctional and metabolically harmful. There are abnormalities in secretion of adipocyte hormones, cytokines, factors, and other molecules that have associated with changes in insulin activity and promotion of insulin resistance and they are shown in Table I. While adipose tissue remains relatively sensitive to insulin, other organs, such as skeletal muscle and liver, become more and more insensitive to insulin causing hyperinsulinemia which may lead to increased adipose tissue and subsequent worsening of adipose function. This is the "obesity metabolic cycle" (13).

Insulin resistance contributes significantly to development of type two diabetes mellitus (2DM) and promotes the combined dyslipidaemia of obesity (16). Dyslipidaemias are strongly related to abdominal obesity and the pattern associated

with childhood obesity consists of a combination of elevated triglycerides (TG), decreased high density lipoprotein cholesterol (HDL-C), and top normal to mildly elevated low-density lipoprotein cholesterol (LDL-C) (16).

Increased waist circumference (≥90th percentile), hypertension (systolic pressure> 130 mmHg; diastolic pressure ≥85 mmHg), low high-density lipoprotein cholesterol (HDL-C < 40 mg/dL), elevated triglycerides (≥ 150 mg/dl), or impaired glucose tolerance (≥100 mg/dL) are criteria they serve to define metabolic syndrome (MS). Therefore, there is no consensus on the definition of MS in children because there are several different sets of criteria (16, 17).

Cardiovascular disease related to childhood obesity

Obesity and related cardiometabolic risk factors have been shown to be associated with vascular changes. These derangements combined with the presence of inflammatory indexes, markers of oxidative stress, altered levels of adipokines, and endocrine abnormalities, particularly insulin resistance, contribute to endothelial dysfunction and initiation and acceleration of early atherosclerosis (18).

Atherosclerosis begins in the first years of life and it is characterized by the intimal medial thickening in elastic and muscular arteries due to the accumulation of lipidic materials. In children and young adults, atherosclerosis is usually subclinical and progression is slow. Yet in some cases, lipid striae can evolve rapidly and give rise to more advanced atherosclerotic lesions that appear early (18). For diagnosis of early atherosclerosis, it could be displayed a carotid intima-media thickening (IMT), using specialized ultrasonography. It is a marker for generalized atherosclerosis and heart disease in adulthood (19).

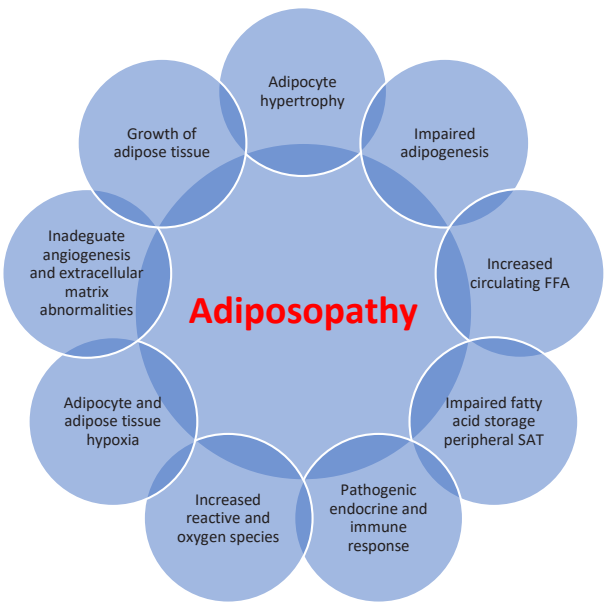

Fig. 1. *Pathophysiological manifestations of adiposopathy.*

Childhood obesity negatively affects cardiovascular remodelling and cause subclinical systolic and diastolic myocardial dysfunction. A new echocardiographic parameter, global longitudinal strain (GLS) is a reliable index of myocardial contractility, able to early identify subclinical myocardial alterations: an impaired GLS has been reported in overweight or obese children (12, 20). Moreover, Epicardial Adipose Tissue (EAT) is a metabolically active adipose tissue strictly related to visceral and subcutaneous fat and BMI seems to be a strong predictor of EAT amount; for this reason, echocardiographic assessment of EAT is a sensitive marker of visceral adiposity and could be used to estimate the CVD risk in obese children (21).

HMGB1 can be passively secreted from damaged or necrotic cells. It plays an important role in the inflammatory process associated with childhood obesity. This peptide may be an important diagnostic marker for obesity-related complications (22). HMGB1 plays a role in several medical conditions (23) The abovementioned pro-inflammatory properties of HMGB1, acquired upon its cellular release and consequent receptor stimulation,contribute to the pathogenesis of several acute and/or chronic noninfectious and infectious human diseases (24-28).

Prevention and treatments

For obese children is very important to prevent accelerated atherosclerosis and other cardiovascular risk factors. The American Heart Association's Committee on atherosclerosis, hypertension, and obesity in the young has published a statement to provide strategies for promoting cardiovascular health (29).

Table I. *Adipocyte hormones and cytokines and changes in insulin activity.*

Hormones and cytokines	Selected Functions
Leptin and adiponectin	Increase insulin sensitivity with decrease hepatic glucose production
Resistin, TNF- a, IL-6	Increase insulin resistance

A recent study (2019) has identified patients with high risk of metabolic complications and it designs targeted and effective prevention strategies. The study of Guzzetti et al confirms that gender and pubertal status influence the prevalence and severity of CVRF in children and adolescents with obesity. CVRF are, in fact, already present in prepubertal age (30).

Recommendations for prevention provide healthy diet and daily physical activity (31). The guidelines for diet are essentially based on reduction of fats and, in particular, saturated fatty acids. It is moreover recommended regular consumption of fruit, vegetables and high-fat fish like called also "omega three fats", which can reduce triglycerides levels by 20-30%. Other treatment approaches include reduction of sedentary life such as the use of computers, video games and increase of active healthy living. Physical activity has benefits not only on weight loss, but also on the lipid structure and on the reduction of oxidizing and inflammatory molecules that activate with obesity (29). Much attention has focused on prevention, especially during the perinatal period. Breastfeeding is also a possible protective factor for obesity in childhood (32).

Pharmacological therapies for obesity should be limited to severe cases and in very high-risk patients. The only anorexic agent approved in adolescence is "sibutramine", inhibitor of the reuptake of serotonin, norepinephrine and dopamine. Unfortunately, its benefits are limited to the first six months of treatment. Another weight loss drug, in addition to the treatment of type 2 DM, is "metformin" that reduces the stimulus of hunger (29).

In some cases, especially in the United State of America, bariatric surgery is also approved. The indications of surgery are limited in case of BMI> 40 Kg/m^2, metabolic syndrome, important comorbidities, or strong familiarity for cardiovascular diseases. The most used techniques in adolescents are gastric banding and gastric bypass (29).

DISCUSSION

This review aims to underline the epidemic problem of obesity and related diseases in the pediatric population. Obesity childhood can be considered the "disease of the century" because it is a global and recent phenomenon with a significant impact on the quality of life. The early and long-term implications of childhood obesity are extremely serious. Adipose tissue is an endocrine organ producing several adipokines that are the key regulating actors of inflammation and tissue injury involved in obesity associated complications in children and adults (33, 34). Awareness of the problem and the continuous healthy campaigns will reduce the obesity problem in the world and related cardiovascular consequences (35, 36).

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Genetics and cardiovascular disease

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In recent years, comprehensive developments in genetics improved our knowledge of inherited diseases, including hereditary cardiac disorders. During the past, only one to several candidate genes could be studied to search for disease-causing mutations; currently Next Generation Sequencing (NGS) techniques enable the analysis of all variants within an individual genome, including those predisposed to disease.

These new genotyping techniques provide the possibility to identify variants which influence disease development, either by leading to more severe symptoms or protecting carriers of pathogenic variants from getting seriously ill.

Cardiogenetics is a branch of medical genetics in which molecular and cellular biology is applied to search for an alteration of cellular functions during development and for variants inside the genes that can explain this dysfunction. Based on these findings, it is important to establish a comparison between patient's phenotype and genotype and try to find a connection between them.

Over the past two decades, researchers in the field of cardiogenetics have acquired a complex understanding of the pathophysiological basis of inherited cardiac disease. Since the discovery of the first cardiomyopathy-associated gene in 1990 and the first cardiac channelopathy-associated genes in 1995, genetic testing for these heritable diseases has advanced from basic scientific level to clinical application (1).

Inherited cardiac diseases, distinguished in Cardiomyopathies and Channelopathies, are genetic disorders that often cause sudden cardiac death

Key words: cardiovascular disease, Next Generation Sequencing, NGS, cardiogenetics

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(SCD) at young age. SCD must be well investigated because it may occur from different causes like also Nemaline myopathy (2). Clinical genetic testing may improve risk stratification among patients affected by cardiomyopathy or channelopathy, especially for arrhythmia and SCD (3).

Cardiomyopathies

Inherited cardiomyopathies are characterized by structural and functional abnormalities of the ventricular myocardium that are unexplained by coronary artery disease or abnormal loading conditions (4). This group of cardiovascular disorders is classified based on ventricular morphology and function and include hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM) and arrhythmogenic right ventricular cardiomyopathy (ARVC) (1). Common complications of cardiomyopathies can include heart failure and SCD (5).

Hypertrophic Cardiomyopathy

Hypertrophic Cardiomyopathy (HCM) has a population prevalence estimated of 1:500 (6). HCM is typically characterized by the presence of unexplained left ventricular hypertrophy (LVH) with a maximum wall thickness ≥15 mm in adults or a z-score >3 in children. If there is a family history of HCM, or if genetic testing confirms that a relative has inherited the family's pathogenic variant, a maximum LV wall thickness ≥13 mm supports diagnosis.

The diagnosis of HCM is established with cardiac imaging, including echocardiography and/or cardiac magnetic resonance imaging (cardiac MRI) and rests on the detection of increased LV wall thickness, but the disease phenotype also includes myocardial fibrosis, morphologic abnormalities of the mitral valve apparatus, abnormal coronary microcirculatory function and electrocardiographic abnormalities.

While asymmetric septal hypertrophy is the most common pattern of hypertrophy, the degree and location of hypertrophy vary. It can be concentric or confined to other walls or the LV apex.

Clinical manifestations of HCM are highly variable, from asymptomatic LVH to arrhythmias to refractory heart failure. Moreover, manifestations can vary even within the same family. An important but relatively rare consequence of HCM is SCD, most likely related to ventricular tachycardia or ventricular fibrillation.

Pathogenic variants in one of the genes encoding a component of the sarcomere, are found in approximately 50-60% of probands with a family history of HCM, and 20-30% of probands without a family history of HCM. Approximately 3-5% of affected individuals have more than one sarcomere gene variant, although fewer than 1% will have more than one pathogenic or likely pathogenic variant (7-9). Double mutations (compound or double heterozygotes) usually produce a more-malignant phenotype than single mutations (1).

Most variants (80%) identified in patients with HCM occur in two genes encoding β-myosin heavy chain 7 (MYH7) or cardiac myosin-binding protein C (MYBPC3). Other genes encoding sarcomeric proteins are also implicated in HCM, including cardiac troponin T2 (TNNT2), cardiac troponin I (TNNI3), α-tropomyosin (TPM1), myosin regulatory light chain (MYL2), myosin essential light chain (MYL3), and cardiac a-actin (ACTC1) (7, 10). Genetic testing is recommended to confirm the diagnosis in individuals with clinical evidence that is suggestive of HCM and to allow cascade screening of at-risk relatives (11, 12).

Dilated cardiomyopathy

Dilated Cardiomyopathy (DCM) has a population prevalence estimated of 1:250 (13). DCM is typically characterized by ventricular dilatation and depressed myocardial contractility, clinically evident by a reduced ejection fraction, without a clear cause such as coronary artery disease.

The diagnosis of DCM is established by the presence of left ventricular enlargement and systolic dysfunction with a reduction in the myocardial force of contraction (8). Frequently, adults manifest in the fourth to sixth decade, although it may present at any age. DCM is genetically heterogeneous with many affected cellular proteins and diverse pathologic mechanism leading to specific phenotype. (9) Pathogenic variants have been reported in more than 30 genes in 40-50% of probands. The detection rate of variants is about 27% (14-16).

The main gene involved in DCM is TTN, encoding titin, the largest protein expressed in the heart. Rare variant in TTN are estimated to account for 10-20% of DCM. Other important genetic causes include variants in LMNA gene, encoding nuclear envelope protein lamins A and C. Rare variant in LMNA are estimated to account for 6% of DCM and is mostly associated with arrhythmic risk. Missense mutations of the LMNA gene can affect nuclear function and may also result in apoptosis and premature cell death of adipocytes, thereby causing lipodystrophy (17).

Other genes encoding sarcomeric, nuclear envelope and cytoskeleton proteins are also involved in DCM (10). Molecular genetic testing should be offered to individual of any age with non-ischemic DCM, including those with peripartum or pregnancy-associated cardiomyopathy (PPCM/PACM).

Molecular diagnosis of asymptomatic first-degree family members of an affected individual can allow early detection of DCM, initiation of treatment and improvement in long-term outcome. Genetic risk assessment and cardiac surveillance of at-risk relatives are important for detection of early treatable manifestations of DCM (16).

Arrhythmogenic right ventricular cardiomyopathy

Arrhythmogenic right ventricular cardiomyopathy (ARVC) has a population prevalence estimated of 1:1000-1250 in the general population. ARVC is characterized by progressive fibrofatty replacement of the myocardium that predisposes to ventricular tachycardia and SCD. It primarily affects the right ventricle and it may also involve the left ventricle. The presentation of disease is highly variable even within family members.

The common genetic causes known to be associated with ARVC are genes encoding components of desmosome: plakophilin2 (PKP2), desmoplakin (DSP), desmoglein-2 (DSG2), desmoscollin-2 (DSC2), and junctional-plakophilin (JUP). Rare variant in PKP2 are estimated to account for 30-70% of ARVC. Less common genetic causes include variants in TMEM43, CTNNA3, DES, LMNA, PLN, RYR2, TGFB3 and TTN (18).

Molecular genetic testing should be considered in individuals who are suspected of having ARVC. If the pathogenic variant have been identified in an affected family member, it is appropriate to offer molecular genetic testing to relatives at risk for ARVC (even those age <18 years) because morbidity and mortality can be reduced by early diagnosis and treatment.

Cardiac channelopathies

Cardiac Channelopathies are a group of primary electrical disorders, resulting from the dysfunction of ion channels, associated with a risk of arrhythmia and SCD. These include Brugada Syndrome (BS), Long QT Syndrome (LQTS) and Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT). A few rare channelopathies characterized by clinical overlap have been associated also with pathogenic variant in ion-channel genes. These conditions include short QT syndrome, early repolarization syndrome, premature cardiac conduction disease, idiopathic ventricular fibrillation, familial atrial fibrillation, sinus node disease and multifocal ectopic Purkinje-related premature contractions (1).

Brugada syndrome

Brugada syndrome (BS) is caused by genetic changes in transmembrane ion channels which create action potentials, leading to an increased risk of cardiac arrhythmia. BS is characterized by cardiac conduction abnormalities (ST-segment abnormalities in leads V_1-V_3 on ECG and a high risk for ventricular arrhythmias) that can result in sudden cardiac death. It presents during adulthood, although age at diagnosis may range from infancy to late adulthood. The mean age of sudden death is approximately 40 years. Clinical presentations may also include sudden infant death syndrome (SIDS; death of a child during the first year of life without an identifiable cause) and the sudden unexpected nocturnal death syndrome (SUNDS).

Other conduction defects can include first-degree AV block, intraventricular conduction delay, right bundle branch block, and sick sinus syndrome (19). The prevalence of the disease manifesting with clinical symptoms is estimated to be 1:5000-10000, but the prevalence of clinically silent of type 1 Brugada ECG pattern is likely much higher (20).

The diagnosis of BS is established in a proband with Type 1 Brugada ECG pattern (elevation of the J wave ≥2 mm with a negative T wave and ST segment that is coved type and gradually descending) in more than one right precordial lead (V_1-V_3) with or without administration of a sodium channel blocker (i.e. flecainide or ajmaline) and at least one of the following manifestations: documented ventricular fibrillation, self-terminating polymorphic ventricular tachycardia, family history of SCD, coved-type ECGs in family members, electrophysiologic inducibility, syncope or nocturnal agonal respiration, and may include identification of a pathogenic variant in one of the genes involved (19).

In approximately 75% of probands, the diagnosis is established based on clinical history and ECG results. The most common gene involved is *SCN5A*, which encodes the α subunit of $Na_v1.5$ sodium channel. Rare variant in *SCN5A* are estimated to account for 15-35% of BS. Less common genetic causes include pathogenic variants in ABCC9, CACNA1C, CACNA2D1, CACNB2, FGF12, GPD1L, HCN4, KCND2, KCND3, KCNE5, KCNE3, KCNH2, KCNJ8, PKP2, RANGRF, SCN1B, SCN2B, SCN3B, SCN10A, SEMA3A, SLMAP, and TRPM4 genes (19).

Molecular genetic testing can confirm the diagnosis of BS. The genetic testing to search a pathogenic variant in family members of affected patient is useful to exclude clinical phenotypes in genotype-negative individuals to allow discharge from follow-up. Identifying pathogenic mutation carriers can have an important role in predicting the development of conduction disease and risk stratification. Genetic testing is not indicated in the case of an isolated type 2 or type 3 Brugada ECG pattern (1).

Long QT syndrome

Long QT syndrome (LQTS) is a cardiac electrophysiologic disorder, characterized by QT prolongation and T-wave abnormalities on the ECG associated with tachyarrhythmias, typically the ventricular tachycardia *torsade de pointes* (TdP). TdP is usually self-terminating, thus causing a syncopal event, the most common symptom in individuals with LQTS. Such cardiac events typically occur during exercise and emotional stress, less frequently during sleep, and usually without warning. TdP can degenerates to ventricular fibrillation and causes aborted cardiac arrest (if the individual is defibrillated) or SCD. (21)

Cardiac events are most common from the preteen years through the 20s but may also occur from infancy through middle age. Diagnosis of LQTS is established by prolongation of the QTc interval in the absence of specific conditions known to lengthen it and/or by molecular genetic testing that identifies a diagnostic change in one or more genes known to be associated with LQTS. Pathogenic variants in KCNH2 (LQTS types 2), KCNQ1 (LQTS types 1), and SCN5A (LQTS types 3), are the most common. Genotype-phenotype correlations have established circumstances associated with risk and ECG characteristics. Less common genetic causes include AKAP9, ANK2, CACNA1C, CALM1, CALM2, CAV3, KCNE1, KCNE2, KCNJ2, KCNJ5, SCN4B, SNTA1. Approximately 20% of families meeting clinical diagnostic criteria for LQTS do not have detectable pathogenic variants in a known gene (21).

Catecholaminergic polymorphic ventricular tachycardia

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an inherited arrhythmogenic disease characterized by cardiac electrical instability exacerbated by acute activation of the adrenergic nervous system. CPVT is characterized by episodic syncope occurring during exercise or acute emotion in individuals without structural cardiac disease. The underlying cause of syncope is the onset of ventricular tachycardia (bidirectional or polymorphic). Spontaneous recovery may occur when these arrhythmias self-terminate.

In other instances, ventricular tachycardia may degenerate into ventricular fibrillation and cause SCD if cardiopulmonary resuscitation is not readily available. The mean age of onset of symptoms (usually a syncopal episode) is between seven and twelve years; onset as late as the fourth decade of life has been reported. If untreated, CPVT is highly lethal, as approximately 30% of affected individuals

experience at least one cardiac arrest before the age of 30 years, and up to 80% one or more syncopal spells. SCD may be the first manifestation of the disease. (22). The prevalence of CPVT in the population is not known. An estimate prevalence is 1:10,000. Approximately 50-55% of probands carry a mutation in RYR2 gene, which encodes the cardiac ryanodine receptor. Pathogenic variants in RYR2 gene are associated with early onset of symptoms. CASQ2 gene encoding calsequestrin account for a small number of rare autosomal recessive cases (2-5%). Cardiac ryanodine receptor and calsequestrin are involved in intracellular and sacroplasmic reticulum, which leads to intracellular calcium overload. This dysregulation can result in triggered activity and subsequently bidirectional ventricular tachycardia (TV). Other genetic causes include CALM1 and TRDN. RYR2 and CALM1-related CPVT are inherited in an autosomal dominant manner. CASQ2 and TRDN-related CPVT is typically inherited in an autosomal recessive manner. Identification of heterozygous pathogenic variants in RYR2 or CALM1, or of biallelic pathogenic variants in CASQ2 or TRDN can establish the diagnosis (22).

Hereditary cardiac disorders share characteristics that have a direct relevance to the clinical utility of genetic analysis and family screening. Because of variable expression and reduced penetrance, clinical phenotype can vary within family members sharing the same mutation, from a patient having no clinical manifestation to severe disease.

Genetic testing for inherited cardiac diseases need to be considered as one component of a multidisciplinary cardiogenetic evaluation in which the certainty of diagnosis, the probabilistic nature of genetic testing and the need for pre-test genetic counselling to inform the patient of the intrinsic uncertainties of genetic testing, and the need to obtain a family history to evaluate disease penetrance and expressivity, are addressed with care (1). Genetic analysis result has the potential to influence clinical decisions, so it is important to communicate it carefully during genetic counselling, the communication process that deals with the risk of occurrence of a genetic disorder in the family and should be regarded as an integral part of the genetic testing process, even in the field of cardiogenetics (23-26).

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Cardiac defects in RASopathies: a review of genotype- phenotype correlations

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RASopathies are one of the largest groups of developmental diseases, caused by mutations in genes of the RAS- MAPK pathway. They include Noonan syndrome (NS; MIM ♯163950) and NS-related disorders (NSRD), such as Cardiofaciocutaneous syndrome (CFCS; MIM ♯115150), Costello syndrome (CS; MIM ♯218040), type I Neurofibromatosis (NF1; MIM ♯162200), Legius syndrome (LS; MIM ♯611431), NS with Multiple Lentigines (NSML; formerly called LEOPARD syndrome; MIM ♯151100), Noonan-like syndrome with loose anagen hair (NS-LAH; MIM ♯607721). These syndromes share many clinical features such as specific facial features, heart defects, growth and skeletal abnormalities, developmental delay, mental retardation, cancer predisposition. In this review, we will provide a clinical overview of RASopathies and an update of principle heart defects with genotype- phenotype correlations.

Clinical presentation

RASopathies share many phenotypic features, like short stature, cardiovascular anomalies, developmental delay and characteristic facial features. In addition, skeletal, hematologic and cutaneous alterations can be associated (1).

Noonan Syndrome

Noonan syndrome is the second most common syndromic cause of congenital cardiopathies after trisomy (2-5). Its prevalence is between 1:1000 and 1:2500 living births (Calcagni et al, 2017). NS is an autosomal dominant multisystem pathology characterized by a phenotypic triad (facial dysmorphic features, cardiopathies, growth retardation), to which can be associated many other developmental defects, such as mild to moderate developmental delay, skeletal abnormalities, cryptorchidism in males, cancer predisposition, endocrine and metabolic imbalances (5).

In patients with NS specific facial features include hypertelorism, down-slanting palpebral fissures, ptosis, low-set posteriorly rotated ears and short neck. Frequently can occur multiple pigmented nevi, café au lait spots, lentigines, keratosis pilaris of the upper arms and face, and curly hair (6) 80%

Key words: RASopathies, Noonan syndrome, cardiovascular anomalies, RAS/ MAPK signaling pathway

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of patients with NS have congenital heart defects, such as pulmonary valve stenosis (PVS, 60%) and hypertrophic cardiomyopathy (HCM, 20%) (5). Another important NS sign is the growth retardation; although birth length is usually normal, final adult height is around -2 SD in both sexes. These patients can present growth hormone deficiency or resistance and neurosecretory dysfunction.

Children with Noonan syndrome are predisposed to several haematological abnormalities, such as transient monocytosis, thrombocytopenia and myeloproliferative disorder. Lymphatic diseases, generally peripheral lymphoedema, are present in less than 20% of patients but can cause morbidity. In patients with Noonan syndrome haematological cancers have been described, such as juvenile myelomonocytic leukaemia, acute myelogenous leukaemia and B-cell acute lymphoblastic leukaemia (6).

Cardiofaciocutaneous syndrome

Cardiofaciocutaneous syndrome (CFCS) shares the most important signs of Noonan syndrome (facial features, heart defects, growth retardation), but this syndrome differs from NS mostly in the high frequency of ectodermal anomalies (thin, curly and friable hair; hyperkeratotic skin changes) (5). Characteristic craniofacial features are high forehead, relative macrocephaly, hypertelorism, telecanthus, down-slanting palpebral fissures, epicanthal folds, ptosis, short nose, ear lobe creases, low-set posteriorly rotated ears, high-arched palate, micrognathia. Nails may be dystrophic. Some patients present cancer diseases, mostly acute lymphoblastic leukemia (7). All affected children have mental retardation (mild to severe) (8).

Costello syndrome

Prevalence of Costello syndrome (CS) is estimated at 1:300000 live births (9). Patients show skin anomalies like soft and loose skin with deep palmar and plantar creases and curly hair. A common feature in CS is the presence of benign cutaneous papillomata typically in the perinasal area or/ and perianal region. Most patients have mental retardation. Congenital heart defects are common, generally hypertrophic cardiomyopathy (HCM) and arrhythmias (5).

Neurofibromatosis type 1

Type I Neurofibromatosis (NF1) is the second most frequent RASopathy (1:3000 live births). In 1882, Friedrich von Recklinghausen first described NF1 as a multisystem disease (10). This condition is an autosomal dominant disorder that affects multiple organs and systems and has variable clinical manifestations (11, 12).

Several benign cancers may affect NF1 patients (neurofibromas, optic nerve gliomas, Lisch nodules). Other characteristic features are multiple café-au-lait spots, bone malformations and mild learning disabilities (5, 13).

Legius syndrome

Legius syndrome (LS), a rare condition, is an autosomal dominant RASopathy. It shares many phenotypic features with NF1 (multiple café-au-lait spots, bone malformations and mild learning disabilities) (7). Occasionally, we can find in patients with LS other malformations, such as pectus excavatum or carinatum, and lipomas. Behavioural problems are also possible manifestations of the syndrome (14).

Noonan syndrome with multiple lentigines

Noonan syndrome with multiple lentigines (NS-ML; previously called LEOPARD syndrome) is a rare autosomal dominant disorder. In 1971 Golin, Anderson and Blaw first used the term "Leopard". The syndrome is characterized by multiple lentigines, electrocardiogram anomalies, hypertelorism, pulmonary stenosis, reproductive abnormalities, growth retardation and deafness (15). Patients with NS-ML have a high prevalence of sensorineural hearing deficits. 80% of patients display hypertrophic cardiomyopathy (5).

Noonan-like syndrome with loose anagen hair

Noonan-like syndrome with loose anagen hair (NS-LAH, also known as Mazzanti syndrome) is phenotypically similar to NS, but patients display distinctive hyperactive behaviour and pathognomonic hair anomalies (loose anagen hair). Usually, other features are described, like macrocephaly, growth hormone deficiency, mental retardation (16). The

most common heart anomalies are mitral valve and septal defects (5).

Heart defects and genotype- phenotype correlations

Numerous heart defects occur in RASopathies with different characteristic and prevalence among different diseases. The altered signaling of the RAS-MAPK pathway determinate heart anomalies.

The RAS- MAPK pathway

There are more than 15 genes encoding components of the RAS/ MAPK signaling pathway. This pathway plays an important role in controlling several cellular processes, such as survival, proliferation and differentiation (17). RAS encodes a guanosine nucleotide- bound GTPase protein (18). Growth factors activate RAS guanine nucleotide exchange factors (RAS-GEFs, e.g. SOS1, SOS2), and stimulate nucleotide exchange and formation of active RAS-GTP. Once in the active state, RAS can bind to several effector proteins to transmit its downstream signals, including the RAF kinases and PI3 kinase. RAS-selective GTPase activating proteins (RAS-GAPs; e.g., NF1, RASA1, RASA2, SYNGAP1) typically stimulate GTP hydrolysis to return RAS to its inactive state (19).

Genetic of RASopathies

The RASopathies are a distinct group of disorders caused by germline pathogenic variants in genes that encode components of the RAS/MAPK pathway (19). RASopathies affect 1:1000/ 1:2500 live births (17). In 2001, Tartaglia and coworkers discovered the first Noonan syndrome disease gene, PTPN11. In the last decade, other genes were found, such as SOS1, KRAS, RAF1, MAP2K1, BRAF, SHOC2, NRAS, CBL, RIT1, RRAS, RASA2, LZTR1, SOS2, SPRED1 and PPP1CB (Table I). These genes occur in roughly 85% to 90% of cases (Calcagni et al, 2018). In Noonan syndrome mutations in PTPN11 are the most recurrent (approximately 50%), with SOS1 (10–15%), RAF1 (5–15%), KRAS (<5%), NRAS (<1%), BRAF (<1%), SHOC2 (<1%), SPRED1 (<1%), and MAP2K1 (<1%) mutations are also described (1).

Table I. *Variants in genes that encode components of the RAS/MAPK pathway.*

Disease name	Disease gene (%)
Noonan syndrome	PTPN11 (50%), SOS1 (10- 13%), RAF1 (5%), RIT1 (5%) KRAS (< 5%), MAP2K1 (< 2%), BRAF (< 2%), SHOC2 (< 1%), NRAS (< 1%), SPRED1 (< 1%), CBL, RRAS, RASA2, LZTR1, SOS2, PPP1CB.
Cardiofaciocutaneous syndrome	BRAF (75- 80%), MAP2K1 and MAP2K2 (25%), KRAS (2- 3%)
Costello syndrome	HRAS (> 90%)
Type I Neurofibromatosis	NF1 (> 90%)
Legius syndrome	SPRED1 (> 90%)
NS with multiple lentigines	PTPN11 (90%), RAF1 (< 5%), BRAF, MAP2K1
NS with loose anagen hair	SHOC2, PPP1CB

PTPN11 encodes the non-receptor protein tyrosine phosphatase (PTP) SHP-2 (20). SHP2 is composed of N- and C-terminal SH2 domains and a catalytic PTP domain. Most missense mutations in PTPN11 involve the interaction between the N-SH2 and PTP domains. Mutations in this region cause an amplified signaling of the RAS/MAPK pathway (7).

SOS1 encodes the RAS guanine nucleotide exchange factor (RasGEF) protein SOS1, which promotes the conversion of RAS from the inactive state (GDP-bound form) to the active state (GTP-bound form). The majority of SOS1 missense mutations are set in codons encoding residues responsible for steadying the protein in an inhibited conformation. These mutations cause a SOS1 gain of function, an increase of active form of RAS and an increased RAS/MAPK pathway signalling (7).

RAF1 encodes the protein RAF1, a serine/ threonine kinase. Most of RAF1 mutations in NS patients are in two gene regions: conserved region 2 and conserved region 3. These mutations cause a RAF1 gain of function (7).

KRAS encodes two different variants, KRAS-A and KRAS-B. The new KRAS mutations that cause NS increase signaling of the RAS/MAPK pathway through one of two alternative mechanisms: mutations that decrease the GAP-stimulated GTPase activity; mutations that inhibit the binding of KRAS and guanine nucleotides (7).

NRAS mutations are within or near the switch II region of NRAS. These mutations interfere with GTPase function and cause an enhanced phosphorylation of ERK and MEK (7).

BRAF is a serine/ threonine protein kinase. Mutations in BRAF cluster mostly in conserved regions of CR1 and CR3. The mutations enhance ERK activation (21).

SHOC2 is a regulatory subunit of PP1C (protein phosphatase 1C). In NS patients, SHOC2 mutations cause a continued RAS- MAPK pathway activation (7).

MAP2K1 encodes the MEK protein, a kinase with a major protein kinase domain activating the extracellular- signal- regulated (Erk) mitogen- activated protein (MAP) kinases. Mutations in MEK protein kinase domain enhance MEK kinase activity (21).

CBL encodes a E3 ubiquitin ligase that inhibit the RAS/ MAPK signalling downstream of RTK. Mutations in CBL increase ERK activation (7).

Costello syndrome is caused by heterozygous mutations in HRAS. More than 80% of patients have a p.G12S substitution. HRAS encodes the oncogene HRAS. Patients with CS have de novo germline mutations in HRAS causing gain of function in the gene product and constitutive activation of RAS protein (9).

Cardiofaciocutaneous syndrome is transmitted in an autosomal dominant manner. This syndrome is genetically heterogeneous, with described mutations in four genes (Table I): BRAF (75%), MAP2K1 and MAP2K2 (25%), KRAS (2-3%) (7). The most common *BRAF* mutation in CFC syndrome patients is p.Q257R. In this syndrome, most of BRAF mutations increase kinase activity (7). MAP2K1 (MEK1) and MAP2K2 (MEK2) are threonine/ tyrosine kinases that activate ERK1 and ERK2. 25% of CSC syndrome patients have mutations in these two genes (7).

Neurofibromatosis type 1 is inherited in an autosomal dominant manner. Heterozygous pathogenic variants in NF1 are responsible for neurofibromatosis 1 (Table I). NF1 was the first syndrome associated with a germline mutation in the RAS/ MAPK pathway. *NF1* encodes neurofibromin, a GTPase-activating protein (RasGAP) that is a negative regulator of RAS. Mutations in NF1 cause neurofibromin loss of function and enhance in active GTP- bound RAS. However, NF1 is an oncoprotein and Neurofibromatosis type 1 is a cancer predisposition syndrome.

In 2007, Brems and colleagues first described Legius syndrome (Benelli et al, 2015). This syndrome is caused by heterozygous inactivating mutations in SPRED1 (Table I). SPRED1 encodes the protein SPRED1, a negative regulator of RAS-mediated RAF1 activation (22). Most of SPRED1 mutations in Legius syndrome patients cause truncation of the protein, resulting in SPRED1 loss of function (7).

Noonan syndrome with multiple lentigines is an autosomal dominant disorder (Table I). Noonan syndrome and Noonan syndrome with multiple lentigines are allelic disorders (Rauen et al, 2013) and 90% of NS-ML patients display PTPN11 mutations

(15). Other genes were found, such as RAF1 (<5%), BRAF and MAP2K1 occurring in a small percentage of subjects (Gelb et al, 2015). The majority NSML-associated PTPN11 mutations affect the catalytic PTP domain, resulting in PTPN11 loss of function (6).

Noonan syndrome with loose anagen hair is an autosomal dominant disorder (Table I). SHOC2 and PPP1CB are the two mutated genes found in this syndrome. In 2009, Cordeddu et al. discovered the association between a SHOC2 mutation promoting N- myristoylation of its protein product and NS-LAH (23). PP1 is a serine/ threonine phosphatase

Table II. *Genotype- phenotype correlation.*

Syndromes	Heart defects	Mutated genes
Noonan syndrome	Pulmonary stenosis	PTPN11 (exon 8), SOS1, RIT1, SHOC2, NRAS, LZTR1
	Atrial septal defect	PTPN11 (exon 3), SOS1, RAF1
	Hypertrophic cardiomyopathy	RAF1, SHOC2, RIT1, LZTR1, PTPN11
	Mitral regurgitation	CBL
	Aortic valve disease	CBL
	Atrioventricular canal defect	PTPN11 (exons 2,3)
NS with Multiple Lentigines	Hypertrophic cardiomyopathy	PTPN11 (exon 7), RAF1
	Pulmonary stenosis	PTPN11
	Rhythm Disturbances	PTPN11 (exon 12, 13)
NS with loose anagen hair	Septal defects	SHOC2
	Pulmonary stenosis	SHOC2
	Mitral valve anomalies	SHOC2
Cardiofaciocutaneous syndrome	Pulmonary stenosis	BRAF, MAP2K1, MAP2K2
	Atrial septal defect	BRAF, MAP2K1, MAP2K2
	Hypertrophic cardiomyopathy	BRAF
Costello syndrome	Pulmonary stenosis	HRAS

having three catalytic subunits: alpha, beta (encoded by PP1CB) and gamma. PP1C and SHOC2 produce a complex that dephosphorylates RAF, resulting in activation of the RAS- MAPK signalling (9).

Cardiac manifestations and gene mutations

Noonan syndrome is characterized by congenital heart disease whose frequency is estimated between 50% and 80%. The most common cardiovascular anomalies are pulmonary valve stenosis (PVS, for about 50% to 60%), mild to- severe hypertrophic cardiomyopathy (HCM, for 20%), atrioventricular defects (AVCD) and atrial septal defects (in less than 10% of patients), atypical electrocardiographic patterns (in approximately 90% of individuals with NS). Pulmonary valve stenosis usually displays some distinctive common features: a dysplastic pulmonary valve, with thickened and redundant cusps (22). Other structural defects commonly observed include branch pulmonary artery stenosis and tetralogy of Fallot. NS-associated hypertrophic cardiomyopathy arises early in life, with a median age of 5 months. About genotype- phenotype correlation (Table II), pulmonary valve stenosis is more common (approximately 70%) in subjects with PTPN11 and SOS1 mutations, and it is less common (approximately 20%) in patients with RAF1 lesions.

An association between pulmonary valve stenosis and codon 308 in PTPN11 was described. Patients with KRAS mutations have more common pulmonary valve stenosis, although hypertrophic cardiomyopathy and atrial septal defect were described. Atrial septal defect is more common in patients with mutations in exon 3 PTPN11 (Pierpont et al., 2018). HCM prevalence is moderately low in patients with PTPN11 or SOS1 mutations, but it is overexpressed in patients with RAF1 mutations (approximately 75%) (22).

Pathologic cardiomyocyte hypertrophy probably occurs because of increased RAS signaling. Cardiovascular defects are also very common among patients with RIT1 mutations, with a prevalence of pulmonary valve stenosis similar to the NS population (65%) but high incidence of HCM (70%) (24) Individuals with biallelic pathogenic variants in LZTR1 have an increased prevalence of hypertrophic cardiomyopathy. Association between atrioventricular canal defect and several PTPN11 mutations were found, but the only recurrent mutation is c.124A>G (25).

A congenital heart defect was noted in 44% of individuals with Costello syndrome. Non-progressive valvar pulmonic stenosis is the most common pathologic finding. Infrequently, atrial septal defects are seen. HCM including characteristic subaortic septal hypertrophy was described in 61% patients. Heart defects are associated with HRAS pathogenic variants (9).

Cardiofaciocutaneous syndrome is characterized by heart abnormalities that occur in approximately 75% -80% of individuals (pulmonic stenosis and other valve dysplasias, septal defects, hypertrophic cardiomyopathy, rhythm disturbances). Pulmonic stenosis is described in 50% of CFC individuals with a BRAF pathogenic variant (7).

Patients with Neurofibromatosis type 1 may present a wide spectrum of heart diseases, such as pulmonary valve stenosis, atrial and ventricular septal defects, coarctation of the aorta (thoracic and abdominal) and hypertrophic cardiomyopathy. Prevalence of cardiovascular defects is low (2.3%) (26). Individuals with a missense mutation affecting codon 1809 within exon 29 of the gene NF1 present a high incidence of pulmonary valve stenosis.

About 85% of individuals with Noonan syndrome with Multiple Lentigines have heart defects. Hypertrophic cardiomyopathy is detected in up to 70% of subjects with heart defects. Pulmonary valve stenosis is noted in approximately 25% of patients. ECG abnormalities include conduction defects (23%) (27). A specific missense mutation in PTPN11 (c.1528C > G; p.Q510E) is strictly associated to NSML and displayed an early onset of hypertrophic cardiomyopathy and a worse clinical outcome. The two RAF1 pathogenic variants (c.770C>T, p. Ser257Leu; c.1837C>G, p. Leu613Val) are strongly associated with hypertrophic cardiomyopathy (27). In patients with Noonan syndrome with loose anagen hair SHOC2 mutations are associated with high prevalence of heart defects, including mitral valve dysplasia and septal defects.

RASopathies are rare diseases with a high

incidence of clinical morbidity and mortality. Individuals with RASopathies have overlapping but heterogeneous clinical presentations, some of which are life threatening, including heart defects. Initial echocardiographic evaluation should be arranged as soon as possible when RASopathy is diagnosed because of the high risks of critical heart defects. Electrocardiographic evaluation is required due to frequent arrhythmias (28, 29). In this review, we tried to summarize the most important mutated genes correlated to RASopathies and we reported the principle phenotype- genotype correlations about heart defects typical of these syndromes.

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Congenital heart disease in Down syndrome

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Children with DS are at an increased risk of multiorgan comorbidities. Congenital heart defects occur in over 60% of infants with Down Syndrome (particularly septal defects). The 5-34% of infants develop persistent pulmonary hypertension of the new-born irrespective of a diagnosis of congenital heart disease. This review aims to expose the latest scientific evidence on the molecular and genetic mechanisms that underlie congenital heart disease in Down syndrome.

Down syndrome (DS) or Trisomy 21 is the most common chromosomal abnormality accounting for 8% of all registered cases, with an incidence of 1 in 700 live births. DS is caused by the presence of a third copy of chromosome 21, or part of it. This extra genetic material is responsible for the classical facial characteristics, multiple malformations, intellectual disability (ID), immune and endocrine dysfunction associated with DS like Autoimmune thyroid disease (ATD). ATD is a multifactorial disease in which autoimmunity against thyroid antigens develops against a genetic background facilitated by exposure to environmental factors (1).

DS has a variable phenotype, but there are many common physical manifestations: hypotonia, epicanthic folds, flat nasal bridge, single palmar crease and sandal toe gap (2). Children with DS have an increased risk of congenital anomalies including congenital heart defects, increased risk of hearing loss, Recurrent Respiratory Infections (3). ophthalmological problems, obstructive sleep apnoea as well as hip dysplasia and pes planus (2-5) and obesity. The hormone, leptin, may contribute to the known higher risk of obesity among children and adults with Down syndrome. Leptin is responsible from long term regulation of metabolism and ghrelin functions as an appetite stimulatory signal. (6) and it is also synthesised and secreted in breast milk, especially in colostrum (7).

In Children with DS is known that immune function is abnormal. They have a higher incidence of autoimmune disorder (8). The increased

Key words: Down syndrome (DS), Congenital heart disease (CHD), Atrioventricular septal defects (AVSD), pulmonary hypertension (PH)

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susceptibility to infections is due to atypical immune function associated with various non-immune related medical and anatomical co-morbidities (4-8). The prevalence of leukaemia is 20 times more frequent in children with DS, and it is still a major cause of mortality in children with DS (9).

The implementation of medical guidelines with preventative health care programmes screening for associated multiorgan involvement continue to help improve life expectancy and quality of life (2). These guidelines have been well established internationally via the Down Syndrome Medical Interest Group (DSMIG) and American Association of Paediatrics (AAP) (10-11).

Cardiac involvement

Congenital heart disease (CHD) is frequently diagnosed in infants born affected by DS, with significant impact on morbidity and mortality. The 54-66% of infants born with DS will have a diagnosis of CHD (12). The AAP's guidelines include cardiology review and echocardiogram in the first 6 weeks of life, however in most centres this occurs within the first few days of life (10, 11). Early surgical correction is necessary to prevent the development of Eisenmenger syndrome and pulmonary hypertension. In unselected populations, septal defects are mostly associated with DS, particularly atrioventricular septal defects (AVSD), followed by ventricular septal defects (VSD), and atrial septal defect (ASD) (Table I).

Patent Ductus Arteriosus and Tetralogy of Fallot are common in children with DS but the rate of conotruncal defects are overlapping compared to the general population (13). CHD in DS is more commonly associated with females particularly AVSD and VSD (14). In children affected by DS and CHD is present the five-year survival rates of 92% compared to 94.2% in children with structurally normal hearts. Mortality is highest if CHD was associated with other extra-cardiac malformations, 93% of deaths occurring in the first year of life. Mortality of children with DS and CHD compared to children with normal chromosomes and CHD was similar (13).

Persistent pulmonary hypertension of a new-born (PPHN) has an incidence of 5-34% in the DS population, in comparison to 0.1% in the general population; pulmonary hypertension (PH) is associated with increased morbidity and mortality (15). Oxidative stress is important in the pathogenesis of PPHN. (16) High mobility group box 1(HMGB1) is a useful marker of oxidative stress (17-21).

Children with uncorrected septal defects will develop shunt of systemic blood to the pulmonary circulation, so there is increased exposure to left to right shunt flow which can cause a sheer stress on the endothelium, induce endothelial dysfunction and, in conclusion, lead to pulmonary arterial hypertension.

Cardiac involvement	Management
- Septal defect AVSD 42% VSD 22% ASD 16% - Pulmonary Hypertension CHD in 45% Respiratory causes in 18%	- Cardiovascular examination with in 24 hours of birth and pulse oximetry - ECG - Ecocardiography as soon as possible (within 6 weeks of age) - If corrective cardiac surgery is required a long cardiological follow-up - Regular cardiological follow-up is necessary for young people and adults to exclude valvular dysfunction

Children with DS and CHD have a greater risk of developing PH than children with CHD, but without DS. Adults with DS, with and without CHD, are at an increased risk of valvular disease. Adults who have previously had a surgical repair for AVSD, have an increased risk of Eisengmenger syndrome and/or left ventricular outflow obstruction with Aortic valve dysfunction, so serial cardiology follow up post corrective surgery is essential (22).

Molecular mechanisms of congenital heart disease

In DS two hypotheses are used for explaining the DS associated CHD:

- Gene dosage amplification hypothesis claims that an increased dosage of genes on human chromosome 21 in DS may increase the level of gene expression (23).
- Gene mutation hypothesis holds that in the trisomy 21 background, certain locus mutations can cause CHD (24).

Genes on chromosome 21

Different genes have been considered as candidate genes for the increased CHD risk in children with DS. DSCAM, located on 21q22.2, is a member of the immunoglobulin superfamily of cell adhesion molecules (Ig-CAMs) and it is important in the nervous system development. Several studies have suggested that, due to the gene dosage multiplier in trisomy 21, the expression of intercellular mucoprotein increases abnormally before endocardial cushion development, so enhanced the adhesion between cells and affected the fusion of endocardial and causing AVSD (25). Collagen VI is expressed in fetal hearts (5-18 weeks of development) and involved in the formation of the original atrioventricular septum.

Also including the middle and lower part of the atrioventricular septal valve and the membrane. Overexpression of type VI collagen (COL6A1, COL6A2) plays a critical role in the pathogenesis of AVSD in DS. It is an interesting phenomenon that AVSD, VSD, PS and TOF could be observed if both DSCAM and COL6A1 were co-expressed, while only DSCAM was copied, just TOF was seen. It indicates that DSCAM and COL6A1 may have a synergistic effect on the overall cardiac defects (26).

Genes on another chromosome

Other genes, not localized on chromosome 21, may play an important role in the development of these cardiac anomalies. The genes CRELD1, CRELD2 and ALK2 seem to increase susceptibility to DS-CHD (27, 28). The CRELD family of proteins have two members: CRELD1 and CRELD2. CRELD1, located on 3p25, is the first find to be involved in the pathogenesis of isolated AVSD (with or without DS).

Congenital Heart Disease (CHD)[13]	**%**
Atrioventricular septal defects (AVSD)	**45%**
Ventricular septal defects (VSD)	**35%**
Isolated secundum atrial septal defects (ASD)	**8%**
Isolated persistent patent ductus arteriosus (PDA)	**7%**
Tetralogy of Fallot (TOF)	**4%**
Other	**1%**

It is expressed during endocardiac cushion development and encodes a cell surface protein that acts as cell adhesion molecule. Studies have found missense mutations in CRELD1 in DS patients with AVSD, these several results indicate that mutations in CRELD1 may contribute to the pathogenesis of AVSD in the context of trisomy 21 (29, 31).

MicroRNA might participate in AVSD in DS patients by negatively regulating AUTS2 and KIAA2022 gene expression. Overexpression of the miR-99a/let-7c cluster subsequently decreased their targets in fetal DS heart tissue. SNP and CNVs probably contributes to the different predisposition to specific CHDs in the various ethnicities.

In this review, we summarized the possible molecular mechanism of DS associated CHD. Gene dosage imbalance hypothesis and gene mutation hypothesis are the main pathogenesis. Different genes are related to CHD in children DS: DSCAM, COL6A1w2, CRELD1, CRELD2. Among these genes, DSCAM, COL6A1w2, located on CHR 21 cause abnormal heart development through different mechanisms by gene dosage effect. CRELD1, CRELD2 lied in other chromosome results in CHD, due to gene locus mutation.

In addition to the factors mentioned above, there may be other new mechanisms that have not yet been discovered, and we believe that the CHD phenotype in DS patients is a combination of multiple factors that interact with each other synergistically or antagonistically and participate in the occurrence of DS-CHD. Our comprehension of the molecular pathogenesis of DS associated with CHD is significantly insufficienct. Many important future research goals need to be achieved.

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Myocarditis in children - from infection to autoimmunity

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The aetiology of myocarditis in children remains often unknown. Myocarditis is an inflammatory disease of the myocardium (inflammatory cardiomyopathy) classified among the acquired primary cardiomyopathies. It is caused primarily by numerous infectious agents, but it may also accompany autoimmune disease, hypersensitivity reactions and toxins. This special issue aims to provide an overview of aetiology and pathophysiology with main target pharmacological properties of myocarditis in children. A comprehensive search of published literature using the PubMed (http://www.ncbi.nlm.nih.gov/pubmed/) database was carried out to identify all articles published in English from 2000 to January 2019, using the following key terms: Myocarditis, Infection, Autoimmunity and Children.

Myocarditis, according to the World Health Organization/International Society and Federation of Cardiology (WHO/ISFC) definition, is an inflammatory disease of the myocardium associated to myocyte necrosis or degeneration of non-ischaemic event (1, 2). It is uncommon in childhood and it may be isolated or part of systemic diseases. The incidence of myocarditis is approximately 2 in 100,000 patient-years, with a male predominance in children older than 6 years, but the true incidence is underestimated because the disease can be subclinical (3).

Clinical presentation of this inflammatory disease includes a very wide symptomatology ranging from dyspnoea, palpitation, chest pain to forms of acute or chronic congestive heart failure, sudden cardiac death or fulminant cardiogenic shock (4). Many cases of myocarditis are, however, asymptomatic (5).

The diagnosis is established by histological, immunological and immunohistochemical criteria (6). The first diagnostic criteria ("*Dallas* Criteria") were proposed in 1986 (2). The diagnosis is, however, difficult and often placed in case of acute myocardial dysfunction. The clinical history, the physical examination, chest X-ray, electrocardiogram (ECG) and echocardiogram associated with an elevation of cardiac enzymes such as creatinine phosphokinase MB fraction (CK-MB) and cardiac troponin I, can lead to diagnosis (6). Cardiac magnetic resonance imaging (MRI) maybe helpful imaging tool, but the gold standard remains the myocardial biopsy (5).

Over the last decade, cardiac magnetic resonance imaging (MRI) has become the gold standard for myocardial tissue analysis in several cardiac diseases (7). Patients with myocarditis displayed increased level of High mobility group box 1 (HMGB1), and, the evaluation of concentrations of HMBG1 could explain how an initial inflammation can trigger the condition of meta-inflammation (8). HMGB1 can be

Key words: myocarditis; infection; autoimmunity and children

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passively secreted from damaged or necrotic cells (9). It plays an important role in the inflammatory process associated with childhood obesity. This peptide may be an important diagnostic marker for obesity-related complications (10). HMGB1 plays a role in several medical conditions (11). The abovementioned pro-inflammatory properties of HMGB1, acquired upon its cellular release and consequent receptor stimulation, contribute to the pathogenesis of several acute and/or chronic non-infectious and infectious human diseases (12-17).

The etiopathogenetic background includes a wide range of infectious and autoimmune mechanisms. There are viral, autoimmune and both forms of myocarditis. The type of etiopathogenetic mechanism influences the clinical presentation, the therapeutic strategies and the prognosis of the affected patients (survival, sudden cardiac death or cardiac transplantation) (6, 17).

Infection-induced myocarditis

The commonest aetiology of myocarditis in children remains infection. Modern molecular techniques, such as hybridization and the polymerase chain reaction (PCR) testing on endomyocardial biopsy samples, makes possible to recognize the main infectious agents responsible for myocardial damage. Among these, viral agents are primarily involved, but there are also, with less evidence, bacteria, fungi and parasites (18).

In Central and South America, the most common cause of myocarditis is Trypanosoma Cruzi, the causative agent of Chagas' disease (19). In North America and Europe, the microbes involved are DNA and RNA viruses. The most frequent DNA virus are Adenoviruses, Parvovirus B19, Cytomegalovirus, Human Herpes virus-6, Epstein-Barr virus, Varicella-Zoster virus, Herpes Simplex virus. Among RNA virus, there are Enteroviruses (Coxsackieviruses A and B), Echoviruses, Influenza A and B viruses (17). Diphtheria often causes myocarditis in countries without widespread immunization (5).

Virus are responsible of heart damage because they have trophism not only for cardiomyocytes but also for endothelial cells (19).

Studies on murine models showed that Enterovirus and some Adenovirus may cause the myocardial damage through three phases. Virus enters the cardiomyocyte and they activate innate immunity (phase I). Some agents, like Coxsackievirus B and Adenoviruses, enter using a transmembrane receptor on the surface of myocardial cells, called CAR (Coxsackievirus and Adenovirus receptor). To demonstrate this, knockout mice for the gene coding for CAR were created. After exposure to the viral agent this type of knockout mice did not developed myocarditis (18). Then, the virus replication in the myocytes induces cell necrosis and the release of intracellular antigens (including myosin) into the circulation that act as triggers of innate immunity (natural killer cells, macrophages, T cell) of the host. The phase II is, therefore, activated, and there is production of specific T cells and cytokines (mainly tumour necrosis factor-α, interleukins 1a, 1b, 2 and interferon gamma) (6, 18). These cytokines with antibodies produced against viral and cardiac proteins, cause cytotoxic events with imbalance of the contractile apparatus and/or interstitial cells, heart damage and systolic dysfunction. The result may be the resolution with total recovery of cardiac function or evolution to dilated cardiomyopathy (DCM) is phase III (19).

Besides, there are viruses, like Parvovirus B19, with trophism for endothelial cells of venules, small arteries and arterioles. It does not induce necrosis of myocytes but causes endothelial dysfunction through T cells and inflammatory cytokines (such as interleukin 6 and tumour necrosis factor) involvement. The endothelial dysfunction triggers coronary vasospasm and then impaired perfusion, cardiac ischemia, myocardial necrosis and finally ventricular dysfunction (19).

Autoimmunity-induced myocarditis

Although the causes of autoimmune disease are complex and multifactorial, one generally accepted model is that immune dysregulation, secondary to an infectious process or excessive exposure to an inciting or cross-reactive antigen, promotes a Th1 response leading to progressive inflammation and autoimmunity (20).

Tissue injury and cardiac dysfunction may

be a consequence not only of direct injury due to infectious agents but also of host immune system hyperactivation with production of autoantibodies against cardiac tissue (21). The presentation of viral antigens evokes an antiviral immune response that can continue despite virus' elimination. The virus' persistence feeds the auto-immune response against the hearth. Activated B lymphocytes produce antibodies that can cross-react with myocardial antigens and may also contribute to impairment of cardiac contractility. Numerous autoantibodies have been identified in patients with myocarditis and dilated cardiomyopathy (22).

The first evidence of autoimmunity in children with clinically diagnosed myocarditis has been reported by Simpson et al. in a manuscript published on Journal of Cardiac Failure (2016) (23). They demonstrated a possible mechanism of autoantibody mediated cellular damage. The autoantibodies directly target heart protein (myosin) and cross-react with beta-adrenergic receptor on cardiac tissue, with downstream effects on cyclic adenosine monophosphate (cAMP) - dependent activation of protein kinase A in heart cells in culture (22). The increase of protein kinase A and the passive transfer of the autoantibodies causes the heart cells apoptosis (24, 25). To confirm this, scientific literature has, in fact, shown that patients with more severe myocarditis and without total recovery of cardiac function had higher levels of protein kinase A than patients with the same disease but with subsequent total recovery of cardiac function. In children with myocarditis, therefore, the downstream activation of beta-adrenergic receptor and the subsequently increase of protein kinase A activation, can be considered as a marker of myocarditis severity (24).

Finally, also muscarinic receptor antibodies have been reported to induce dilated cardiomyopathy phenotype in murine models and they are associated with arrhythmias (25). Although the evidences of autoimmunity in pediatric patients with clinical myocarditis, prospective studies in children are needed to understand the complex relationship of autoimmune activation with patient outcomes and identify potential target-therapy (22).

Myocarditis therapy

The viral genome finding is useful in the clinical practice to use a more rational and targeted therapeutic approach with antiviral agents, aimed at the cure of acute myocarditis and persistent cardiac dysfunction unresponsive to supportive therapy. Supportive therapy includes the use of diuretics for preload reduction, angiotensin converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARB) for afterload reduction and β blockers. Patients with fulminant or acute myocarditis often require intubation with ventilator support and usually hemodynamic support with vasopressors, inotropes, and intravenous diuretics (3). Immune therapy is still characterized by great controversy in the treatment of pediatric myocarditis. Intravenous immunoglobulin (IVIG) has many antiviral, immunomodulatory and anti-inflammatory effects at higher doses (2 g/kg over 2 days for children) (26).

In a previous study it showed a positive response of therapy by immunoglobulin injection in the treatment of multiple oral ulcers in Stevens–Johnson syndrome (27).

The beneficial effect of IVIG leads to a more rapid clearance of myocardial viral infection via targeting specific antibodies to viruses and can also modulate immune responses, which potentially cause decreased cardiac inflammation (28). A 2019 meta-analysis, however, demonstrated that IVIG treatment was not associated with better survival. Neither IVIG or corticosteroids individually or combined demonstrated any benefit in myocarditis. The use of IVIG therapy, therefore, in acute myocarditis in children cannot be routinely recommended based on current evidence (29-30).

Myocarditis remains an uncommon diagnosis in the pediatric population, with significant variation in clinical presentation. Its treatment is not yet standardized and randomized clinical trials in the pediatric population are essential to determine the subset of patients who would benefit from immunoglobulin therapy, immunosuppression and both. The hope is to reduce the mortality and morbidity of myocarditis in children.

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Cardiovascular complications in children with chronic kidney injury

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Chronic kidney disease (CKD) is associated with type 4 cardiorenal syndrome (CRS type 4). The term cardiorenal syndrome (CRS) is used to emphasize the tight interaction between the cardiovascular and renal systems in acute and chronic disease. In CRS type 4 the primary chronic kidney disease (CKD) leads to an impairment of cardiac function, with ventricular hypertrophy, diastolic dysfunction, and increased risk of adverse cardiovascular events. The rate of fatal cardiac events is indeed highly increased in children with CKD compared to the general population (1). The most common disease that causes chronic kidney disease (CKD) is primary glomerular disease (GD), followed by congenital abnormalities of the kidney and urinary tract, cystic, hereditary, or congenital disorders and, more rarely, secondary GD (2).

Cardiovascular death rates are different between children on peritoneal dialysis PD (28%), on hemodialysis HD (32%), and after transplant (22%) (2). The last one has a relatively lower risk of cardiac death. However, even in these patients, the mortality rates are still significantly higher (approximately 10 times) than in the general pediatric population. Recent analysis of long-term survival who received a first kidney transplant during childhood showed that most deaths were from cardiovascular causes (3). Children selected to start dialysis on HD or PD exhibit a similar mortality risk during the first 2 years of treatment (4). (Table I)

The development of cardiovascular complication during young adulthood is due to a combination of traditional and uremia-related risk factors. Children, even with early stages of CKD, have high prevalence of traditional cardiovascular risk factors such hypertension (54%), dyslipidemia (45%) obesity in (15%) hyperinsulinemia (9%) and insulin resistance (19%) (5). Obesity and attendant co-morbidities are an emergent problem in public health. Much attention has focused on prevention, especially during the perinatal period. Breastfeeding is also a possible protective factor for obesity in childhood

Key words: chronic kidney disease, CKD, cardiorenal syndrome, CRS, adverse cardiovascular events

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(6). Increased levels of aldosterone may be responsible for hypertension and for cardiovascular complications associated with obesity (7).

A prospective study, with a cohort of 586 children with CDK, indicate that a decline in blood pressure may predict a decline in left ventricular hypertrophy and suggests to use early antihypertensive medications as angiotensin-converting enzyme inhibitor (ACEI) or an angiotensin receptor blocker (ARB) in these patients (8). However, these risk factors do not fully explain the high rates of cardiac death in children with CKD. To better understand the increased risk of cardiac disease in these children we should analyze uremia-related risk factors. It is known that these patients have a high prevalence between abnormal mineral metabolism, anemia, inflammation, increased level of oxidative stress and toxins.

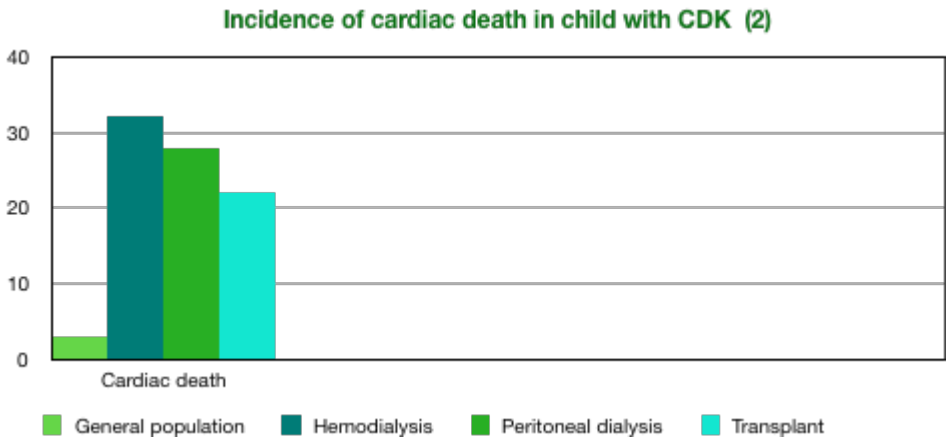
These findings become more frequent as kidney function decreases and seems to have a role in the pathogenesis of uraemic cardiomyopathy (9). The disorders of mineral metabolism are due to alterations in the levels of parathyroid hormone (PTH), serum phosphate, vitamin D. Vitamin D3 (cholecalciferol) is the form that is stored in tissues such as the liver and adipocytes. The prohormone requires two hydroxylations-the first in the liver, and the second in the kidney-to become the biologically active form (1.25 [OH] 2D3; calcitriol (10)).

Also, FGF23 and Klotho protein's levels are altered. The levels of circulating phosphate and fibroblast growth factor 23 (FGF23) are elevated, whereas serum levels of 1.25-dihydroxy-vitamin D (vitamin D) and soluble Klotho are reduced. Alterations in the levels of all four of these factors might independently contribute to cardiac hypertrophy by directly and/or indirectly interfering with cardiac remodelling. The hight level of phosphate might lead to endothelial cell damage and thereby indirectly induce vascular calcification. Elevated phosphate levels can also lead to hyperparathyroidism.

Increased levels of parathyroid hormone (PTH) cause cardiac hypertrophy via activation of the PTH type 1 receptor (PTH1R) on cardiomyocytes. FGF23 can directly target the myocardium via binding to FGF receptor 4 (FGFR4) and thereby induce hypertrophic growth of cardiomyocytes and potentially subsequent damage, including cardiac fibrosis. Klotho protein is a transmembrane protein with three isoforms, αKlotho is the one which we refer. It is expressed in the kidney and parathyroid glands; forms complexes with FGFR1, FGFR3 and FGFR4; and is a co-receptor for FGF23. αKlotho can directly target the heart and prevent the development of left ventricular hypertrophy via blocking transient receptor potential cation channel, subfamily C, member 6 (TRPC6) and/or an unknown receptor.

Soluble Klotho can also block the activation of the renin–angiotensin–aldosterone system (RAAS), the sympathetic nervous system (SNS), transforming

Table I. *Incidence of cardiac death in child with CDK (2).*



growth factor-β (TGFβ) and the reactive oxygen species (ROS)–insulin–insulin-like growth factor I (IGF1) signalling pathway and thereby protect the myocardium from pathological stress-induced cardiac hypertrophy and fibrosis. Vitamin D can directly target the heart via the vitamin D receptor (VDR) and downregulate cardiomyocyte proliferation and hypertrophy. As phosphate, vitamin D, FGF23 and soluble Klotho regulate each other's expression, they might also be able to amplify each other's cardiac actions. For example, FGF23 expression is increased by phosphate and reduced by vitamin D (11).

Also, uraemic toxins have a critical role in the development and progression of the cardiomyopathy. For example, indoxyl sulfate and p-cresyl sulfate, has been shown to produce pro-hypertrophic and pro-inflammatory effects and to increase arrhythmias in cardiac tissues by increasing oxidative stress (12). Some of these uraemic toxins are difficult to remove via haemodialysis. This finding can explain the hight prevalence of cardiovascular events in patient on hemodialysis in comparison with patient on peritoneal dialysis.

Although mortality is still high, to optimize dialysis prescription, great care should be aimed at improving both fill volume and treatment duration, at preventing peritonitis and at preserving peritoneal membrane function (13, 14). Increased levels of oxidative stress and inflammation are also prevalent in patients undergoing haemodialysis and are associated with increased cardiovascular mortality. To date, the beneficial effects of antioxidant therapy in patients with CKD in general and in those with uraemic cardiomyopathy has not been demonstrated. Anemia is one of the most important factors along with hypertension for the development of LVH in CKD patients. Therefore, correction of anemia is an essential strategy in the CRS type 4 population. Targeting the mechanisms that are involved in the development of uraemic cardiomyopathy is in fact a logical approach to potentially improve outcomes in these children. The studies on pathophysiological mechanisms involved in CRS type 4 are very important because will allow the development of more appropriately therapeutic strategies to improve outcome in these patients.

Another aim of recent studies is to identify new and early echocardiographic signs of cardiovascular impairment. Children with chronic renal failure frequently develop cardiac abnormalities of left ventricular (LV) structure and function such left ventricular hypertrophy (LVH) with preserved systolic function. Different studies have assessed that LV diastolic dysfunction develops early when renal failure is mild or moderate in children and progresses as renal function deteriorates (15). These findings indicate that abnormalities of LV diastolic function may develop during initial stages of renal impairment in children with kidney disease and may be significant predictors of cardiac impairment in children with CKD.

Doppler examination of mitral inflow velocity has been the most widely used method to determine LV diastolic function (E/A ratio). Using this method, different studies showed that LV diastolic function was impaired in dialyzed children when compared to control patients. Unfortunately, the transmitral Doppler velocities, and therefore the E/A ratio, are affected by several factors, including left atrial pressure and preload. This is particularly important for patients with advanced chronic renal failure because many of them have abnormal volume status. The effect of preload can be corrected by using the combination of TDI of the mitral annuls and mitral inflow velocity from conventional Doppler (E/Em ratio). TDI velocities are independent of LV mass and morphology and provide sensitive additional information about early alterations of the left ventricular function in children with CKD.

Moreover, a recent study shows independent associations of both lower GFR and higher blood pressure with reduced diastolic function measured by TDI E'/A' Z-Score (15). Additionally, diastolic function measured by E'/A' Z-Score tended to be better preserved in patients treated with RAS blockade independently of renal function (16). In conclusion children with CDK require the coordinated efforts of multiple Pediatric specialists throughout treatment and in long term follow-ups to identify new strategies to reduce the cardiovascular risks and improve their quality of life.

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Kawasaki disease and cardiac involvement: an update on the state of the art

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Kawasaki disease (KD) is an acute systemic vasculitis of unknown etiology. It has a self-limiting course and so far, represents the most common cause of coronary heart disease acquired in children aged between 6 months and 5 years. The inflammatory process can involve the coronary arteries with the formation of aneurysms and thrombotic occlusions with the risk of sudden death, especially in infants. Myocardial inflammation and abnormalities of cardiac contractility can occur acutely or many years after the disease onset. Therapy must be started within 10 days after the onset of symptoms to reduce the risk of heart complications. Immunoglobulin and aspirin treatment are effective in reducing heart complications. Recent studies have shown new therapeutic strategies (corticosteroids, immunosuppressive and biological drugs) in case of ineffectiveness of treatment with immunoglobulins.

Kawasaki disease (KD) is the second most common systemic vasculitis of childhood (1) and affects small and medium-sized vessels. It is the most common cause of acquired heart disease in children in developed countries. The incidence varies across the different geographical regions, in Europe is about 5-10 cases /100,000 (2). The highest incidence has been described in Japan with 265 cases/100,000 children under the age of 5 (3). Most patients are under 5 years of age, although the disease can occur in older children and adults (4). The male / female ratio is approximately 1.5 to 1 (5).

KD causes coronary artery aneurysms (CAA) in 25% of patients treated due to coronary vasculitis. Some clinical trials show that this risk drops to 4% with intravenous immunoglobulin (IVIG) treatment (6, 7). Emerging experimental non-clinical trials suggest worse results despite using IVIG, especially under 12 months of life (8). Mortality depends on the population: 0.015% in Japan (6); 0.17% in the United States (6) and 0.36% in the United Kingdom (9).

The complexity and heterogeneity of the

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presentation of KD, the differential diagnosis and the lack of diagnostic tests can make a prompt diagnosis difficult. (8) The cause of KD is still unknown. It is hypothesized a trigger (infectious) that stimulates an abnormal immune response in genetically predisposed children. A previous study measured High-mobility group box protein 1 (HMGB1) levels in children affected by KD, demonstrating higher HMGB1 values than healthy controls. The pro-inflammatory properties of HMGB1, acquired upon its cellular release and consequent receptor stimulation, contribute to the pathogenesis of several acute and/or chronic non-infectious and infectious human diseases (10-13). HMGB1 may be used as a marker of inflammation in vasculitis disease (14).

The infectious etiology is suggested by the symptoms and epidemiology of KD which are similar to that of common childhood infections (young age-group affected, epidemics with wavelike geographic spread of illness, the self-limited nature of the acute febrile illness, and the clinical features. The infectious triggers can be primarily viruses but also bacteria (15).

The trigger probably leads to an upregulated immune response in the susceptible individuals. At the basis of this abnormal immune response there is an imbalance between the pro-inflammatory (16, 17) and anti-inflammatory pathways (18).

Serum cytokine levels reflect the activity of the immune system. Therefore, modulation of cytokine environment and balance of Th1/Th2 are critical points to achieve tolerance and immune suppression (18). The elevation of serum High Mobility Group Box-1 (HMGB1) may play an important role in the development of KD. Recent studies have directed attention toward HMGB1, a 30 kDa nuclear and cytosolic ubiquitous protein, belonging to the alarmins family and promoting an immediate activation of the innate immune response (19).

Genetic factors seem to contribute to susceptibility to KD. In fact, there is an increased risk of KD in children of Asian ethnicity and in family members of subjects with KD history. Moreover, genetic factors seem to increase the probability of developing CAA and to affect the response to treatment. Polymorphisms in multiple genes, such as ITPKC (inositol-triphosphate 3-kinase) (20), CASP3 (21, 22), FCGR2A (23), BLK (24) and CD40 (25) seem to be associated with an increased susceptibility for KD development and CAA.

Specifically, ITPKC acts as a negative regulator of the Ca2 + / NFAT pathway and it seems to act as a second messenger in T cell receptor signaling. This suggests that ITPKC is responsible of a greater and prolonged expansion of inflammation, thus increasing the risk of KD and / or the severity of the disease.

Diagnosis of Kawasaki disease - Classic KD

According to the latest AHA Guidelines, " Classic KD is diagnosed in presence of fever for at least 5 days (the day of fever onset is taken to be the first day of fever) together with ≥ 4 of the 5 following principal clinical features (In the presence of ≥4 principal clinical features, particularly when redness and swelling of the hands and feet are present, the diagnosis of KD can be made within 4 days of a fever, although experienced clinicians who have treated many patients with KD may establish the diagnosis with 3 days of fever in rare cases):

1. Erythema and cracking of lips, strawberry tongue, and/or erythema of oral and pharyngeal mucosa.
2. Bilateral bulbar conjunctival injection without exudate.
3. Rash: maculopapular, diffuse erythroderma, or erythema multiforme-like.
4. Erythema and edema of the hands and feet in acute phase and/or periungual desquamation in subacute phase.
5. Cervical lymphadenopathy (≥1.5 cm diameter), usually unilateral.

A careful history may reveal that ≥1 principal clinical feature was present during the illness but resolved by the time of presentation. During the acute phase, laboratory tests typically reveal a normal or elevated white blood cell count with neutrophilic predominance and elevated acute phase reactants such as C-reactive protein and erythrocyte sedimentation rate. Low serum sodium and albumin levels, elevated serum liver enzymes, and sterile pyuria can be present too. In the second week after fever onset, thrombocytosis is common. Other clinical findings may involve:

- Cardiovascular (Myocarditis, pericarditis, valvular regurgitation, shock, Coronary artery abnormalities, Aneurysms of medium-sized noncoronary arteries, Peripheral gangrene, Aortic root enlargement)
- Respiratory (Peribronchial and interstitial infiltrates on CXR, Pulmonary nodules)
- Musculoskeletal (Arthritis, arthralgia (pleocytosis of synovial fluid))
- Gastrointestinal (Diarrhea, vomiting, abdominal pain, Hepatitis, jaundice, Gallbladder hydrops, Pancreatitis)
- Nervous system (Extreme irritability, Aseptic meningitis, Facial nerve palsy, Sensorineural hearing loss)
- Genitourinary (Urethritis/meatitis, hydrocele)
- Other: Desquamating rash in groin, Retropharyngeal phlegmon, Anterior uveitis by slit lamp examination, Erythema and induration at BCG inoculation site." (6).

Diagnosis of KD before day 5 of the fever

Fever ≥ 5 days as a criterion for diagnosis can lead to delayed treatment. Although the duration of the fever has historically been important for a clear definition, recent studies suggest that clinicians should not delay the diagnosis of KD. Therefore starting the treatment, if five out of six diagnostic KD criteria are present before day 5 of a fever or if CAA (Z-score ≥2.5) or coronary dilatation (Z- score > 2, but < 2.5) are present (8).

Incomplete KD (Atypical)

The 2017 AHA guidelines define KD as incomplete when children have a fever ≥ 5 days and two or three clinical criteria, or infants have a fever for ≥ 7 days with no other explanation. Incomplete KD occurs most commonly in infants who are therefore highly exposed to the risk of developing coronary aneurysms. In these situations, early echocardiography is recommended. This may reveal evidence of coronary vasculitis, confirming the diagnosis of KD. However, coronary artery aneurysm is not generally detected in the first week of disease and therefore a normal echocardiogram in this period cannot exclude the diagnosis of KD (8, 6).

Cardiac involvement and Long-Term Follow-up

KD-related cardiac complications can lead to significant morbidity and mortality.

Therefore, KD patients need careful cardiologic follow-up. Due to the rapidly evolving nature of KD, all the patients should undergo an intermediate echocardiogram, 2 weeks after the first IVIG administration, including those whose initial echocardiography was normal and those whose disease activity has decreased (3, 6, 26). All patients should undergo echocardiography 6-8 weeks after disease onset (3, 6). In addition to CAAs, other complications can include changes in myocardial contractility and heart failure, arrhythmias, myocardial infarction and peripheral arterial occlusions. Several cases of KD associated with hemodynamic instability during the acute phase of the illness (Kawasaki disease shock syndrome-KDSS) have been described.

KDSS is a rare and severe condition defined by the presence of systolic hypotension or clinical signs of peripheral blood circulation perfusion disorder with accompanying features of KD (27). It should be emphasized that KD-related myocarditis is more common than CAAs from a histological perspective. Specifically, most patients with KD in the acute phase have subclinical myocarditis, which has mild electrocardiographic changes or mild clinical symptoms. It was also noted that a "gallop" was auscultated in 13% of patients within the first 20 days of illness. Other common clinical findings included tachycardia out of proportion to fever and hyperdynamic precordium.

Myocarditis typically resolves after the acute phase of KD, but the long-term effects of the initial insult can lead to fibrosis and to a reduction of the number of myocytes with the implications that follow. These long-term complications that do not concern coronary artery abnormalities are not yet fully delineated (7). Several studies have also suggested that in a small subset of patients with KD, myocardial fibrosis generated by myocarditis can lead to long-term systolic or diastolic dysfunction. This complication requires further investigation, as most of the previous research has focused on the complications of coronary artery aneurysm.

Treating Kawasaki disease

As soon as a patient is diagnosed with complete or incomplete KD treatment should be started (8). As shown by several studies, early diagnosis and treatment with IVIG and aspirin in the first 10 days of the onset of KD reduces the risk of developing CAA (27).

Initial Treatment - IVIG

The standard initial treatment is single high-dose IVIG (2 g/kg) administered over 10–12 h plus aspirin. Some of the affected people can be treated with IVIG even after 10 days of illness if their fever persists or they have coronary artery abnormalities together with elevations of ESR or CRP.

Aspirin

Patients with complete or incomplete KD should initially receive aspirin at a dose of 30-50 mg / kg / day, divided into 3-4 doses, as described in the latest articles (6-8). Aspirin should be reduced to an antiplatelet dose of 3-5 mg / kg / day if clinical characteristics are improving for 48 hours and CRP levels are decreasing. Aspirin can then be stopped if the echocardiogram performed at 6-8 weeks remains normal. However, if CAA persists in the convalescent phase continuation of a long-term low-dose aspirin is recommended, at least until its resolution (6, 7).

Adjunctive Therapies for Primary Treatment

Up to 20-40% of patients are resistant to IGIV with an increased risk of developing CAA (28). Therapeutic delay seems the main factor contributing to these results, but some authors hypothesize that the IVIG response may vary between populations, probably due to genetic differences (29, 30).

Several scoring systems have been created to stratificate the IVIG non-response risk in Japan. Kobayashi et al. (31) developed a model to predict unresponsiveness to IVIG in Japanese children with KD based on a cut-off point ≥ 4 calculated as per below: sodium ≤ 133 mmol/L (2 points); days of illness at initial treatment ≤ 4 (2 points); aspartate aminotransferase ≥ 100 IU/L (2 points); percentage of neutrophils ≥ 80% (2 points); CRP ≥ 100 mg/L (1 point); age ≤ 12 months (1 point); platelet count ≤ 300 x 10 9/L (1 point).

Treatment with corticosteroids

According to the latest European consensus-based recommendations for the diagnosis and treatment of Kawasaki disease (8), corticosteroid treatment could be given to patients with severe KD:

- who are IVIG resistant, with on-going fever and/or persistent inflammation or clinical signs ≥ 48 h after receiving a single dose of IVIG (7) (a second dose of IVIG alongside corticosteroids is at the discretion of the treating physician (7)).
- Who have a Kobayashi score ≥ 4 (31).
- With features of HLH.
- With features of shock.
- Who are under the age of 1 year.
- Who present with coronary and/or peripheral aneurysms.

If there are indications to use steroids, 2 treatment schemes can be followed:

1) methylprednisolone 0.8 mg/kg BD i.v. for 5-7 days or until CRP normalizes; then convert to oral prednisone/prednisolone 2 mg/kg/day and wean off over next 2-3 weeks.

2) methylprednisolone 10-30 mg/kg (up to maximum of 1g/day) once daily for 3 days followed by oral prednisone/prednisolone 2 mg/kg per day until day 7 or until CRP normalizes; then wean over next 2-3 weeks (8).

TNF-alpha blockers and other treatments

The use of anti-TNF-alpha drugs (eg Infliximab) should be considered in patients with persistent inflammation despite treatment with IVIG, aspirin and corticosteroids, even after consulting a specialized center (32, 33). The use of DMARDs such as ciclosporin, cyclophosphamide and methotrexate, along with anakinra and plasma exchange, cannot be recommended, except on an individual basis after consultation with a specialist unit (8).

Anti-coagulation and anti-aggregation

In the presence of giant aneurysms (internal diameter 58 mm, or Z 5-10 score, and / or coronary

artery stenosis), warfarin (in addition to aspirin) should be administered after initial heparinization. Low molecular weight heparin represents a valid alternative, especially in young children where safe warfarinization can be difficult.

According to the latest AHA Guidelines, it would be reasonable to administrate systemic anticoagulant therapy with low-molecular-weight-heparin (LMWH) or warfarin, in addition to low-dose ASA, to patients with rapidly expanding coronary artery aneurysms or a maximum Z score of ≥10.

In addition, it should be taken into account the administration of a "triple therapy" with ASA, a second antiplatelet agent and anticoagulant therapy with warfarin or LMWH to patients with large or giant aneurysms (≥8 mm or Z score ≥10), who are at increased risk of thrombosis, and with a recent history of coronary artery thrombosis. It should always be considered that ibuprofen and other nonsteroidal anti-inflammatory drugs acting on the cyclooxygenase pathway can be harmful in patients taking ASA for its antiplatelet effects.

Long-term anticoagulation

A careful follow-up is extremely important. The class stratification risk proposed by the American Heart Association (AHA) is very useful for the correct timing of therapeutic interventions:

- Class I (no abnormality of coronary arteries in the various phases of the disease): treatment with ASA for the first 8 weeks and in any case until a documented normalization of both platelet count and inflammatory markers is recommended.
- Class II (Transient coronary artery ectasia that disappears within 8 weeks): treatment with ASA until normalization of both platelet count and inflammatory markers and disappearance of coronary artery lesions (also tortuosity/ stiffness of the vessel walls) documented by two subsequent controls is recommended.
- Class III (Single aneurysm of small-medium caliber between +3 and +7 SD in one or more arteries): administration of ASA until complete aneurysm regression documented by two subsequent negative controls is recommended.
- Class IV (One or more aneurysms ≥7 SD, including multiple and complex giant aneurysms without any obstruction): antiplatelet treatment (ASA + possible association with clopidogrel in selected patients) and any anticoagulant (warfarin in the giant aneurysms or LMWH in selected cases) is recommended.
- Class V (Coronary artery obstruction at the angiography), antiplatelet treatment (ASA + possible association with clopidogrel in selected patients) and anticoagulation with warfarin (or LMWH in selected cases) is recommended (34, 35).

It is essential to suspect a KD (typical or atypical) in a child with prolonged fever, especially in infants under the age of 6 months who have increased risk of developing cardiac complications. In fact, the treatment with immunoglobulins administered between 6-10 days of illness reduces the risk of cardiac complications which can also be fatal. Although complete knowledge of KD is limited due to the lack of identification of a responsible etiological agent, many authors in the past decades have helped to improve the management of complicated KD cases and to identify new therapeutic strategies for high risk patients. It should be emphasized also the importance of adequate long-term follow-up to address possible cardiac complications that can occur even years after the onset of the disease.

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Genetic cardiac channelopathies and SIDS

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Sudden infant death syndrome (SIDS) is the leading cause of postneonatal (28 days to 1 year of age) mortality in developed countries, characterized by the unexplained death of seemingly healthy babies, without prior warning, also known as "crib death", because the infant often dies in its crib. A few deaths that are diagnosed as SIDS are found, with further specialized investigations, to be attributable to metabolic disorders or arrhythmia-associated cardiac channelopathies. This special issue aims to provide an overview of genetic cardiac diseases proposed as the substrate for an infant's critical vulnerability in a small subset of SIDS cases. A comprehensive search of published literature using the PubMed (http://www.ncbi.nlm.nih.gov/pubmed/) database was carried out to identify all articles published in English from 2000 to January 2019, using the following key terms: "SIDS", "cardiac abnormalities", "inherited arrhythmia syndromes" and "cardiomyopathies".

According to the widely accepted "San Diego definition of SIDS", Sudden infant death syndrome (SIDS) is defined as "The sudden unexpected death of an infant <1 year of age, with onset of the fatal episode apparently occurring during sleep, that remains unexplained after a thorough investigation, including performance of a complete autopsy and review of the circumstances of death and the clinical history". If these criteria are not fulfilled the cases can be termed "unclassified infant death" (USID) or as sudden unexpected infant death (SUID). In the USA, SUID has an incidence of 87.0/100.000 live births and 45.6% are due to SIDS (1).

Around 90% of SIDS cases occur in the first 6 months of life, with a peak between 1 and 4 months of age and it is uncommon after 8 months of age (2). Risk reduction campaigns have decreased the incidence by 50–90% in the 1980s and 1990s (3, 4); in the 1994 the 'back to sleep' campaign advocated a supine sleep position for infants, after discovery that the prone sleep position tripled the risk of SIDS5. Although SIDS rates have declined by >50% since the early 1990s, SIDS remains the leading cause of postneonatal (28 days to 1 year of age) mortality. SIDS risk-reduction recommendations from the American Academy of Pediatrics include supine sleep position, use of a firm sleep surface, avoidance of soft objects

Key words: sudden infant death syndrome, SIDS, cardiac channelopathies, crib death, inherited arrhythmia syndromes

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in the crib, smoke cessation during pregnancy, room sharing without bed sharing, use of a pacifier at nap time and bedtime, and avoidance of overheating. Despite these efforts, in the United States, over 2500 infants die suddenly and unexpectedly each year with nearly 100 deaths annually in Australia (6).

Pathogenesis of SIDS

There have been multiple theories over the years regarding the etiology and mechanisms of SIDS. In recent years, there has been growing consensus among scientists that SIDS is multifactorial in origin. The Triple Risk Hypothesis (Fig. 1) proposes that when a vulnerable infant, such as one born preterm or one exposed to maternal smoking, is at a critical but unstable developmental period in homeostatic control and is exposed to an exogenous stressor, such as being placed prone to sleep, then SIDS may occur.

Exogenous stressors that place an infant at risk of asphyxia include prone and side sleep position, soft bedding, head covering, overheating, bed sharing, perinatal smoke exposure, ethanol intake, drug use and/or socioeconomic disadvantages, mild infections, and prematurity (1, 5-7). Underlying vulnerability includes genetic susceptibility to SIDS and nongenetic factors, such as antenatal factors (placental abnormalities, poor fetal growth, preterm labour, *in utero* exposure to cigarette smoke and alcohol) and intrinsic factors (ethnicity and demographic factors). The injury mediated by oxidative stress (OS) is one of the major pathogenic protagonists in the onset of several conditions relating to new-borns, (8). Increased OS levels and reduced antioxidative capacities may contribute to the pathogenesis of perinatal and postnatal disorders (9).

It is known that many inflammatory mediators, among which High Mobility Group Box-1 (HMGB1), are involved from the beginning of pregnancy to birth of the infant (10). HMGB1 is one of the most important DAMP molecules, initiating and perpetuating immune responses both in non-infectious and/or infectious inflammatory processes (11, 12). It is a sensitive biomarker in children and it also seems to have an inverse correlation with lung function (13). Over the years, research on HMGB1 has been quite thorough and other properties have been revealed. HMGB1 has resulted to be a leading mediator in both acute and chronic inflammation, and plays a crucial role in several medical conditions, including autoimmune diseases, cancer, hepatitis, malaria, myocardial ischemia, infection-elicited inflammatory diseases even pregnancy (14, 15), and it may be linked to infant vulnerability.

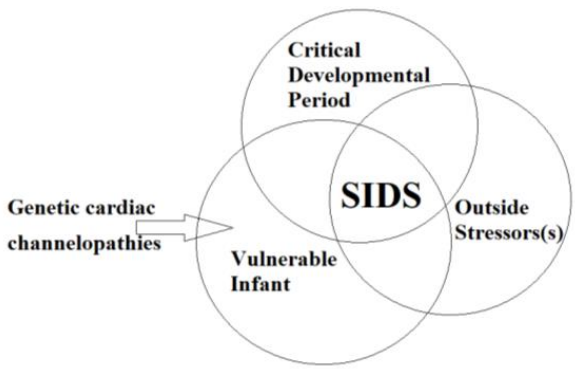

Fig. 1: *Triple risk hypothesis*.

Genetic cardiac channelopathies

Cardiac channelopathies have been found to be implicated as a causative factor of sudden infant death syndrome (SIDS) by numerous studies. The main inherited arrhythmia syndromes relevant to SIDS and sudden cardiac death (SCD) in the young include Long QT Syndrome (LQTS), Brugada syndrome, catecholaminergic polymorphic ventricular tachycardia (CPVT), and short QT syndrome (SQTS). A range of mutations in other minor genes encoding other cardiac ion channels and channel-interacting proteins has been associated with SIDS. SCD must be well investigated because it may occur from different causes, also like Nemaline myopathy (16).

LQTS

Long QT syndrome (LQTS) is a group of inheritable primary electric diseases of the heart. It is characterized by the presence of QTc interval prolongation in repeated ECGs (after exclusion of secondary causes) and an increased risk of *torsades de pointes* leading to syncope and sudden cardiac death. QTc interval prolongation results from ion channel

dysfunction that prolongs cellular repolarization (17). There are currently 15 genes known to cause congenital LQTS: however, the main 3 genotypes account for >90% of genetically confirmed LQTS.

LQT1 is caused by loss-of-function mutations affecting KCNQ1, the gene encoding for IKs (slow) channel. LQT2 is caused by loss-of-function mutations in KCNH2, the gene encoding for the IKr (rapid) channel. Gain-of-function mutations in SCN5A, the gene encoding for INa, causes failed inactivation and increased late current leading to LQT3. Although most LQTS is inherited in an autosomal dominant way, the rare recessive form LQT1 (Jervell and Lange-Nielsen syndrome) leads to a severe LQTS phenotype and associated sensorineural deafness (17) Tester and Ackerman estimated that approximately 10% of all SIDS cases were actually caused by cardiac channelopathies resulting in LQTS (18).

Brugada syndrome

(BrS) is an arrhythmogenic disorder. This disease is a channelopathy (Sodium channel proteins) characterized by ST-segment elevations in the right precordial leads (V1 - V3) and is associated with an increased risk for sudden death in the absence of overt structural heart disease. The prevalence of BrS has been reported in the ranges of 5-20 per 10 000 people (19). Diagnostic criteria, from a recent consensus report, require the patient to have a spontaneous type 1 Brugada pattern ECG characterized by a coved ST segment elevation ≥2 mm, in the right precordial lead V1 or V2 in either second, third, or fourth intercostal space. Three ECG patterns have been described in BrS but only type 1 is diagnostic of BrS.

The electrocardiographic aspect only, is not associated with further conditions, (such as personal history of recurrent syncopes, family history of sudden death at age ≤45 years, documented episodes of ventricular tachycardia), it is referred as "Brugada pattern". BrS is genetically determined; it has been associated with loss-of-function mutations in one major ion-channel gene, *SCN5A*, in approximately 20% of index cases. In BrS patients, sudden death often occurs at rest or while sleeping especially in children, and although risk of ventricular arrhythmias is generally low in children, these circumstances might be especially pertinent to SIDS.

CPVT

Characterized by exercise or stress-induced bidirectional and polymorphic ventricular tachycardia. The prevalence in Europe is 1/10.000 live births. The pathophysiology of CPVT is determined by abnormal intracellular calcium handling proteins leading to triggered activity. The most responsible genes are RYR 2 and CASQ2, whose mutations cause anomalies in the functioning of the calcium receptors within the cardiac myocytes. CPVT predominantly affects children and is associated with a mortality of 50% in untreated individuals aged <20 years, supporting a role of CPVT in SIDS.

SQTS

An extremely rare condition of unknown prevalence. The diagnosis requires the presence of a very short QTc ≤330 ms or a QTc ≤360 ms in association with one of the following: family history of SQTS, pathogenic mutation, family history of sudden death at age ≤40 years, or survival of a VT/VF episode in the absence of heart disease. SQTS is associated with gain-of-function mutations in K+ channel genes, including *KCNQ1* and *KCNH2* leading to syncope or SCD.

Variants in genes encoding Nav (*Cardiac sodium current)* proteins have been identified in SIDS case-reports and cohort studies. Ackerman *et al.* were first to undertake a systematic molecular autopsy of *SCN5A* in a cohort of 93 SIDS cases. Gain-of-function missense variants were identified in two SIDS cases. Another crucial study including 201 SIDS cases from Norway identified nine distinct rare *SCN5A* variants in 13 cases (6.5%). Most of these variants were associated with an increased persistent inward Na+ current (*I*Na), either under baseline conditions, when expressed under specific conditions. These findings were consistent associated with the LQT3 phenotype and suggested an important role of *SCN5A* variants in a small subset of SIDS cases. In adults, cardiac events are less frequent in LQT3 patients than in LQT1 – LQT2, but more likely to be lethal and related to sleep, likewise to SIDS.

Several variants in genes encoding major K+-

channel proteins have been associated with SIDS (*Cardiac potassium currents*). Ackerman et al. were the first to screen for mutations in the *KCNQ1* and *KCNH2* genes in their series of 93 population-based, unrelated SIDS cases. In the aforementioned Norwegian cohort of 201 SIDS cases, screening for mutations in the K+-channel genes *KCNQ1*, *KCNH2*, *KCNE1*, *KCNE2*, and *KCNJ2* identified rare variants in nine cases, although only five were considered as likely contributors to SIDS on the basis of available functional data.

Cardiac channelopathies have been found to be implicated as a causative factor of sudden infant death syndrome (SIDS) by numerous studies. However, no solid criteria based on genetic testing have been established to recognize high-risk infants. Family genetic screening is recommended for all first-degree relatives of a member affected by LQTS, BrS and CPVT but no universal neonatal screening is discussed or mentioned. Other factors might be more important in causing infant vulnerability. In addition, the genetic risk in SIDS might be polygenic and, therefore, harder to identify. More work is needed to improve our understanding of the genomic background of vulnerable infants.

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Group A beta-hemolytic Streptococcus and heart in children

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Streptococcus pyogenes, also known as group A beta-hemolytic Streptococcus (GAS), is a human-specific pathogen that commonly colonizes the human respiratory tract. Although St. pyogenes can cause a wide range of diseases, both in pediatric and the adult population: from the most common pharyngitis or skin infections (e.g. erysipelas, cellulitis) to necrotizing fasciitis or toxic shock syndrome (1). Besides GAS can trigger some autoimmune diseases such as acute rheumatic fever (ARF) and rheumatic heart disease (RHD) (2). ARF and RHD are closely related, since multiple and recurrent episodes of ARF over years, are responsible for an accumulation of heart valve damage, eventually hesitating in RHD. This article aims to perform an updated overview of the literature about the immunological strategies of GAS in causing heart damage and the novelties in relation to it.

Genetic Factors

Over the years, many genes that predispose to RHD have been studied. Human leukocyte antigen (HLA class II) was found to be most closely associated with RHD. Specifically, HLA-DR2, DR3, DR4, DR6, DR9, DR11, DR53 and mainly HLA-DR 7 have been associated with RHD. Other studies have also reported an association with alleles DQB1 * 08, DQB1 * 16, DQB1 * 03 and DRB1 * 04. In the past, many other non-HLA genes responsible for the pathogenesis of HD were studied, for example mannose/mannane-bound (MBL2), ficolin-2 (FCN2), serine protease-associated serine Mannose (MASP2), tumor necrosis factor alpha (TNF-α), tumor growth factor beta (TGF-β), toll-like receptor 2 (TLR2), interleukin-1 (IL-1R) and cytotoxic T lymphocyte-associated protein-4 (CTLA4).

Virus "Weapons" and host response

Three surface St. Pyogenes proteins (named M, T, R) are virulence factors. M protein is the most important one, existing in different serotypes (3, 5, 6, 14, 18, 19, 24, 27, 29) and configuring different rheumatogenic behavior (3). M protein owns a structure similar to human proteins like myosine, laminine, vimentine which explains the mimicry process that leads to antibodies production against self-proteins (localized into heart, brain, joint, skin). At first, anti-endothelial cell antibodies (AECA) against self-proteins are produced. These types of antibodies are implicated in several autoimmune rheumatic diseases such as systemic lupus erythematosus, vasculitis and scleroderma. AECA has been shown to play pathogenic roles in multiple ways: by producing

Key words: Streptococcus, group A beta-hemolytic Streptococcus, GAS, autoimmune diseases, proinflammatory cytokines

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proinflammatory and procoagulant effects (increased expression of adhesion molecules and tissue factors, increased cytokine release) in endothelial cells, or, relating to ARF, binding valvular vimentin and increasing VCAM-1 production and complement activation with a consequent cellular damage (4).
Consequently, other self-antigens are exposed and presented by antigen-presenting-cells (APC) to MHC class II molecule on T-helper (Th) cells' surface, with a subsequent activation of cellular immunity. Activated T lymphocytes contribute to produce a consistent amount of proinflammatory cytokines with pleiotropic effects: amplification of immune response by recruiting other immune cells, creation of "fertile ground" for autoimmunity, infiltration of cardiac layers and valvular damage, valvular fibrosis (5). Multiple Th profiles activated during the progression of the disease (Th1 and Th2 mostly) and in the last several years are the studies in which these profiles have been analyzed.

As the pathology progresses over the years, immunological response seems to evolve, going from a Th1 activity toward a Th2 response (3, 6). Th1 cells increase into the heart valves during the acute phase, determining secretion of cytokines like IL1, TNF-a, IL2, IL 6, IL-8. Both IL-1 and TNF- lead to maintenance of a pro-inflammatory environment during the acute phase of the disease (recruiting other cytokines) and on the other hand seem to be directly proportional to severity of valvular damage in the chronic phase of RHD (6, 7). IL-2 seems to be able to increase the CD4:CD8 cells ratio, since IL-2 has been demonstrated to be elevated in ARF with a consequent overexpression of CD4+ cells in the valvular tissue in the acute phase. On the contrary, IL-2 is suppressed in the chronic phase of RHD, explaining the progressive lowering of CD4+ cells and on the increase of CD8+ ones (3).

Other important Th-1 cytokines are IL-6 and IL-8, involved in the strengthening of humoral activity, contributing to B cells maturation and consequently to the production of autoantibodies. As previously said, approaching the chronic phase of cardiac damage, there is a change in the immunological pattern with a prevalence of Th-2 cells, demonstrated by higher concentration of cytokines like IL-4, IL-5, IL-10 (7, 8).

According to recent studies, IL-10 has shown to play an important role in the pathogenesis of RHD (9). IL-10 is an important immunoregulatory cytokine with anti-inflammatory action. IL-10 inhibits the production of pro-inflammatory Th-1 cytokines (IL-12 and IFN-γ), regulates immune cells (T cells, B cells, mast cells and antigen presenting cells) and tolerance towards autoantigens. In addition, IL-10 plays an important role in regulating antigen presentation and acts as an immunosuppressive cytokine by inhibiting the expression of MHC II and thus altering antigen-presenting cells. Finally, highly expressed IL-10 inhibits the activity of the CD28 co-receptor present on T cells and acts as an immunosuppressive agent. These immunomodulatory functions of IL-10 explain its role in the persistence of bacterial infections and in the pathogenesis of various autoimmune and allergic diseases 21 (10).

IL-10 appears to be a powerful stimulator of antibody production in many autoimmune diseases, such as systemic lupus erythematosus (SLE), rheumatoid arthritis, diabetes (11) and RHD.

In a study conducted by N. Sharma et al. an important increase of IL-10 levels has been demonstrated in the RHD patient group compared to the control group. As previously explained, it has been seen that both in serum and in cardiac cells a switch from a Th1 type response to a Th2 type one occurs, with consequent disease progression. Indeed, IL-10 inhibits IL-12 and IFN-γ promoting the development of Th-2 type cytokines, consequently, corresponds with an increase in the number of CD8 + T cells. IL-10 also promotes chemotaxis and cytotoxic action of CD8 + T lymphocytes, whose action strongly contribute to valves damage (9). Similarly, two studies have demonstrated the presence of CD4 + and CD8 + cells infiltrating the heart valve tissue of subjects with RHD.

Th-17 lineage has also been studied. IL-17 is up-regulated in autoimmune and allergic diseases 21 (12), and increased levels are related to chronic inflammation (13). Similarly, IL-10 is also observed to be increased in autoimmune diseases and up regulation of IL-10 leads to immunosuppression (14). The balance of these two inflammatory cytokines is crucial for maintaining tolerance.

However, the correlation between IL-17 and IL-10 is still controversial. For example, one study reported IL-10 as an IL-17 suppressant (15) while both IL-10 and IL-17 are up-regulated in patients with RHD. Furthermore, according to a study conducted by Dileepan, the high immune response of TH-17 seems to be promoted by IL-6 and this could explain the supporting role of IL-6 in the IL-17 mediated immune response in RHD (16).

In fact, during the initial phases of the GAS infection there are increased IL-6 and IL-8 levels, while in the chronic phase the ones to be increased are IL-17 and IL-10. Finally, another cytokine involved in the promotion of heart damage is IL-23, important for the development and proliferation of Th-17 cells. The impact of IL-10 on IL-23 activity is not very clear, while the results of a research suggest that IL-10 regulates IL-23 production (17). There is also evidence in the scientific literature that the IL-23 improves IL-10 production and induces IL-17 synthesis (18, 19).

Despite the disease being virtually eradicated from the high-income countries, RHD remains an endemic public health problem in many low- and middle-income countries, among which population it is still hyper-endemic (20). Antibiotic prophylaxis still represents the main therapy in preventing the disease from arising. A better understanding of the genetic predisposing factors would further allow to identify the most susceptible people, applying preventive measures and trying to inhibit the carrier state of S. pyogenes. Above all, further studies on the close relationship between host immune response and GAS, as well as the precise role of specific cytokines and their polymorphism, would lead to the identification of new target therapies.

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The heart in Anderson-Fabry disease

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Anderson-Fabry disease (AFD) is an X-linked lysosomal storage disorder caused by deficiency of the lysosomal enzyme α-galactosidase A, due to mutations in the GLA gene, leading to progressive accumulation of glycosphingolipids, predominantly globotriaosylceramide (Gb3), in lysosomes of various cell types throughout the body. This causes a multisystemic clinical presentation, with frequent childhood onset including neurological, ocular, skin, gastrointestinal, renal, and cardiac manifestations. Cardiac involvement, including hypertrophic cardiomyopathy, valvular abnormalities, arrhythmias and chronic heart failure is frequent in AFD and is one of the most important causes of a reduced life expectancy and disease-related mortality. Early signs of cardiac abnormalities such as left ventricular hypertophy, reduced heart rate variability and arrhytmias, can be found also in pediatric patients with AFD. Laboratory and instrumental tools are of fundamental importance for the identification of cardiac damage, as well as for monitoring the response to Enzyme Replacement Therapy (ERT). ERT has been proven to reduce left ventricular mass, improving regional myocardial function and exercise capacity. AFD patients with proven myocardial fibrosis may have only limited or no benefit from ERT and this suggests that an early start of ERT is always desirable. Another disease specific treatment, oral chaperone therapy showed encouraging results in terms of reducing left ventricular mass. Despite the availability of these specific treatments, several concomitant therapies still play an important role in the management of cardiological manifestations of AFD.

Anderson-Fabry disease (AFD) is an X-linked lysosomal storage disorder caused by deficiency of the lysosomal enzyme α-galactosidase A due to mutations in the GLA gene (1). The worldwideincidence of AFD is reported to be in the range of 1 in 40,000-117,000, although this value may be underestimated according to the highest incidence rates reported in risk populations and provided by screening studies on populations of newborns (2). α-galactosidase A deficiency leads to progressive accumulation of glycosphingolipids, predominantly globotriaosylceramide (Gb3), in lysosomes of various cell types throughout the body causing

Key words: Anderson-Fabry disease (AFD), GLA gene, α-galactosidase A, Enzyme Replacement Therapy (ERT), Left Ventricular Hypertophy (LVH), Cardiomyopathy, Arrhythmias, valve disease

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a multisystemic clinical presentation including neurological (3, 4), ocular, skin, gastrointestinal (5, 6), renal, and cardiac manifestations.

Clinical manifestations of AFD first appear in childhood or adolescence (7) and include acroparesthesias, gastrointestinal symptoms, hypohidrosis, and mild proteinuria, although other signs of renal involvement cannot be excluded even in children (8). In adulthood progressive renal, cardiac, and cerebral involvement (stroke) characterize clinical picture and could be the major causes of death related to AFD, especially in hemizygotic males with a classical form but also in many heterozygous females which can present different degrees of disease severity as well as males. An extreme intra and inter-familial variability of clinical presentation has been described although to date the causes that underlie this extreme variability are still not clear (9, 10).

Beside the classical form of AFD, a late-onset or "atypical variant" has also been described These variants are caused by specific mutations in the GLA gene associated with residual α-galactosidase activity. Usually in patients with this form of AFD clinical manifestation starts later in life, often with prevalent cardiac or renal involvement (2, 11).

The comprehension of the pathophysiological mechanisms that underlie AFD as well as that of other LSDs (12), allowed the realization of a specific cause-therapy for this disease, Enzyme Replacement Therapy (ERT), which became available in 2001, in two different enzyme preparations: agalsidase alfa and agalsidase beta. Clinical studies and emerging evidence suggest that ERT may slow disease progression and improve many of the features of AFD (13, 14), although response to therapy appears to depend heavily on the stage of the disease (14) and on other variables not yet fully understood (15).

Cardiac manifestations are frequent in AFD and are one of the most important causes for reduced life expectancy and disease-related mortality. More than 60% of AFD patients present

Table I. *Signs and symptoms of AFD stratified by age.*

Sign/ Symptom	Childhood	Adolescence	Adulthood
Acroparesthesias	+	+	+
Gastrointestinal symptoms (diarrhea, abdominal pain, vomiting, nausea, postprandial vomiting)	+	+	+
Cornea verticillata	+	+	+
Angiokeratoma	+/-	+	+
Hypo/anhidrosis	+/-	+	+
proteinuria	-	+/-	+
End-stage renal disease	-	-	-
Levt ventriculat Hypertrophy	+/-	+/-	+
Cardiomyopathy	-	-	+
Arrhythmias and conduction abnormalities	+/-	+/-	+
Heart valve disease	-	+/-	+
Strokes	-	-	+

cardiovascular symptoms such as dyspnoea, angina, palpitations, syncope and peripheral oedema (16). Cardiac manifestations including hypertrophic cardiomyopathy, valvular abnormalities, arrhythmias and chronic heart failure occur frequently in patients with AFD.

Early signs of cardiac abnormalities can be found also in pediatric patients with AFD. Left ventricular hypertophy (LVH) with its slight progression over time (17), reduced Heart rate variability and arrhytmias like sinus bradycardia or ectopic atrial rythms (18) have been described in children and adolescents with AFD. Table I shows signs and symptoms of AFD stratified by age.

Pathophysiology of cardiac involvement in AFD

Cardiac dysfunction in AFD is due to Gb3 storage in cardiomyocytes, conduction system cells, valvular fibroblasts, endothelial cells and vascular smooth muscle cells. However, since it has been demonstrated that glycosphingolipid deposition accounts for less than 3% of the total myocardial mass, Fabry cardiomyopathy is mainly caused by activation of other signalling pathways leading to myocyte hypertrophy, apoptosis, necrosis and fibrosis. It has been shown that the accumulation of Gb3 increases oxidative stress, up-regulates adherence molecules' expression in vascular endothelial cells and may lead topro-inflammatory cytokines releasing (19), so that it can be hypothesized that Gb3 storage is the first step of a cascade of events leading to cellular and tissue damage. Oxidative stress stimulates inflammatory responses that can also lead to allergic disorders such as atopic dermatitis, allergic rhinitis, and asthma (20, 21).

Furthermore, a deacylated metabolite of Gb3, lyso-Gb3, seems to stimulate vascular smooth muscle cells proliferation and myocardial ischaemia due to increased oxygen demand of the hypertrophied muscle, decreased capillary density, increased diastolic filling pressures and Gb3 deposition in endothelial and smooth muscular cells of small arterioles and capillaries, may contribute to heart damage progression. All these mechanisms, together with other factors not yet fully known, contribute to the establishment of an initial interstitial fibrosis and a subsequent cell replacement fibrosis which, in addition to worsening the cardiac function, implies obvious limitations in the effectiveness of the ERT on the myocardial damage of the AFD.

Cardiomyopathy

AFD-associated hypertrophy of significant degree, seldom develops in young patients, although left ventricular hypertophy and mildly increased LV mass have been described from many authors in pediatric patients with AFD. In agreement with previous data, more recently Havranek et al. (17) in a retrospective observational study on 22 paediatric patients with AFD, described mildly increased LV mass, more frequent in male patients, with three boys fulfilling the definition of LVH during the follow-up, thus demonstrating the progressive nature of cardiac damage in AFD. LVH was accompanied with ECG and Holter ECG abnormalities in all three cases.

Most adult patients with AFD of both sexes develop left ventricular hypertrophy (LVH) shown by echocardiography. 40% of AFD patients present LVH at diagnosis with this rate increasing to 77% in patients over the age of 75. The LVH pattern is diffuse concentric in most patients, although asymmetric septal hypertrophy has also been reported. Ventricular hypertrophy increases with disease progression. Typical findings in AFD cardiomyopathy is rare, althouh possible, dynamic left ventricular outflow obstruction (21), prominent papillary muscles and a preserved global ejection fraction combined with early stages of diastolic dysfunction (22).

Although conventional studies of left ventricular systolic performance are usually within the normal range, tissue Doppler imaging and strain-rate imaging have showed reductions in systolic function, occurring earlier in the longitudinal than in the radial contraction. This happens because progression of the hypertrophy causes a decrease in ventricular wall motion, particularly at the base of theposterior wall of the left ventricle. In more advanced stages of the disease, also radial function is reduced (22). Right ventricular hypertrophy (RVH) has also been described in 25% of AFD patients with a similar prevalence in both sexes (23). Intramural replacement fibrosis characterizes end-stage Fabry

cardiomyopathy leading to wall motion abnormalities with lower regional systolic deformation values. ECG abnormalities indicative of LVH comprise large voltage QRS complexes, and repolarisation abnormalitieswhich are present in 61% of men and 18% of women over the age of 30 (21).

The role of Magnetic Resonance Imaging (MRI) in Anderson-Fabry cardiomyopathy

The usefulness of Magnetic Resonance Imaging (MRI) in the study of Fabry cardiomyopathy has been widely demonstrated. MRI is helpful in identifying areas of hypertrophy, not visualized by echocardiography, providing a differential diagnosis of hypertrophic cardiomyopathy from other causes of LVH. Fabry's cardiomyopathy is characterized by regional or global myocardial hypertrophy seen on MRI. Glycosphingolipid intra-myocardialstorage can reduce the non-contrast T1 mapping values on MRI. A low T1 occurs in Anderson-Fabrycardiomyopathy associated with an increase of the extracellular volume (ECV), but not in amiloid infiltrative cardiomyopathy. A distinguishing feature of cardiac hypertrophy due to AFD is late enhancement of gadolinium (LGE). In patients with AFD, LGE may be detected particularly affecting the basal inferolateral wall in the absence of coronary artery disease. However, LGE is only present in severe stages of AFD cardiomyopathy, reflecting the extensive fibrosis consequent to Gb3 storage (24).

The assessment of myocardial fibrosis is important to stage AFD cardiomyopathy and is necessary for monitoring ERT efficacy. Consequently, every adult Fabry patient should receive a cardiac MRI scan once a year if possible.

The role of biomarkers

With the aim of identifying a diagnostic tool or screening parameter for high-risk patients, as well as a tool for evaluating the response to ERT, several biomarkers and their correlation with cardiac involvement have been studied, as it was done for the evaluation of other organs (25, 26).

The most studied biomarker in AFD is lyso-Gb3, a degradation product of the stored Gb3. Plasma lyso-Gb3 is dramatically increased in male with classic AFD but is also increased in females and may be reduced, but not normalized following ERT, while it is undetectable in normal human plasma. Another biomarker, a plasmaproliferative factor, sphingosine-1 phosphate (S1P), has been studied by Brakch et al, who demonstrated that plasma levels of S1P correlated with left ventricular mass index and common carotid arteryintima-media thickness in patients with AFD. Moreover recently, high-sensitivity troponin (hs-TnT) has been identified as a useful biomarker for progression in AFD cardiomyopathy.

In different diseases, previous studies have reported on the critical role of high mobility group box-1 (HMGB1), an alarmin promoting an immediate immune response to tissue damage (27-30). The recent discovery of HMGB1 as a critical mediator of inflammation, stimulated an increasing interest in the field of inflammation research, suggesting therapies for viral/bacterial infection or for other inflammatory disease (31, 32), The HMGB1 effects, associated with several critical pro-inflammatory mediators, have stimulated an increasing interest in the field of several inflammation-associated disorders including allergic (33), haematological (34), infective, endocrine, and gastrointestinal (35) diseases.

Arrhythmias and conduction abnormalities

Rhythm and conduction abnormalities are common in AFD due to Gb3 deposition and fibrosis as well as atrial dilatation and relative ischaemia secondary to LVH. Different types of arrhythmias have been described in pediatric patients with AFD. Hopkins et al. described sinus bradycardia or ectopic atrial rhythms in 9 children with AFD. Similar findings have been described more recently by Wilson et al. (18) who evaluated electrocardiographic and clinical findings in 26 pediatric patients with AFD. They detected that sinus bradycardia was the most frequent rhythm abnormality (23%), followed by ectopic atrial rhythm (12%) and premature atrial contractions (8%), confirming what was observed in the Fabry Registry in which sinus bradycardia is the most common pediatric arrhythmia described (18). An important observation in pediatric patients with AFD is reduce Heart rate variability

described from many authors, due to involvement of parasymphathetic and/or symphathetic nerves innervating the heart with consequent impaired autonomic control of heart rate, which is a risk factor for cardiac sudden death in adult age.

ECG abnormalities are frequent in adult patients with AFD. Apart from voltage criteria for LVH various degrees of block as well as short PR interval have also been reportedwithout the presence of a concomitant delta wave suggesting accelerated AV nodal connection in up of 40% of affected males, although other athors reported lower frequency of short PR interval in their cohort of patients. AFD is associated with atrial and ventricular arrhythmias (36). Registry data have suggested that the rate of atrial arrhythmia could be as high as 13%. However, the incidence of ventricular arrhythmia varies widely (5% to 30%).

Resting bradycardia and an impaired heart rate response on exercise are very frequent (37). Tachyarrhythmias including atrial fibrillation (AF), non-sustained ventricular tachycardia (NSVT) or sustained malignant ventricular arrhythmia such as ventricular tachycardia (VT) or ventricular fibrillation (VF) have also been described in patients with AFD. Malignant bradyarrhythmias or tachyarrhythmia can be the causes of sudden cardiac death in patients with AFD (36). Advanced cardiac disease can be associated with an increase in arrhythmia, needing intervention. This includes bradyarrhythmia which requires a permanent pacemaker (PPM), ventricular arrhythmia requiring an implantable cardiac defibrillator (ICD) and atrial fibrillation (AF) requiring anticoagulation. Frequency of arrhythmias and their correlation with morbidity and early death, make very important to screen regularly AFD for rhythm and conduction abnormalities by periodicals 24-h Holter-ECG.

Valvular heart disease

Gb3 storage occurs on cardiac valvular tissues although clinically relevant disease is mostly limited to the aortic and mitral valves, mainly in adult patients, possibly due to higher haemodynamic stress. In early report valves abnormalities have been descrbed in a large proportion of patients, but in general, hemodynamic significant valve abnormalities, are uncommon findings with poor clinical impact. There is no association between the presence of valvular abnormalities and α-galactosidase levels. Although the presence of valvular disease is frequent the incidence of Fabry's patients undergoing valve replacement surgery is low.

Treatment of cardiac disease in AFD

ERT, available since 2001, was the first disease-specific treatment for AFD patients. Several clinical trials and observational studies have been conducted to assess ERT efficacy on different AFD manifestations, including cardiac involvement. In paediatric patients with AFD ERT it has been shown to reduce LV mass and in potentially preventing its increase, although Haranek et al. have described the development of left ventricular hypertrophy during treatment (17).

Many studies documented no overall change from normal baseline values in ECG parameters during ERT in pediatric patients with AFD. Regarding HRV, one study described that at baseline HRV was lower in males than females and that after ERT it increased significantly in males and remained normal in females, another study showed that HRV improvements remained stable after ERT, another one reported no changes in HRV during ERT, which was normal in most cases at baseline.

Regarding adult patients ERT resulted in a significant reduction in left ventricular mass, with improvement in regional myocardial function and in exercise capacity, although some studies didn't show significant changes in left ventricular mass and/or ventricular wall thickness after ERT (38, 39) and this poor response to ERT is highly related to the extent of myocardial fibrosis assessed by the magnetic resonance imaging late-enhancement technique. AFD patients with proven myocardial fibrosis may have only limited or no benefit from ERT and this suggests that an early start of ERT is always desirable.

Recently, another disease specific treatment, oral chaperone therapy became available for AFD. Migalastat is a small molecule able to stabilize mutant α-galactosidase. The use of this new therapy has given encouraging results in terms of reducing left

ventricular mass. Nonetheless, further future studies will be needed to determine the effectiveness of chaperonic therapy on cardiac involvement of AFD.

In association with ERT, AFD patients frequently need concomitant medications for the managment of cardiac involvement. Angiotensin-converting enzyme inhibitor (ACEi) or angiotensin receptor blockers are often used for the effects on cardiac hypertrophy and their antihypertensive effect. Moreover, arrhythmias and conduction abnormalities frequently require the use of b-adrenergic blockers, pacemaker implantation, implantation of a cardio-defibrillator (ICD).

AFD is a multisystemic lysosomal storage disease in which cardiological involvement is one of the most important cause of reduced life expectancy and disease-related mortality. Enzyme replacement therapy, available today, has proven capable of improving or stabilizing cardiac function only if irreversible damage has not yet occurred. This suggests the importance of a correct timing on the beginning of the ERT which presupposes a careful laboratory and instrumental monitoring of patients affected by AFD.

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Dilated cardiomyopathy in mucolipidosis type 2

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Mucolipidosis II and III are lysosomal storage diseases caused by pathogenetic mutations in *GNPTAB* and *GNPTG* genes which cause an impaired activity of the lysosomal hydrolase N-acetylglucosamine-1-phosphotransferase, a key enzyme in the synthesis of the mannose-6-phosphate targeting signals on lysosomal enzymes. Patients with MLII alpha/beta present coarse facial features, cessation of statural growth, important skeletal manifestations, impaired neuromotor development and cardiorespiratory involvement. All children appear to have cardiac involvement, but severe dilated cardiomyopathy is uncommon. In this report we describe the case of an 11-month-old girl who is affected by a MLII. Analysis of the *GNPTAB* gene identified at a heterozygous level the previously described gene variants c. 2693delA p(Lys898Serfs*13) and c. 2956C>T p(Arg986Cys). Her main clinical features were coarse face with gingival hypertrophy, dysostosis multiplex, recurrent respiratory infection and an early onset of dilated cardiomyopathy, an uncommon feature for MLII. To our knowledge, dilated cardiomyopathy has been previously described in literature in only two cases of MLII and in one patient affected by MLIII.

In 1970 Spranger and Wiedemann introduced the term "mucolipidosis" (ML), to describe different conditions with similar features of mucopolysaccharidoses and sphingolipidoses (1). Among these disorders, MLII (I-cell disease OMIM #252500) and MLIII (pseudo-Hurler polydystrophy OMIM #252600 and #252605) are two autosomal recessive diseases which are expressed by two extreme conditions within the same clinical spectrum.

The worldwide estimated incidence of MLII and MLIII varies between 2.5 and 10 cases per 1.000.000 live births (2). They are caused by an impaired activity

Key words: mucolipidosis, GNPTAB gene, GNPTG gene, dilated cardiomyopathy

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of the lysosomal hydrolase N-acetylglucosamine-1-phosphotransferase (GNPT), an hexameric transmembrane enzyme composed of two alpha, two beta and two gamma subunits. The subunits alpha and beta are synthesized as a common alpha/beta precursor and encoded by the *GNPTAB* gene, while the gamma subunit is encoded by the *GNPTG* gene (3). This enzyme mediates the first step of the synthesis of the mannose-6-phosphate, a recognition marker essential to direct newly produced hydrolases to lysosomes (4). In MLII and MLIII, the impaired activity of GNPT results in the massive release of these enzymes into the intercellular space and body fluids (4, 5). In 2007 Cathey et al. proposed a new classification of ML based on the affected gene: MLII alfa/beta or MLIII alfa/beta, in the case that the mutated gene is the *GNPTAB* (c. 12q23.3) which encodes the alfa and beta subunits; MLIII gamma in the case that the mutations are present in the *GNPTG* gene (c.16p13.3) which encodes the gamma subunit (4, 6). This new classification is important for clinicians and patient's prognosis because the mutations of the alfa/beta subunits are associated with an increased phenotype severity.

Patients with MLII alpha/beta at birth are small for gestational age and may present weak cry, generalized hypotonia, restricted joint movement, thick skin with craniofacial abnormalities (flat face, depressed nasal bridge and shallow orbits) (7, 8). In early infancy patients present coarse facial features with gingival hypertrophy, which gradually worsens and seems to be characteristic of MLII (9). Skeletal manifestations are important, including hip dislocation, joint contractures and thoracic deformity (kyphoscoliosis and lumbar gibbus) (8, 9). In infancy, skeletal radiographs reveal dysostosis multiplex. The clinical course is characterized by poor feeding and breathing difficulties due to the progressive mucosal thickening (4, 7). All children appear to have cardiac involvement, thickening and insufficiency of the mitral valve and the aortic valve are the most typical findings. Right side and general ventricular hypertrophy and pulmonary artery hypertension have been reported but severe cardiomyopathy is not common (7, 8). Patients present severe neuromotor retardation and moderate intellectual disability so that mucolipidosis falls into the group of metabolic diseases in which neurological involvement is expected (2, 7, 10). Death occurs in early childhood, due to cardio-pulmonary disease (11).

Diagnosis is based on the assay of lysosomal hydrolases activity which is 5 to 20-fold higher in plasma and in other body fluids; urinary excretion of oligosaccharides is excessive while urinary excretion of glycosamminoglycans is normal. Sequence analysis of the *GNPTAB* gene confirms the diagnosis (8).

At the moment there are no therapies that can change the progression of the disease, although several efforts have been made to understand the molecular mechanisms underlying this and many other lysosomal storage diseases in order to identify specific therapies for each of them (4, 8, 12). Only few reports with hematopoietic stem cell transplantation treatment have been reported so far in MLII patients. However, there is no evidence of improved clinical outcome and patient survival is poor (11). Thus, a multidisciplinary approach is necessary for the symptomatic and supportive management of patients with MLII. We report the case of an 11-month-girl affected by MLII, who presented a dilatative cardiomyopathy, an uncommon feature for Mucolipidosis.

Case report

The 11-month-old girl presented to our service for failure to thrive and dysmorphic features. She was the second child of unrelated parents, born at 39 weeks by caesarean section for oligohydramnios, birth weight was 2.640 kg (SDS -1.69). Familiar history was negative for genetic-metabolic diseases. She had surgery for inguinal hernia at the age of 4 months. She presented several dysmorphic features: coarse and flat face, depressed nasal bridge, gingival hypertrophy, thick skin around the bilateral epicanthal folds of the eyes, hoarse voice and noisy breath, deformed chest, lumbar gibbus and hip dysplasia. (Fig. 1. A, B and Fig. 2) At the age of 11 months her weight was under 5th percentile, length on 10th percentile. She did not start to crawl, she was a poor eater, her diet was mainly liquid, and she suffered from persistent nasal obstruction.

Laboratory investigations showed normal liver, renal and thyroid function. Skeletal radiographies revealed dysostosis multiplex with hypomineralization, periosteal cloaking, diaphyseal expansion of the long tubular bones and short anteroposterior diameter of the vertebral bodies with anterosuperior hypoplasia of the supper lumbar bodies. (Fig 3. A, B)

Abdominal ultrasound was negative for organomegaly. Ophthalmic evaluation showed exotropia and bilateral +4.50 with clear crystalline lens. Thorax radiology revealed severe cardiomegaly and cardiac ultrasound showed a dilated cardiomyopathy characterized by a dyskinetic septum, a normal septum thickness, an ejection fraction of 38%, a mild aortic insufficiency and a secondary mitral regurgitation; pulmonary pressions were normal. (Fig. 4 and Fig.5) She started a therapy with beta blockers.

A mucopolysaccharidosis was suspected but urinary excretion of glycosamminoglycans was normal. The assay of lysosomal hydrolases on serum/plasma detected a marked increase ranging from 10 to 40-fold of normal values (n.v.). In particular alpha-mannosidase was 3552 nM/ml/h (n.v. 14-80),

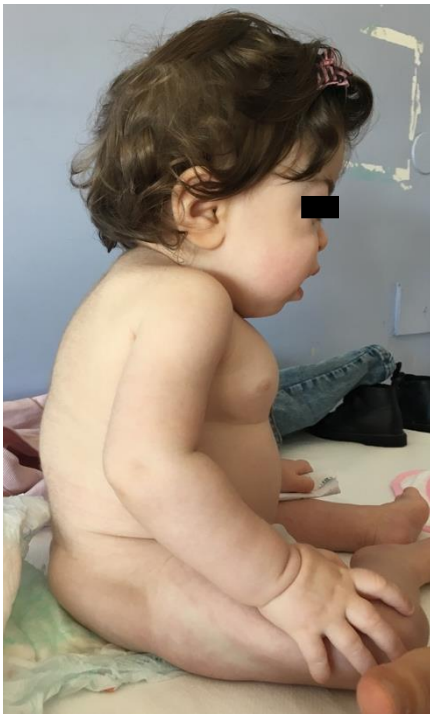


Fig. 1. *Phenotypic features of our patient at 13 months of age.*

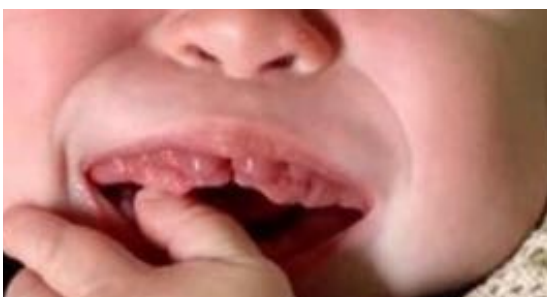
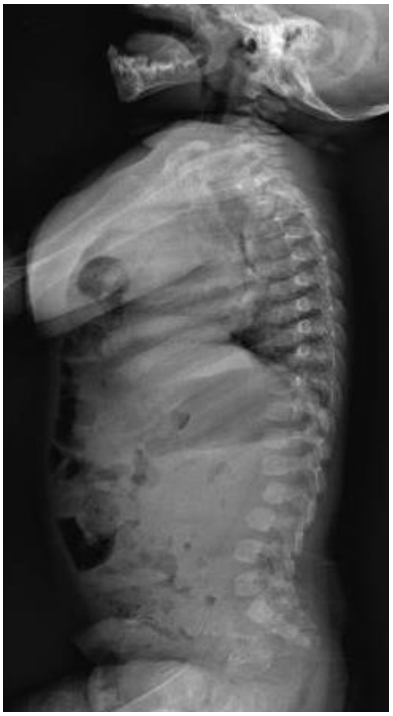


Fig. 2. *Gingival hypertrophy.*



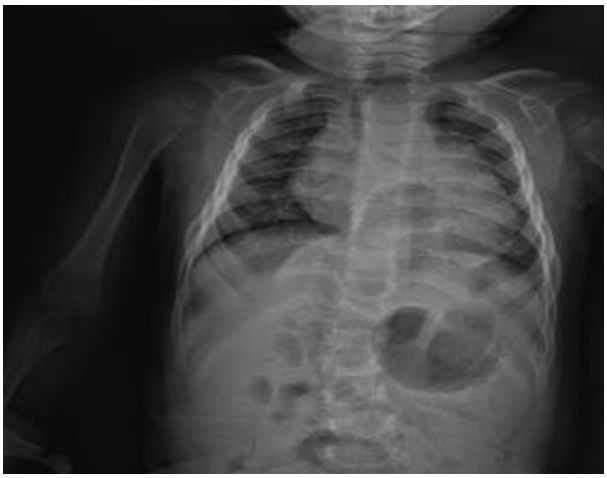


Fig. 3. *Skeletal radiographies revealing a dysostosis multiplex and large cardiac silhouette.*

Fig. 4. *Left ventricular dilatation.*

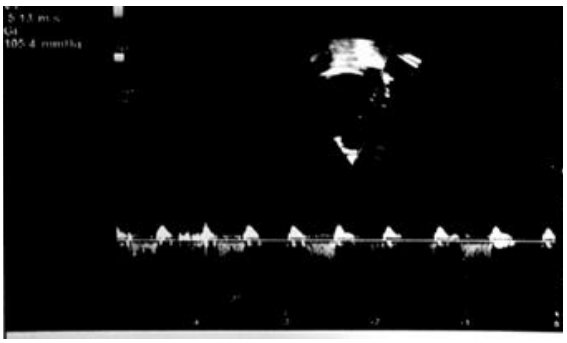


Fig. 5. *Mitral regurgitation due to dilated cardiomyopathy, jet velocity 5,13 m/sec.*

alpha-L-fucosidase was3979 nM/ml/h (n.v. 100-600), arylsulfatase A was 414 nM/ml/h (n.v. 10-32.9) and beta-D-glucuronidase was 4427 nM/ml/h (n.v. 40-250). Next generation sequencing analysis for lysosomal storage disorders, using a panel of 64 genes, led to the identification of the reported gene variants c. 2693delA p(Lys898Serfs*13) and c. 2956C>T p(Arg986Cys) in the *GNPTAB* gene at a heterozygous status, that confirmed the diagnosis of MLII alpha/beta.

After the diagnosis was confirmed, a multidisciplinary follow-up was carried out. At the age of 20 months cardiac ultrasound imaging confirmed a persistent dilated myocardiopathy, but with a kinetic improvement (ejection fraction of 40-45%) and an improvement of the aortic insufficiency was noticed. Beta blockers therapy was continued. The patient presented a global psychomotor delay, but she showed little improvement in every area of psychomotor development. Recurrent respiratory infections were still reported.

DISCUSSION

ML II alpha/beta is a very rare and often misdiagnosed disease. In a recent update on *GNPTAB* and *GNPTG* mutation Velho et al. underlined that establishing a genotype-phenotype correlation is complicated because of the rarity of the disease, the high proportion of individual mutations and the variable expressivity in patients carrying the same mutation (2). The severe phenotype is correlated with absent or strongly reduced GNPT activity, while the presence of a *GNPTAB* allele associated with residual enzymatic activity seems to be protective and results in a clinically less progressive disease (2). The authors report that 65% of all known variants of *GNPTAB* gene represent frameshift and nonsense mutations which lead to a premature stop codon (2).

Patients carrying in both *GNPTAB* alleles genetic variants leading to loss-of-function mutation are generally affected by the severe MLII disease, although up to now 25 out of 66 missense mutations have been identified in severely affected patients (2).

In our case, clinical features and laboratory data were suggestive of MLII and diagnosis was confirmed by the genetic analysis of *GNTAB* which revealed two heterozygous mutations.

Otomo et al. described for the first time the mutation c. 2693delA p (Lys898Serfs*13) which leads to a premature stop codon. Authors described this mutation in one patient who presented duplication of exon 2 in association. The age of the patient and the degree of the cognitive involvement were not specified; however, the authors reported that the patient was able to stand alone, walk without support and speak single words. Moreover, they described a heart murmur, inguinal hernia, hepato and splenomegaly but no sign of dilatative cardiomyopathy was detected in this patient. To make a genotype-phenotype correlation the authors considered that the frameshift mutations contribute to the severe phenotype but at the same time they hypothesized that the duplication of exon 2 leads to an attenuated phenotype (13).

The pathogenetic variant c.2956C>T p (Arg986Cys), leading to the replacement of an arginine by a cysteine at position 986 of the protein

(R986C) was described by Coutinho et al. (14). Their patient had a homozygous missense mutation and presented as a neonate with a coarse face and gum hypertrophy. In early infancy he presented severe developmental delay, a deformed chest, broad wrists, finger contractures, clear cornea, dysostosis multiplex and no hepato-splenomegaly (14). The same mutation was also reported in a Turkish 4-year-old boy by Cobos et al. but the clinical features were not described. The authors analyzed the activity of the enzymes alpha-iduronidase, iduranate-2-sulphatase, aryshulphatase B and beta-galactosidase and carried out the molecular genetic analysis in dried blood spots collected from patients who presented a "MPS-like phenotype".

Our patient presented almost the same clinical characteristics excepting the severe development delay and the dilated cardiomyopathy, that was not described in the Coutinho report.

However, since both the *GNTAB* genetic variants identified in our patient are pathogenetic, this may explain her phenotype. We consider our case particularly relevant because she presented an early onset of dilated cardiomyopathy, that is not a typical feature of ML.

Patients with ML often present a cardiovascular involvement. Alegra et al. described 27 Brazilians patients with ML. In their report, 8 patients presented at cardiac auscultation a systolic murmur who was related to mild valvular insufficiency, patent foramen ovale (1/8, a patient with MLII), mitral thickening (4/8, 3 with MLII), mild mitral regurgitation (7/8) and aortic valve stenosis (2/8, both with MLIII) and pulmonary valve insufficiency (8/8). Left ventricular hypertrophy was seen in two patients with MLII, one presented a concentric hypertrophy and the other one presented a discrete increase in the left ventricle (9).

Cathey et al. reported mitral and aortic valve thickening as the most common cardiac problem and they found that hypertrophy of ventricular walls was a consistent feature in the longer surviving probands (7). To our knowledge, dilated cardiomyopathy has been described in literature only in two cases of MLII (16, 17, 18) and in one patient affected by MLIII (19) (Table I).

Table. I. *Cases of dilated cardiomyopathy in MLII described in literature.*

Case report	**Mutation**	**Age**	**Clinical features**
Schulz et al. 1987	Not described	10 weeks	dilatative cardiomyopathy and endocardial fibroelastosis without signs of cardiac insufficiency
Muller et al. 2000	Not available	9 moths	coarse facies and skin, gum hyperplasia, rough voice, joint contractions, hip luxations, and dysostosis multiplex dilatative cardiomyopathy with atypical origin of coronary arteries
Our case	c.2693delA p(Lys898Serfs*13)/ c.2956C>T p(Arg986Cys)	11 months	coarse and flat face, depressed nasal bridge, gingival hypertrophy, thick skin around eyes bilateral epicanthal folds, hoarse voice and noisy breath, deformed chest, lumbar gibbus and hip dysplasia, inguinal hernia, dysostosis multiplex

Dilated cardiomyopathy is a progressive disease, characterized by left ventricle dilation and reduced systolic function, leading to heart failure, with a 20% mortality rate at 1 year and a 56% mortality rate at 4 years. The etiology of dilated cardiomyopathy is varied. In children the causes of dilated cardiomyopathy may be infections (viral and bacterial), metabolic diseases (such as endocrine disorders or storage diseases), systemic diseases (connective tissue diseases), neuromuscular disorders, toxic causes and tachyarrhythmias. In 70% of cases it has an idiopathic origin and diagnosis is made excluding all the causes previously reported (20). Symptoms may be non-specific; a chest X-ray screen shows an enlarged cardiac silhouette and echocardiography is used to confirm diagnosis and to assess the progression and the severity of the disease. Mortality in children is higher than in adults and the risk factors are age at diagnosis, presence of dilated cardiomyopathy in the family and severity of ventricular systolic dysfunction at baseline (21).

Schultz et al. described a patient with MLII who presented at the age of 10 weeks a dilatative cardiomyopathy and endocardial fibroelastosis without signs of cardiac insufficiency. No more information could be known about this patient because only the abstract was available on PubMed (16). Muller et al. reported the case of a child with MLII who developed a cardiac insufficiency at the age of 9 months. Echocardiogram revealed a dilatative cardiomyopathy with extremely reduced systolic function of the left ventricle; valves were normal. Coronarography showed an atypical origin of coronary arteries. The patient was treated with digitalis and diuretics and was stabilized, but the echocardiographic parameters remained unvaried (17). Her condition remained poor due to cardiac dystrophy and she died suddenly at the age of 30 months of an atypical pneumonia (18). Genetic analysis was not performed in this patient.

The patient described by Kwak et al. is a 32-year-old woman who underwent to a heart transplantation due to a dilated cardiomyopathy diagnosed two years before. She was the third child of nonconsanguineous parents, and she had a sister who died at the age of 30 of heart failure due to dilated cardiomyopathy. She presented flexion contracture in fingers, which began in her early teens, her height was between the 3rd-5th percentiles and at 24 years of age she underwent lumbar spine surgery for deformities. She also had a short neck and coarse facial features with a broad nose, prominent forehead and big mouth, elbow and shoulder stiffness. Her psychomotor development was normal. Genetic analysis identified the c. 2715+1G>A and c. 3173C>G *GNPTAB* gene variants and she was diagnosed with MLIII alpha/beta (19).

Unfortunately, we do not have the genetic analysis of the two patients described who presented MLII and dilated cardiomyopathy. Our case is the first to report the genetic variants associated with this uncommon clinical feature. Although, as previously said, a precise genotype-phenotype correlation is not possible in this rare group of metabolic diseases, it would be interesting to know the exact molecular analysis of the two patients with MLII and dilated cardiomyopathy previously described, in order to verify whether this particular aspect of cardiac involvement can only be expected in certain types of mutations.

ML II alpha/beta is a very rare and often misdiagnosed disease. Phenotypes are variable and it is difficult to establish a genotype-phenotype correlation. Our case is for the very early onset of the dilatative cardiomyopathy, a condition not often described in MLII patients. A multidisciplinary follow-up is mandatory to ameliorate the clinical course of these patients and to assess the severity or their disease.

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PPHN and oxidative stress: a review of literature

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Persistent pulmonary hypertension of the neonate is a multifactorial condition characterized by maladaptive pulmonary vascular remodeling and abnormal contractile reactivity. This review evaluates the role of oxidative stress and antioxidant treatment on the persistent pulmonary hypertension of the neonate.

Persistent pulmonary hypertension of the newborn (PPHN) is a clinical syndrome that occurs in 2-6 per 1000 live births, caused by an abnormal transition at birth, resulting in high pulmonary vascular resistance (PVR), right-left extrapulmonary shunt of the deoxygenated blood and severe hypoxemia, which carry a significant risk of short and long term morbidity and death (1, 2).

Risk factors for PPHN can be divided into "prenatal" and "perinatal" factors. The first are the black race, gestational diabetes, obesity, advanced maternal age, urinary tract infections, fever, drug use during pregnancy and cesarean section; instead the perinatal factors are gestational age from 34 to <37 weeks, big or small babies by gestational age, congenital diaphragmatic hernia (CDH), meconium aspiration syndrome (MAS), respiratory distress syndrome, and other respiratory tract anomalies (3). Since the oxidative stress (OS) has been recognized as a crucial factor in the pathophysiology of various neonatal lung diseases, the purpose of this review is to evaluate the role of oxidative stress on the pathogenesis of PPHN.

Perinatal circulatory transition and PPHN pathogenesis

During the transition from fetal to neonatal life, the lungs undergo dramatic changes. In fact, while the fetus is accustomed to its intrauterine hypoxic environment, at birth it must quickly adapt to a new environment in which the oxygen concentration is five times higher than that in the uterus (4, 5). Usually, the perinatal circulatory transition is characterized by a rapid drop in PVR during the first breath and a marked increase in systemic vascular resistance (SVR) secondary to clumping of the umbilical cord. A successful postnatal transition is facilitated by complex physiological and nitric oxide (NO)-mediated biochemical processes. In

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fact, NO stimulates vasorelaxation by activating soluble guanylate cyclase (sGC) to generate cyclic guanosine monophosphate (cGMP), while type 5 phosphodiesterase (PDE5) compromises vasorelaxation by degrading cGMP.

The production of NO by endothelial NO synthasese (eNOS) is regulated by protein phosphorylation, as well as by the availability of substrate and numerous cofactors including calcium-calmodulin, and tetrahydrobiopterin (BH4). Instead, mechanisms that inhibit eNOS activity or attenuate downstream NO signaling can induce vasoconstriction. However, if one or more pulmonary vascular system abnormalities happen (table 1), the adaptation mechanisms fail, the PVR exceeds the SVR and a right-to-left hemodynamic shunt occurs through the oval foramen and / or arterial duct as in the fetal circulation (7-9). In fact, PPHN can also be called "persistent fetal circulation" and the result is reduced lung perfusion and systemic hypoxemia. (Table 1. Synthesis of anatomical alterations and risk factors of pulmonary vascular system abnormalities).

Oxidative stress in PPHN

Oxidative Stress can be caused by multiple events, including inflammation, hyperoxia and mechanical ventilation, which contribute to prolonged lung damage up to impaired lung function. Reactive oxygen species (ROS), such as superoxide anions and hydrogen peroxide, produced by the electron transport chain (ETC) in mitochondria due to exposure to hyperoxia, are key signaling molecules for regulating tone and pulmonary vascular function by stimulating the PDE5 and cGMP decomposition and reducing the vasodilator effect of NO. Indeed, the vascular tone depends on the balance between the free radicals production of endothelium, mitochondria, xanthine oxidase and NADPH oxidase (Nox) and antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase (10-11).

Animal model studies suggested that ROS may play a key role in the pathogenesis of PPHN. Indeed, reduced expression and activity of sNOS and sGC and increased expression and activity of PDE5 and ET-1 levels have been shown in fetal lambs with PPHN caused by ligation of the arterial duct (12-15). In vitro studies have shown that smooth pulmonary artery muscle cells (PASMC) isolated from PPHN lambs have elevated cytosolic ROS, higher expression of Nox4 and reduced extracellular superoxide dismutase (ecSOD) activity compared to controls (16). In addition, PPHN PASMC also exhibits a high ROS in mitochondrial matrix, associated with an increase in mitochondrial SOD activity (MnSOD), an increase in PDE5 activity and a reduction in cGMP reactivity to NO (17, 18). Other surveys have also shown that ROS stimulate vascular growth of SMCs, suggesting that altered ROS generation and scavenging also contribute to pulmonary vascular remodeling in PPHN (19, 20).

About preterm babies, several studies have also shown that antenatal exposure to oxidative stress is associated with a subsequent onset of bronchopulmonary dysplasia (BPD) and PPHN. Indeed, the analysis of the metabolic profiles of the cord blood revealed that oxidative stress markers were strongly associated with the subsequent onset of PPHN and chronic lung disease in preterm infants (21). Recently, a meta-analysis of 12 studies also showed that PPHN was associated with fetal growth restriction (22). These results suggest that vascular development in premature babies may be abnormal long before postnatal external lung insults.

Furthermore, postnatal stresses, such as nutritional deficiency, sepsis, ventilation and hyperoxia, may also amplify the damage on pulmonary vascularization (23). It was demonstrated that the combination of postnatal growth restriction (PNGR) with exposure to 75% oxygen for the first 14 days of life produces a more severe PPHN phenotype with reduced pulmonary vessel density, thickening of the pulmonary arteriolar walls and right ventricular hypertrophy. In fact, PNGR and hyperoxia cause a reduced expression of angiogenesis and vascular tone modulators including VEGF, VEGF 2 receptor, HIF1α, HIF2α, eNOS and NOS (24). In addition, hyperoxia not only increases oxidative stress by promoting the production of free radicals by the mitochondrial matrix and the enzymatic sources in the cytosol, but also reduces the response to pulmonary vasodilators such as inhaled NO (iNO) through the

activation of PDE5 which reduces cGMP generation. In 2012, a study evaluating the pro-inflammatory effects of hyperoxia documented that exposure of infants with PPHN to high concentrations of O2 increased proinflammatory interleukins (IL-6, IL-8, TNF-α), while the administration of inhaled NO with low O2 reduced the cytokines and improved the oxygenation index (25).

Therapies

The use of antioxidant therapy to prevent or address PPHN is supported by the fact that oxidative stress and ROS generation are critical to the pathophysiology of this condition (26). Several studies suggest that antioxidant therapy may increase oxygenation in PPHN lambs by improving NO-mediated vasodilation at many points of the signaling pathway. Indeed, intratracheal administration of antioxidants increases the expression of eNOS and cGMP and reduces the activity of ROS and PDE5 in the pulmonary arteries of ventilated PPHN lambs. (27-29). In addition, both the administration of prenatal betamethasone and postnatal hydrocortisone significantly improved oxygenation in PPHN lambs (30-32).

Among antioxidant drugs, melatonin is an emerging therapy with antioxidant, anti-apoptotic, anti-inflammatory and analgesic properties, which showed promising results in pre and clinical studies (33-35). Indeed, a study evaluating the effect of melatonin on 10 lambs with PPHN, showed that its administration reduced pulmonary blood pressure after the first 4 days of treatment and the contractile response to vasoconstrictors K, TX2 and ET-1 after 1 month, while endothelium-dependent and muscle-dependent vasodilation of the pulmonary arteries increased. In addition, the treatment induced antioxidant enzymes and reduced pro-oxidant factors (36). However, to date, clinical studies on the administration of antioxidant therapy in infants had marginal benefit (37, 38).

Oxidative stress is involved in a wide spectrum of newborn disease. OS due to antenatal and postnatal factors, has a key role in the pathophysiology of PPHN. Although the mechanism linking oxidative stress and neonatal lung diseases has been studied for several years, still many steps must be taken to find an effective antioxidant therapy that can support the treatment of PPHN.

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Role of oxidative stress in the pathogenesis of congenital cardiopathies

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Oxidative Stress

In the normal physiology of the human body, a small part of the oxygen metabolized is not completely reduced and this leads to the formation of reactive oxygen species (ROS). (1). ROS embrace both free radicals, such as Hydroxyl (OH), superoxide ($O_2^{-)}$ nitric oxide (NO), nitrogen dioxide (NO_2), peroxyl (ROO) and lipid peroxyl (LOO), and non-free radical oxygenated molecules. The latter group includes hydrogen peroxide (H_2O_2), ozone (O_3), singlet oxygen ($^1O_2$), hypochlorus acid (HOCl), nitrous acid (HNO_2), dinitrogen trioxide (N_2O_3) andlipid peroxide.

Moreover, nitric oxide (NO) may combine with oxygen to form peroxynitrite ($ONOO^-$), a reactive nitrogen species (RNS). Low or moderate concentrations of ROS and NRS are useful for immune response and cells maturation but if present in high doses they can react with molecules as proteins, DNA, RNA, glucids and fatty acid leading to a change in their original structure or functions. (1). Oxidative stress (OS) is the result of a rupture of the balance normally existing between free radicals (FR) production and antioxidant defense mechanism (2, 3).

High-mobility group box 1 (HMGB1) protein, a chromatin-binding nuclear protein and damage-associated molecular pattern molecule, is integral to oxidative stress and downstream apoptosis or survival (4). The HMGB1 effects, associated with several critical pro-inflammatory mediators, (5) have stimulated an increasing interest in the field of several inflammation-associated disorders including allergic (6-8), haematological (9), infective (10), endocrine, and gastrointestinal diseases (11).

The antioxidant system consists of enzymes such as catalase, superoxide dismutases, retinoic acid (vitamin A), glutathione peroxidase, alpha tocopherol (vitamin E), urin acid, glutathione, ascorbic acid (vitamin C). During embryogenesis, the fetus grows in a relative hypoxic environment (12), with 20-25 mmHg PO2 oxygen tension. At birth they meet a normoxic environment of about 100 mmHg PO2 (13). This increased oxygen tension leads to elevated production of ROS. Gestational period is characterised by physiologic free radical

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production in placenta and fetus allowing a normal fetal development (14). During pregnancy, ROS are generated from mitochondrial metabolism and placenta is a site rich in mitochondria and metabolically active. Increased OS in normal gestational age is the result of this mechanism (15).

It has been shown that there is an association between male gender and OS due to the fact that male seem to have a higher concentration of markers for OS compared to women. Different markers can be analysed to evaluate OS: for example, high level of Melatonin may be considered a response to counteract the elevated OS associated with severe conditions. (14) However, in particular stressful conditions, OS with overproduction of FR and a reduced antioxidant defence mechanisms (15) can lead to neonatal and maternal complication as pre-eclampsia, intrauterine growth restriction (IUGR), preterm delivery and neonatal infections (16). As consequence of the increased OS and the decreased antioxidant activities, the embryological and perinatal period can be susceptible of FR- mediated oxidative damages.

New-borns are exposed to OS, in particular preterm infants, due to reasons including: transition from the hypoxic intrauterine environment to an normoxic extrauterine life (13), infections, especially in preterm due to the immunodeficient condition, low levels of antioxidant mechanisms (13), high levels of free iron leading to production of highly toxic hydroxyl radical (17). In preterm newborns an impaired antioxidant capacity can be due to both an insufficient endogenous production and an inappropriate interaction of placental foetal transfer (18). The relation between OS and congenital malformations and neonatal morbidities such as hypoxic ischemic encephalopathy (HIE), intraventricular haemorrhage (IVH), periventricular leukomalacia, bronchopulmonary dysplasia (BPD), chronic lung disease (CLD), retinopathy of prematurity (ROP) and necrotizing enterocolitis (NEC) (19, 20), congenital heart diseases has been investigated in literature (21). In this paper we focused on the role played by OS in cardiovascular disease to better understand the pathomechanism and identify new strategies of therapy.

DISCUSSION

Congenital heart defects (CHDs) are the most common congenital diseases and they are one of the most important healthcare problems in children. Mostly genetics, other environmental factors are implicated in CHDs, such as maternal alcohol, cigarette smoking, industrial chemicals and viral infections. All these factors lead to an excessive production of ROS with a consequent endothelial injury and extracellular/intracellular OS (22).

It has been shown that Homocysteine and Folate pathways alterations are involved in direct and indirect embryo-toxic effect by increasing OS. Homocysteine level increases, while Folate decreases. Elevated levels of homocysteine lead to stress through both an excessive production of ROS and a decrease in the glutathione-dependent antioxidant-defensive mechanism. Since mitochondria are a major source for ROS formation, cardiac dysfunction may be also explained with the high concentrations of them in cardiac tissue (23).

Also upregulated NADPH oxidase and overactive NO-enzymes are involved in OS generation and cardiovascular diseases induction (24). Moreover, it has been found that ROS formation can be induced by oxidized low density lipoprotein (ox LDL) in endothelial cells of the umbilical vein (25), angiotensin II and uremic toxin indoxyl sulfate (26). Furthermore Myeloperoxidase (MPO) containing in neutrophils and monocytes can produce ROS involved in lipid oxidation. Lipid peroxidation is the primary trigger for atherosclerosis process.

A well know teratogenic factor is represented by hypoxia (27). A state of hypoxia, indeed, can induce a severe OS due to the high concentration of oxygen during reperfusion, as occurring after a period of oxygen deprivation (28), with consequent tissue damage. Moreover, it was shown that hypoxia causes the decrease of ATP in the cells leading to a massive formation of xanthine and hypoxanthine, with a consequent release of superoxide radicals (29). Hypoxia is also involved in genes and proteins regulation. Most of these genes are related to angiogenesis, apoptosis and cell proliferation.

A chronic state of hypoxia leads to an increase

in pulmonary vasoconstriction that can result in pulmonary hypertension, increase of the right ventricle afterload and eventually heart failure (30). Hypoxia also regulates the expression of hypoxia-associated miRNA involved in cardiac development and ischemic cardiovascular diseases (31, 32).

Ercan et al (20) focused on the role of OS in children with cyanotic and acyanotic CHD. They observed that the oxidant and antioxidant levels in children with cyanotic CHD were higher than the acyanotic and control group suggesting that hypoxia is dependent on the primary anatomical defects in these patients. Hypoxia induces the increasing of the ROS and the levels of antioxidant substances increase as consequence, to compensate it. It seems that children with acyanotic CHD are under less OS comparing with those with cyanotic CHD.

However, the role of OS in the development of specific heart and vascular structures is still not well understood. It was hypothesized that hypoxia-induced remodelling of the cardiac outflow tract may represent an embyogenic moment of vulnerability to ROS. Fisher et al (33) exposed chick embryos to fixed concentrations of the stable cell permeant oxidant H2O2. The OFT myocardium appears particularly sensitive to this oxidizing effect of H2O2. Moreover, it was shown that the effect of oxidant stress H2O2 induced depends on the concentration and duration of exposure (34). At low concentration H2O2 causes reversible modifications, on the contrary, at higher concentrations H2O2 may induce irreversible alterations concerning proteins structure and cell death.

Moreover, maternal diseases such as diabetes seem to be an important risk factor for heart embryonic malformations and cardiac outflow tract defects (35). The recurrence of CHDs in infants of diabetic mothers is about 5%. It has been shown that diabetes in gestational age represents an independent risk factor for the development of embryonic malformations (36).

This fact can be explained by the role of Glucose in ROS generation through different pathways included mitochondria, nicotinamide, adenine dinucleotide phosphate oxidase, the sorbitol pathway, activated glycation and insulin pathway (24) leading to a proapoptotic signalling in the process of cardiac outflow septation (35). Furthermore, glucose is involved in genetic expression during embryonic development by inducing epigenetic changes (37).

These alterations and those concerning the cardiovascular outflow tract can occur mostly if the mother develops insulin resistance in the third trimester (35). To reduce OS and consequently OS-associated cardiomyopathies, it is possible to act in two different ways: using antioxidants or preventing ROS production. Antioxidants include ROS scavengers such as vitamin A, vitamin C, vitamin E, carotenoids, phenolic acids, flavonoids, acetylcysteine, exogenous selenium, zinc, magnesium, Glucocorticoids, Allopurinol and curcumin. Propofol is an anesthetic used as ROS scavenger (38). Also, Melatonin has been shown to have a high antioxidant power acting as a free radical scavenger (39-41).

Preventing ROS production is possible by the use of Carvedilol and Omega 3.

Carvedilol inhibits mitochondrial complex involved in the mechanisms of anthracycline-induced cardiotoxicity (42,43). Omega 3 Polyunsaturated fatty acids composed of eicosapentanoic acid (EPA), docosahexaenoic acid (DHA) and alpha-linolenic acid (ALA) act by both mechanisms. Fatty acids can electrically stabilize heart cell membranes changing membrane fluidity (44). They can also stimulate post-ischemic functional recovery (45), decrease heart rate and reduce myocardial oxygen consumption (45) by the increased expression of the antioxidants enzymes.

Another possible therapeutic solution is the administration of Hypoxia-inducible miRNA and miRNA mimics. Hypoxia – inducible miRNA promotes cell survival and inhibit cardiomyocyte apoptosis (46). Instead the second group acts by the restoration of miRNA (47). They can both reduce infarction size (48), fibrosis and improve myocardial function (49). Liposomes, Polymers, conjugates, exosomes and bacteriophages are used to improve the bioavailability of microRNAs (50).

Fumaric acid could also be used to activate of the Nrf2 pathway. Nox and MPO can modulate ROS and so they can used to treat and prevent hearth diseases. Viral delivery gene therapy (51) and cell therapy are

still studied as potential strategies: stem cells can be preconditioned to exert an antioxidant effect in target tissue (52).

Oxidative stress plays a key role in the pathogenesis of different types of congenital cardiovascular diseases. In physiological conditions, ROS are involved in developing embryos, but if antioxidant capacity is deficient and ROS are over-produced, they may have important toxic effects in fetuses. Fetal tissues are particularly sensitive to oxidative damage because of the rapidly growing nature of their cells.

Oxidative stress is so related to the induction of foetal heart malformations. New discovers in the pathomechanism of oxidative stress-related congenital heart diseases, represents the basis of possible clinical applications in screening, prevention, and therapies. Potential therapeutic strategies for ROS include various radical scavengers, inflammatory mediator antagonists and antioxidants. Antioxidants include vitamin A, vitamin C, vitamin E, carotenoids, phenolic acids, flavonoids, acetylcysteine, exogenous selenium, zinc, magnesium Glucocorticoids, allopurinol and Propofol. On the other hand, Carvedilol and Omega 3 can prevent ROS production. Other studies are required to better define the OS mechanisms related to cardiovascular diseases and the potential therapeutic strategies to prevent a possible congenital heart disease.

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Cardiac malformations in children with congenital hypothyroidism

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Congenital hypothyroidism (CH) is the most common endocrine disease in children, according to literature, infants with CH have an increased risk of associated congenital malformations (CM), especially cardiac defects (CD), compared to the general population. We retrospectively analyzed medical records of 255 patients with a positive screening result for CH in the period 1991-2016 followed at our Center. At the time of enrollment, the clinical examination included looking for the presence of heart murmurs and dysmorphic features. In all patients an echocardiography with cardiological evaluation were performed. Of all patients, 191 were included in the final analysis. Of these, 51.3% (98/191) presented an eutopic normally sized thyroid gland while 48.7% (93/191) showed a thyroid dysgenesis. Among the studied infants, 13.6% (26/191) presented CD. The most frequent cardiac anomaly was atrial septal defect (ASD) which was found in 65.4% (17/26) of patients with CD. Other defects were ventricular septal defect (VSD), patent ductus arteriosus (PDA), pulmonary valve stenosis (PvS), transposition of the great vessels (TGV), aortic valve stenosis (AvS). Six patients had multiple defects. In the analysed group, there was no significant relation with sex, type of CH, median blood-TSH (b-TSH) and serum-TSH (s-TSH) values and frequency of CD. There is a high prevalence of CD in CH, indicating the need of routine echocardiography in these patients to achieve an early diagnosis and management of CD.

Congenital hypothyroidism (CH), the most frequent endocrine disorder in children, is one of the common and readily preventable causes of mental retardation (1). Endocrine dysfunctions are related in some case with some metabolic disorders (2). Prevalence of sporadic CH is 1 in 2500-4000, but this frequency varies by geographic location and by ethnicity (3, 4). However, in the last 20-30 years an increase in frequency up to 1:2000 newborns have been observed (5, 6). Several factors likely account for this change. The most important factor is the lowering of the thyroid-stimulating hormone (TSH) screening cut-off occurred in many newborn screening (NBS) programs (7, 8).

Key words: congenital hypothyroidism, cardiac defects, malformations

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According to data from current literature, infants with CH have an increased risk of associated congenital malformations (CM) as abnormalities of the gastrointestinal system or genitourinary tract compared to the general population (14.6%-59%) (9-11). Olivieri et al. reported that prevalence of additional CM was more than fourfold higher than that reported in the Italian population. The cardiac defects (CD) were described as the most frequent congenital malformations associated with CH (12). Echocardiography is known to be a powerful technique for the assessment of preclinical markers of cardiac dysfunction (13, 14). The aim of this study was to define the frequency and type of concomitant CD in a cohort of patients with primary CH, followed at our center.

MATERIALS AND METHODS

We retrospectively analyzed medical records of 255 patients with a positive screening result for CH in the period 1991-2016 followed at our Center, without syndromic disorders. Of all patients, 64 were excluded because of incomplete medical records or diagnosis of transient form of CH. Finally, 191 (79 M, 112 F) patients were included in the analysis.

Thyroid ultrasonography was performed to recognize patients with eutopic thyroid gland and to define the thyroid volume based on the anterior-posterior diameter of the gland at diagnosis (normal diameter between 5 mm and 9 mm) and at re-evaluation (normal diameter between 7 mm and 12 mm). Scintigraphy was performed in patients with non eutopic thyroid gland on ultrasonography.

The NBS program for CH is primarily based on the measurement of blood-TSH (b-TSH) and blood-thyroxine (b-T4) levels on neonatal Guthrie cards. The blood sample was collected after 48 hours of life and our regional threshold is 7 μIU/ml and 7 μg/dl for b-TSH and b-T4 respectively. If the b-TSH level is between 7 μIU/ml and <15 μIU/ml a second NBS sample is requested between 1-2 weeks of age. If this repeat measurement is positive (b-TSH >3 μIU/ml) or b-TSH concentration is >15 μIU/ml on the first screen sample, serum TSH (s-TSH) and serum free T4 (s-FT4) concentrations are measured. Serum thyroglobulin (Tg), antithyroid peroxidase antibodies (TPOAb), antithyroglobulin antibodies (TgAb) and TSH receptor antibodies (TRAb) are also measured. In patients with s-TSH >20 μIU/ml or s-FT4 <0.7 ng/dl and in patients with a persistent elevation of s-TSH levels between 6-20 μIU/ml, levothyroxine (L-T4) treatment was started at a dose of 10–15 μg/kg/day and it was prolonged until 3 years.

At the time of enrollment, a detailed record of the information regarding the neonate as well as further physical examination were made. The clinical examination included looking for the presence of heart murmurs, dysmorphic features and other CM. In all patients an echocardiography with cardiological evaluation were performed.

Two-dimensional echocardiography, M-B mode, pulsed-wave and continuous-wave Doppler, and color flow mapping were done. A search for associated CM, any valvular lesion or myocardial involvement with routine measurement of left ventricular dimensions, function and other cardiac chamber dimensions was done. Among CD we included atrial septal defect (ASD), ventricular septal defect (VSD), patent ductus arteriosus (PDA), pulmonary valve stenosis (PvS), transposition of the great vessels (TGV), aortic valve stenosis (AvS) while patients with patent foramen ovale (PFO) were not comprised in this group.

All the patients were regularly followed-up at our Center, according to a therapeutic protocol which provides adjustments of L-T4 doses based on s-TSH values (reference values: 0.5-2.5 μIU/ml). S-TSH and s-FT4 levels were measured by chemiluminescent microparticle immunoassay. S-TSH and s-FT4 levels for thyroxine dosage adjustment were determined every 2-3 months in the first year, every 3-6 months until 3 years and subsequently every 6 months.

Statistical analysis

Groups were compared using the Mann-Whitney U-test for continuous data with nonparametric distribution and the Yates's chi-square test for categorical variables. Normal distribution was assessed by the Shapiro-Wilk test. Continuous variables were reported as median and interquartile range (IQR) and categorical variables were reported as count and percentage. P-values <0.05 were considered statistically significant and all tests were two-sided. Statistical analyses were performed using GraphPad Prism version 8 for Windows. The study was conducted in accordance with the ethical standards of the Helsinki Medical Declaration and its later amendments.

RESULTS

Among 255 patients with a positive screening result for CH in the period 1991-2016 followed at our Center, 191 (79 M, 112 F) were included in the final analysis. The average duration of pregnancy was 38.44 weeks (range: 28-42), average birth weight was 2935 g (range: 890-4350 g). The median (IQR) screening b-TSH and s-TSH values were 15 (103.03) mIU/mL and 55 (154.03) mIU/mL, respectively. Of all, 51.3% (98/191) presented an eutopic normally sized thyroid gland while 48.7% (93/191) showed a thyroid dysgenesis. Particularly, in the latter group 75 patients presented a hypoplasic thyroid, 9 patients a thyroid agenesis, 6 patients an ectopic gland while 3 patients an hemiagenesis.

Among patients, 13.6% (26/191) had CD of whom 25 patients had an isolated cardiac defect while one patient had also a hypospadias. The most frequent cardiac anomaly was ASD which was found in 65.4% (17/26) patients. Other defects were VSD, PvS, AvS, PDA, TGV. Of these, six patients had multiple cardiac defects (Table I).

In the cohort study, one patient showed a horseshoe kidney. Girls are more likely to have congenital CD. The difference in sex ratio between infants with and without CD was not significant (p=0.59). Median (IQR) values of gestational age were 38.5 (1) and 39 (2) weeks in patients with and without CD respectively with no significant difference between the two groups (p=0.67). Median (IQR) b-TSH levels in CH infants with and without CD were not significantly different: 28 (185.53) mIU/mL versus 15 (79.3) mIU/mL (p=0.28). Median (IQR) s-TSH levels in CH infants with and without CD were 75.23 (281.43) mIU/mL versus 47.36 (147.22) mIU/mL respectively (p=0.33). In the analysed group, there was no significant relation (p=0.72) with the type of CH (thyroid dysgenesis or dyshormonogenesis) and frequency of CD. The results were summarized in Table II.

Table I. *Frequency of cardiac defects in infants with congenital hypothyroidism.*

Cardiac defects	Frequency	Percent
ASD	13	50%
VSD	4	15.4%
PvS	1	3.85%
AvS	1	3.85%
PDA	1	3.85%
ASD + VSD	3	11.5%
ASD + PvS	1	3.85%
VSD + TGV + PvS	1	3.85%
VSD + PvS	1	3.85%

*Atrial septal defect (**ASD**); ventricular septal defect (**VSD**); patent ductus arteriosus (**PDA**); pulmonary valve stenosis (**PvS**); transposition of the great vessels (**TGV**); aortic valve stenosis (**AvS**).*

DISCUSSION

In our work the frequency of congenital heart diseases was 13.6%. This data is consistent with other previous studies and revealed that congenital CD are more frequent among infants with CH than expected. Congenital heart defects are described as the most frequent malformations at birth in Italy (15) and as the most common extrathyroidal congenital defects in patients with CH (12).

Balestrazzi et al. (16) retrospectively analyzed the Italian National Registry for CH for the years 1987-1992. They found that the birth defect incidence was higher in patients with CH than in the general population (4.3% versus 2.5%–3%) and especially the incidence of CD (2.1% versus 0.3%–0.8%). The most frequent CD observed by the authors were septal defects and PvS. In a study by Stoll et al. (17) among 129 CH infants, 6.9% had congenital CD. Olivieri et al. (12) carried out a retrospective review of the CH infants during the period 1991-1998 and reported that the prevalence of additional CM (8.4%) was more than fourfold higher than that reported in the Italian population. Particularly, CD were the most frequent malformations associated with CH with a prevalence of 5.5%. In a prospective clinic-based study Kreisner et al. (18) evaluated the presence of major CM in CH infants from Brazil. Among the 76 patients identified, 8 (10.5%) had CD. In a prospective study by Sabri et al. (19) the prevalence of congenital CD was 6.25% in patients with CH versus only 1.7% in euthyroid controls after the exclusion of PFO. Gu et al. (20) reported co-occurrence of congenital CD and CH at a level of 8.9% and Reddy et al. (9) at a level of 29%. In the analysis of Razavi et al. (10) the frequency of CD in CH patients was 4.9% while Bas et al. (21) reported a frequency of 8%. The most common observed

Table II. *Characteristics of patients with and without cardiac defects affected by congenital hypothyroidism.*

Characteristics	**Pt with CD (n=26)**	**Pt without CD (n=165)**	**p-value**
Sex:			
M, n (%)	9 (34.6%)	70 (42.4%)	0.59
F, n (%)	17 (65.4%)	95 (57.6%)	
Gestational age (weeks), median (IQR)	38.5 (1)	39 (2)	0.67
US findings:			
Eutopic normally sized gland, n (%)	12 (46.2%)	86 (52.1%)	
Agenesis, n (%)	2 (7.7%)	7 (4.3%)	
Hemiagenesis, n (%)	1 (3.8%)	2 (1.2%)	0.72
Hypoplasic, n (%)	11 (42.3%)	64 (38.8%)	
Ectopic, n (%)	0 (0%)	6 (3.6%)	
b-TSH (mIU/mL) 1° NBS, median (IQR)	28 (185.53)	15 (79.3)	0.28
s-TSH (mIU/mL), median (IQR)	75.23 (281.43)	47.36 (147.22)	0.33

CD: *cardiac defects;* ***Pt:*** *patient;* ***M:*** *male;* ***F:*** *female;* ***US:*** *ultrasonography;* ***n:*** *number;* ***b-TSH:*** *blood-TSH;* ***NBS:*** *newborn screening;* ***s-TSH:*** *serum-TSH;* ***IQR:*** *interquartile range.*

defects are ASD, PDA and VSD (8, 10, 21).

More recently, Wędrychowicz et al (22) demonstrated the presence of congenital CD in 18.5% of 54 children with CH. In this study the most common heart defects were PDA (30%) and ASD (30%). In this regard, the most common heart anomalies in our cohort were septal defects, particularly ASD, while only one patient presented PDA. Among patients, 51.3% presented an eutopic normally sized thyroid gland while 48.7% showed a thyroid dysgenesis of whom one patient had both thyroid agenesis and resistance to thyroid hormone (23). Our data showed that there was no correlation between type of CH (thyroid dysgenesis or dyshormonogenesis) and frequency of CD. Moreover, other factors as sex, gestational age, b-TSH and first s-TSH were not statistically related to CD.

These data suggest that CH may not be an isolated condition but rather a part of a larger malformative spectrum resulting from some unknown insults during embryogenesis. Multiple hypotheses are taken into consideration (9, 10, 21, 24). The cause of various extrathyroidal abnormalities in patients with CH is often attributed to the lack of metabolic activity of the thyroid hormone which are essential in the growth and maturity of cells and tissues in the foetal period. Particularly, a lack of thyroid hormones leads to defects in the tissues of the nervous and skeletal systems. However, this hypothesis does not appear to explain the co-occurrence of CH and defects in other organs and systems because in the first 12 weeks of life the foetus is dependent on the mother's production of thyroid hormones (22, 25).

On the contrary, there are evidences indicating that gene mutations causing CH can be a cause of increased incidence of CM in this disease (26). Particularly, Dentice et al (27) identified NKX2-5 as a gene involved in thyroidogenesis. Given that NKX2.5 is also expressed in cardiac tissues, this may explain the increased incidence of CD in CH patients. This gene is important in regulating the division of the heart during its morphogenesis and development and function of the atrioventricular node (28). Foetal development of the thyroid and heart are closely associated. In fact, the thyroid is pulled to the base of the neck due to the migration of the heart during the early stage of development of the gland. Other studies, based on experimental models, suggest the role of the HEX homeobox gene in the development of the cardiovascular system and thyroid (9).

Our study showed a high prevalence of congenital heart disease in CH, indicating the need of routine echocardiography in these patients to achieve an early diagnosis and management of CD. However, our study has some limitations such as a retrospective design with a relatively small group of patients and the lack of complete data regarding the long-term outcomes of the patients, in some cases.

Therefore, further prospective studies are needed. With this perspective we believe that our data, supported by other studies, could have important clinical implications for patient management preventing diagnostic delays and could allow a better understanding of the mechanisms underlying association between CH and cardiac diseases.

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Prevalence of elevated pulmonary artery systolic pressure in Down Syndrome young patients with and without congenital heart disease

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This study examined the prevalence and distribution of elevated systolic pulmonary arterial pressure, measured by echocardiography, in young patients with down syndrome associated or not with congenital heart disease and surgical correction during childhood. Pulmonary artery systolic pressure, computed by regurgitant tricuspid flow velocity evaluation, is the most frequently used parameter for the screening of pulmonary hypertension. Down syndrome and congenital heart disease often coexist and the probability to detect elevated systolic pulmonary arterial pressure in this setting is high. However, little is known about the evaluationofpulmonary arterial pressure during growth of patients with down syndrome with or without congenital heart disease. We enrolled 47young patients (55% of male sex; mean age: 18.4 ± 6.0 years), 40 with congenital heart disease and 7 without a cardiac defect. Systolic pulmonary arterial pressure was assessed by echocardiography. No difference was found in the population dichotomized by presence or absence of CHD. Only male sex (p=0.000), highly sensitive troponin-T (P=0.027), tricuspid annular plane systolic excursion (TAPSE, p=0.045) and sPAP (p=0.004) were elevated in surgical group. The ASD was found as, the most common structural abnormality in our patients (50%), followed by VSD (27.5%) and complex CHD (such as complete atrioventricular canal defect, CAVC = 25% and Fallot disease = 15%). Furthermore, about 45% of patients had the combined defect. Only 37.5% of patients underwent to corrective surgery during the first months of life. We observed a significantly increase of sPAP values in patients with complex CHD, such as CAVC (p=0.019) and Fallot disease (p=0.001) but, in the following multivariate analysis performed in the patients with CHD, only Fallot disease remains as independent predictors of elevated values of sPAP (p=0.022). An elevated systolic pulmonary arterial pressure may represent the key screening tool in the diagnostic assessment of suspect pulmonary arterial hypertension in high risk population with down syndrome regardless the presence of congenital heart disease.

Down syndrome (DS) is the most common chromosomal abnormality, with an estimated incidence of approximately 1.1 per 1.000 live births (1). It is associated with several medical morbidities, especially congenital heart disease (CHD), intellectual disability (ID), Recurrent Respiratory Infections (2), Autoimmune thyroid disease (ATD). ATD develops facilitated by exposure to environmental factors

Key words: Down syndrome, systolic pulmonary arterial pressure, pulmonary arterial hypertension

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(3). High mobility group box-1 (HMGB1), possibly contribut (in a cumulative fashion) to ID predisposition in these patients (4).

The pro-inflammatory properties of HMGB1, contribute to the pathogenesis of several acute and/or chronic human diseases (5-9). The CHD is present in 50% of children with DS and is a major cause of both morbidity and mortality in these patients. The atrioventricular septal defect (AVSD) is found as the most common structural abnormality in patients with DS, followed by ventricular septal defect (VSD) and atrial septal defect (ASD). One of the most important complication of CHD in patients with DS is the development of pulmonary arterial hypertension (PAH). Oxidant stress is important in the pathogenesis of PAH (10).

It is estimated that about 50% of children with DS and CHD develop PAH, especially if they have AVSD (89%) (11). However, pulmonary hypertension (PH) can occur as a consequence of a number of underlying diseases, including left heart, respiratory and pulmonary vascular diseases or as isolated condition. PAH is a rare disease with increased mortality and morbidity, defined by a mean pulmonary artery pressure (mPAP) of $\geq$ 25 mmHg at rest and pulmonary wedge pressure (PWP) $\leq$ 15 mm Hg, typically recorded during right heart catheterization (RHC) (12). However, the invasive nature of this method renders it unsuitable in population-based studies with evident scarcity of epidemiological data both in the general population and in a setting of patients even if high-risk, such us children with DS. Therefore, echocardiography is a non invasive key screening tool in the diagnostic algorithm and prognostic assessment of PH. Pulmonary artery systolic pressure (PASP), computed by regurgitant tricuspid flow velocity evaluation according to the simplified Bernoulli equation, is the most frequently used parameter. We sought to describe the CHD related distribution and the prevalence of elevated sPAP measured by echocardiography in a sample of young patients affected by DS with or without CHD, who underwent or not to previous surgical intervention.

MATERIALS AND METHODS

In this study were enrolled children with DS over the age of 10 years, followed by our department, with or without CHD. The diagnosis of DS was suspected after birth on the basis of clinical features and then confirmed with the demonstration of chromosomal abnormality on peripheral blood cell karyotype. The diagnosis of CHD in these patients was made through routine echocardiography at birth.

All subjects underwent a clinical interview and a physical examination, and laboratory investigations (alanine aminotransferase [ALT], aspartate aminotransferase [AST], coagulation, blood count, thyroid and kidney function, inflammatory and metabolic markers, cardiac markers, uric acid) and blood gas analysis. Exercise capacity was evaluated through the 6-minute walking test (6MWT) where the following variables were assessed: rest and peak exercise transcutaneous oxygen saturation (SpO_2), heart rate, systemic blood pressure, 6-minute walking distance (6MWD). Lung function was assessed by spirometry and anatomical cardio-pulmonary abnormalities by chest X-ray. Each patient underwent a complete cardiological evaluation with electrocardiogram (ECG) and transthoracic echocardiography (TTE) study.

All echocardiograms were obtained by experienced sonographers using a GE Vivid 7 or Vivid E9 (GE Vingmed Ultrasound AS, Horten, Norway) cardiac ultrasound machine. The standardized protocol included 2-dimensional scanning in the parasternal long and short axis views, apical and subcostal views as recommended by the American Society of Echocardiography (13). A standard imaging protocol was used based on apical 4- and 2-chamber views; two-dimensional echocardiograms of the LV short axis were recorded from the left parasternal region at three levels: mitral valve, mid-papillary muscle level and apex. M-mode echocardiographic features were used for measurement of LV and left atrial dimension, while LV ejection fraction and LV volumes were estimated from apical four chamber view, using the biplane modified Simpson method (14).

The peak early transmitral filling velocity during early diastole (E), late diastole (A) and deceleration time (DT) were imaged in the apical 4-chamber view at the tip of the mitral leaflets. Color-coded tissue Doppler imaging (TDI) was applied to the apical 4-chamber view to determine mean early (E') and late (A') velocity at the septal mitral annulus. PASP was calculated as the sum of the estimated right atrial pressure (RAP) by inferior vena cava diameter and the peak velocity of the tricuspid regurgitant jet (TRV)

as: sPAP = 4*TRV^2 + RAP. For standardization, a RAP of 10 mm Hg was assumed for all patients unless, clear features were present, that suggested otherwise. A written informed consent was obtained from each participant before initiating any study-related procedure. The study protocol was approved by the research review board of the participating hospital units. This study conforms to the STROBE recommendations for reporting cohort studies (15).

Statistics

Data are presented as means, standard deviations, and frequency of occurrence. Continuous variables were compared with the paired or unpaired Student's t-test, ANOVA with post-hoc Tukey test, or with simple Pearson correlation as appropriate. Cross tabulations using the χ^2 statistics were used with non-parametric Kruskale – Wallis test to compare differences. Missing data was imputed with

Table I. *Differences of variables according to congenital heart disease.*

	All (47 pts)	CHD (40 pts)	no CHD (7 pts)	P
Male sex (%)	55,3	62,5	14,2	0,000
Age (year)	18,4 ±6,0	17,8 ± 5,6	22,3 ± 7,4	NS
Body-mass index (kg/m^2)	23,7 ± 5,3	23,5 ± 5,5	25,1 ± 3,3	NS
Systolic BP (mmHg)	107,2 ± 11,7	107,7 ± 12,5	104,9 ± 6,0	NS
Diastolic BP (mmHg)	65,4 ± 10,1	65,7 ± 10,0	64,4 ± 11,8	NS
Heart rate (beats/min)	79,2 ± 14,0	79,0 ± 14,3	80,4 ± 13,5	NS
O$_2$ saturation (%)	98,2 ± 1,2	98,3 ± 1,3	98,3 ± 1,4	NS
6 minute walking test (m)	441,4 ± 71,7	444,5 ± 72,4	424,2 ± 70,2	NS
FEV$_1$/FVC (%)	103,7 ± 12,1	101,8 ± 12,7	109,7 ± 10,4	NS
Hemoglobin (g/dl)	14,5 ± 1,2	14,5 ± 1,2	14,6 ± 1,1	NS
Total Cholesterol (mg/dl)	156,3 ± 30,1	156,5 ± 32,1	155,1 ± 16,6	NS
HDL Cholesterol (mg/dl)	48,9 ± 11,0	48,2 ± 11,1	53,5 ± 10,7	NS
Triglyceride (mg/dl)	77,8 ± 28,3	77,7 ± 29,6	77,9 ± 21,3	NS
Fasting glucose (mg/dl)	86,9 ± 20,9	86,8 ± 22,5	87,7 ± 8,6	NS
HOMA – IR	2,4 ± 1,7	2,3 ± 1,7	2,9 ± 1,3	NS
Uric Acid (mg/dl)	5,3 ± 1,4	5,4 ± 1,4	4,7 ± 1,4	NS
Creatinine (mg/dl)	0,7 ± 0,1	0,7 ± 0,2	0,7 ± 0,2	NS
C reactive protein (mg/dl)	4,7 ± 3,6	4,7 ± 3,7	4,3 ± 1,9	NS
Creatine kinase (U/L)	85,7 ± 29,4	88,9 ± 29,2	69,9 ± 27,4	NS
High sensitive troponine-T (ug/l)	0,0043 ± 0,002	0,0044 ± 0,002	0,0032 ± 0,0005	0,027
Aortic diameter (cm)	2,2 ± 0,3	2,2 ± 0,3	2,0 ± 0,2	NS
Left atrial diameter (cm)	2,8 ± 0,6	2,8 ± 0,6	2,6 ± 0,3	NS
End-diastolic volume (mL/m^2)	56,8 ± 26,2	55,8 ± 24,8	62,3 ± 34,5	NS
End-systolic volume (mL/m^2)	15,9 ± 10,5	15,1 ± 9,4	20,7 ± 15,4	NS
Index LV mass (g/m^2)	77,0 ± 33,0	74,2 ± 34,3	66,6 ± 26,6	NS
Ejection Fraction (%)	64,0 ± 7,6	63,9 ± 7,7	64,6 ± 7,3	NS
TAPSE (cm)	2,0 ± 0,3	1,9 ± 0,3	2,3 ± 0,5	0,045
sPAP (mm Hg)	28,7 ± 11,0	30,0 ± 11,4	22,0 ± 4,3	0,004
E/A ratio	2,0 ± 0,8	2,0 ± 0,8	2,2 ± 0,9	NS
E/E' ratio	7,7 ± 2,1	7,8 ± 2,2	7,1 ± 2,1	NS
Follow-up (months)	221,4 ± 72,4	236,5 ± 56,5	214,3 ± 78,5	NS

BP: *blood pressure;* ***sPAP:*** *Pulmonary artery systolic pressure;* ***LV:*** *Left ventricular;* ***TAPSE:*** *Tricuspid annular plane systolic excursion.*

a multiple imputation procedure (5 imputations) using the Markov Chain Monte Carlo method. A test for normality was carried out on all the variables, by Kolmogorov-Smirnov test, and non-normally distributed variables transformed for the purpose of regression analysis.

To evaluate the reproducibility of the EF and sPAP estimation, we evaluated the variability of individual methods between two observers, and between two analyses by the same observer. Inter-observer variability was studied by comparing pairs of results analyzed by two observers. Intra-observer variability was studied by comparing pairs of results from the repeated analyses by the same observer. Intra- and inter-observer agreement in Simpson's method and sPAP evaluation were assessed by linear regression with Bland-Altman analysis, showing respectively a correlation of 0.91 and 0.88 for the first measurement, and 0,92 and 0,89 for the second measurement. Independent predictors of elevated sPAP values were assessed by multiple linear regression by adjustment for age, sex and body mass index. Significant or trend variables on bivariate analysis were entered in each multivariable model. P value <0.05 was considered statistically significant. The SPSS 20 statistical software was used for the analysis (Statistical Package for Social Sciences, Chicago, IL, USA).

RESULTS

A sample of 47 young patients with DS (55% of male sex; mean age: 18,4 ± 6,0 years), regardless of the presence or absence of CHD, were recruited from January 2013 to June 2015 during their follow-up visit at our Hospital (mean: 221.4 ± 72.4 months). The baseline characteristics of the 47 enrolled patients are summarized in Table I. There was no difference between the patients dichotomized by presence or absence of CHD for all variables with the exception of male sex (p=0.000), high sensitive troponine-T (P=0.027), tricuspid annular plane systolic excursion (TAPSE, p=0.045) and sPAP (p=0.004). The distribution of all forms of CHD in

Table II. *Distribution of different types and exposure time of CHD and the related values of Spap.*

	Prevalence (%)	sPAP (mm Hg)	Exposure to CHD (months)
All	85,1	28,7 ± 10,9	51,8 ± 65,9
ASD o.p.	15,0	24,4 ± 7,3	15,0 ± 14,6
ASD o.s.	35,0	26,7 ± 6,1	84,8 ± 83,2
PFO	22,5	25,8 ± 5,0	34,7 ± 49,7
VSD	27,5	32,6 ± 6,4	66,5 ± 79,7
CAVC	25,0	36,7 ± 17,6	10,9 ± 9,9
PDA	17,5	30,2 ± 7,8	33,2 ± 44,7
Fallot Disease	15,0	42,4 ± 20,1	31,7 ± 44,7
Multiple defects	45,0	26,9 ± 7,8	61,4 ± 68,8
Surgery	37,5	35,0 ± 13,9	21,4 ± 32,6
Residual CHD	10,0	35,0 ± 2,0	122,4 ± 92,0

ASD o.p.: *atrial septal defect ostiumprimum;* ***ASD o.s.:*** *atrial septal defect ostiumsecundum;* ***PFO:*** *patent formamenovale;* ***VSD:*** *ventricular septal defect;* ***CAVC:*** *complete atrioventricular canal defect;* ***PDA:*** *patent ductusarteriosus.*

our population was showed in the Table II.

The ASD is found as the commonest structural abnormality in our patients (50%), followed by VSD (27.5%) and complex CHD (such us complete atrioventricular canal defect = 25% and Fallot disease = 15%). Furthermore, about 45% of patients had combined defect. Only 37.5% of patients underwent corrective surgery during the first months of life with an exposure to the congenital defect shorter than the groups with spontaneous resolution and without actual persistence of residual CHD (22.8±33.4 vs 57.4±68.0 months, P=NS).

However, while comparing the group of patients surgically treated with the patients that did not undergo surgery, we observed a significant increase of specific echocardiographic parameters, potentially unfavorable (Table III), on the other side the timing of the corrective surgery does not seem to be associated with an increase of the sPAP in our observation period. We observe, in fact, a significant increase of sPAP values and ratio between transmittal inflow and tissue velocity. Any other matched variables differ significantly between these two groups of patients.

An expected result (Table IV) is the significantly increasing of sPAP values in patients with complex CHD, such us CAVC (p=0.019) and Fallot disease (p=0.001) but, in the following multivariate analysis performed in the patients with CHD, only Fallot disease remain independent predictors of elevated values of sPAP (p=0.022).

Table III. *Differences of echocardiographic variables according to corrective surgery of CHD.*

	Surgery	No Surgery	P
Left atrial diameter (cm)	3,1 ± 0,6	2,5 ± 0,4	0.005
End-diastolic volume (mL/m^2)	60,3 ± 29,9	52,8 ± 21,0	NS
Index LV mass (g/m^2)	91,7 ± 34,0	62,8 ± 29,9	0.009
Ejection Fraction (%)	64,5 ± 8,4	63,4 ± 7,3	NS
TAPSE (cm)	1,8 ± 0,3	2,0 ± 0,1	NS
sPAP (mm Hg)	35,0 ± 13,9	26,4 ± 7,7	0.024
E/E' ratio	9,1 ± 2,6	7,0 ± 1,4	0.026

TAPSE: *Tricuspid annular plane systolic excursion;* ***sPAP:*** *Pulmonary artery systolic pressure;* ***LV:*** *Left ventricular*

Table IV. *CHD-related predictors of elevated sPAP in bivariate correlation and stepwise linear regression analysis.*

	Elevated sPAP values			
	R	*P*	*β*	*P*
ASD o.p.	-0,223	NS	-0,152	NS
ASD o.s.	-0,206	NS	-0,104	NS
PFO	-0,182	NS	-0,228	NS
VSD	0,135	NS	0,150	NS
CAVC	0,346	0,019	0,266	NS
PDA	0,010	NS	0,004	NS
Fallot Disease	0,493	0,001	0,478	0,022
Surgery	0,376	0,012	0,041	NS
Residual CHD	0,133	NS	-0,012	NS
Exposure to CHD	0,092	NS	0,271	NS

ASD o.p.: *atrial septal defect ostiumprimum;* ***ASD o.s.:*** *atrial septal defect ostiumsecundum;* ***PFO:*** *patent formamenovale;* ***VSD:*** *ventricular septal defect;* ***CAVC:*** *complete atrioventricular canal defect;* ***PDA:*** *patent ductusarteriosus.*

DISCUSSION

DS is the most common chromosomal abnormality. CHD and obstructive airways disease are common in infants with DS, both of these can lead to the development of PAH. Over the past few decades there has been a substantial increase in the life expectancy of children with DS, so that long term follow-up of such patients is important. Even were the heart disease is 'corrected' at an early age, it is not sure that upper airway obstruction or development of additional complications will not lead to pulmonary hypertension in the future. So, patients with DS are more prone to developing PAH earlier, despite this, however, a recent registry study found that patients with DS received significantly less PAH-specific treatment, for the lack of clinical trials in these patients.

As patients with DS represent most patients with PAH and CHD it is necessary that they be managed in an optimal manner. Given the difficulties associated with standard measures of prognosis such as functional class and Six Minute Walking Test

(6MWD), there is also the need to establish new prognostic indicators and tailor QoL questionnaires to better evaluate treatment effect in these patients. In this study, while confirming some established data, particularly that CHD is often concomitant in infants with DS, we demonstrated that when DS and CHD coexist, especially when cardiac defect is complex, the probability to detectelevated systolic pulmonary arterial pressure is greater, even after many years.

We also showed that surgery is associated with statistical significance, but clinically not relevant for the worsening of specific echocardiographic parameters, such us left ventricular filling pressure and mass. These observations highlighted that echocardiographic monitoring is useful in decision making and prediction of cardiovascular outcomes of this high-risk population. It has been suggested that the children with DS with large left-to-right shunt lesions tend to develop PAH much earlier than normal children with similar defects, probably because there is a subtle endothelial dysfunction (8, 9, 10). A left to right shunt of blood across the heart causes an increase in pulmonary blood flow which, in turn, brings to an increase in shear stress on pulmonary endothelial cells, resulting in a pathological process ultimately ending in irreversible pulmonary vascular disease (PVD).

In cases of AVSD, continuous PH results in a progressive reactive intimal fibrosis which will eventually obstruct the lumen of the artery. The obstructive process, causes an anatomical blockage in the artery, resulting in high pulmonary vascular resistance (PVR). Later, thrombo- obstructive lesions may occur, further increasing resistance to pulmonary flow (16, 17). These pathological processes have been further characterized. Particularly, it has been thought that proliferation of smooth muscle cells into peripheral non-muscular arteries (18) together with the development of plexogenic lesions and fibrinoid necrosis (19) play an important pathogenic role leading ultimately to vessel luminal obliteration.

Treatment of choice for CHD is the corrective cardiac surgery. In most cardiac units' congenital cardiac defects are usually referred to at 3-6 months of age, to reduce the incidence of PAH caused by left to right shunt. Children with DS and AVSD or VSD are often referred for surgery earlier than a non-Down syndrome child with the same heart defect (20). Even if the heart disease is "corrected", there is no guarantee that these patients do not develop PH in the future. This may reflect the lack of clinical trials in patients with SD and PAH – CHD. Moreover, several other known causes of PAH have a high frequency in children with DS: a greater incidence of upper airway obstruction, structural lung disease, gastro-esophageal reflux (21) is described and, during the adolescence, patients with DS can develop additional complications which include, most commonly, mitral valve prolapse (46%) and, less commonly, aortic regurgitation (17%) (22). Upper airway obstruction may result from obstructive sleep apnea, a condition well recognized to be associated with DS (23). It has been estimated that obstructive sleep apnea affects just 3% of the general population compared with 30–50% of patients with DS.

In addition, there is evidence that the incidence of Persistent Newborn Pulmonary Hypertension (PPHN) is higher in DS at 5.2% than in neonates without DS and this may relate to the possible presence of pulmonary hypoplasia in this patient (24). An early aggressive treatment of the underlying cause is required to prevent the development of irreversible PH. If airway disease is thought to be the predominant factor in the etiology of PH, an adequate management of this problem is needed. In conclusion, what is clear is that patients with DS represent a significant proportion of the PAH-CHD population. Moreover, improvements in the diagnosis of CHD and its surgical and medical management have produced a significant increase in the number of patients with DS and CHD surviving into adulthood. These results could lead to an increased number of patients in which PAH may develop during their lives. Therefore, this study, although still ongoing, shows the need for close cardiologic follow-up even in patients with DS who underwent corrective surgery.

sPAP as measured by echocardiography has low prevalence in our population, but estimates may be higher in specific subgroups, especially in those with complex CHD. The gold standard for the measurement of hemodynamic parameters is heart

catheterization. Doppler echocardiography cannot measure hemodynamic parameters but only provide an estimate of them. Therefore, the main limitation of this study is the absence of hemodynamic data. On the other hand, echocardiography is considered an excellent screening test for patients with symptoms and/or risk factors for PH (25) with a sensitivity of 83%, a specificity of 72%, and a correlation of 0.7 with invasively acquired estimates (26). Previous corrective surgery, independently of timing, was associated with elevated sPAP values. The management of these patients in specialized centers, guarantees a high quality of care. Analysis of the registry data could be an instrument for quality control and might help identify weak points in assessment and treatment of these patients.

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Cardiac involvement in Lysosomal Storage Diseases

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Lysosomal storage diseases (LSDs) include a heterogeneous group of rare, inborn, metabolic diseases characterized by deficiency of lysosomal enzymes or of other proteins involved in lysosomal function, leading to multi organ system substrates accumulation, with consequent multi systemic clinical presentation. Cardiac disease is particularly important in some group of LSDs as glycogen storage diseases (Pompe), mucopolysaccharidoses and in glycosphingolipidoses (Anderson-Fabry disease and less frequently Gaucher disease). Various cardiac manifestations may be observed including hypertrophic and dilated cardiomyopathy, coronary artery disease and valvular disease. The availability of enzyme replacement therapy (ERT) has changed the natural history of some LSDs such as Pompe disease, thanks to the significant effects on cardiological involvement. In other LSDs such as MPSs or Fabry disease, ERT has been shown to stabilize or slow the progression of heart damage. This imposes the need for a timely diagnosis that allows a rapidonset of ERT.

Lysosomal storage diseases (LSDs) include a heterogeneous group of rare, inborn, metabolic diseases characterized by deficiency of lysosomal enzymes or of other proteins involved in lysosomal function. Resulting intra lysosomal accumulation of underrated specific substrates leads to multiorgan system damage with consequent multisystemic clinical presentation. LSDs present a progressive clinical course, with a broad spectrum of clinical manifestations and organ involvement depending on the specific substrate and site of accumulation. Cardiac disease is particularly important in some group of LSDs as glycogen storage diseases (Pompe), mucopolysaccharidoses and in glycosphingolipidoses (Anderson-Fabry disease and less frequently Gaucher disease) (Table I).

In this review, we focus on cardiac manifestations of Pompe disease, Mucopolysaccharidoses, Anderson-Fabry (breafly) and Gaucher disease. A more detailed description of cardiac involvement in Anderson-Fabry disease is treated elsewhere in this Review Table I shows the main features of lysosomal storage diseases causing heart involvement.

Key words: Lysosomal storage diseases (LSDs), Pompe disease (PD), Mucopolysaccharidoses (MPSs), Anderson-Fabry disease (AFD), Gaucher disease, Enzyme replacement therapy (ERT), Heart, cardiomyopathy, valvular disease

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Pompe disease

Pompe disease, also known as acid maltase deficiency or glycogenosis type II, is a rare autosomal recessive and progressive genetic disease, caused by biallelic mutations of the acid α-glucosidase (GAA) gene located on chromosome 17q25, leading to reduced activity of the lysosomal enzyme GAA (1). This causes intra- and extra lysosomal glycogen storage especially in the heart, skeletal and smooth muscle, and the nervous system (1). Pompe disease is generally classified considering the age of onset as infantile (IOPD) when clinical manifestations start during the first year of life, and late onset (LOPD) when they start afterwards. Childhood, juvenile and adult-onset Pompe disease are examples of the late onset form. The incidence of all types is approximately 1:40 000, while IOPD alone affects about 1 in 140.000 newborns (1).

IOPD is a multisystemic disease affecting several organs and tissues including the heart (1, 2). Infants presenting with increased levels of creatinine kinase (CK), hypertrophic cardiomyopathy (HCM), failure to thrive and muscular hypotonia during the first six months of life. The rapid disease progression leads most untreated subjects to death within the first year of life due to both respiratory and cardiac failure (1, 2). Classic IOPD can be distinguished from non-classic or late-infantile Pompe disease that also starts during infancy but has less severe cardiac involvement and a more positive prognosis (1). LOPD, which occurs during childhood or adolescence and in adults, is characterized by progressive limb girdle weakness with slowly progressive myopathy, usually, without cardiomyopathy.

Cardiac involvement in Pompe disease

Heart involvement is frequent in Pompe disease although pathophysiology is not well known. Classically, some mutations cause abnormalities of cytoskeleton components leading to a failure of the mechanical link between the sarcolemma and the extra cellular matrix. This defect results in a progressive cardiomyopathy with cardiomyocyte degeneration and fibrosis (3). Moreover, due to substrate storage, infiltrative process can affect conduction system (4) and cardiomyocytes, which present vacuolization.

Cardiac involvement in Pompe disease is typically characterized by hypertrophic or dilated cardiomyopathy. In IOPD, accumulation of glycogen in cardiomyocytes and in the cardiac conduction system causes a severe hypertrophic cardiomyopathy with small ventricular lumen (5), which is predominant on the posterior wall of the left ventricle, and the interventricular septum and may be evident already in the prenatal period. Left ventricular outflow tract obstruction as well as reduced ejection fraction may manifest. This causes tachycardia, weak sucking, failure to thrive, and profound sweating and dyspnea during mild efforts. In some patients a dilative component of the cardiomyopathy may reduce contractility leading to low exercise tolerance and chronic heart failure (6). Moreover glycogen storage may lead to conduction disorders such as non-sustained and sustained supraventricular tachycardia, atrial or ventricular premature beats, severe ventricular arrhythmias (ventricular tachycardia or fibrillation), and to inadequate coronary perfusion with consequent sub endocardial ischemia and risk for sudden death. (7, 8, 9, 10).

Electrocardiography findings include short PR interval, high voltage QRS complexes because of left ventricular hypertrophy. Bradycardia in the newborn may disclose glycogenosis storage (11). Echocardiography provides important information for diagnosis of hypertrophic cardiomyopathy. Right and left ventricular walls are thickened. Left ventricular cavity can be smaller because of severe wall hypertrophy which may cause left ventricular outflow tract obstruction and abnormal ventricular compliance (12). In addition, diastolic function may be altered due to left ventricular hypertrophy. In a Dutch patient group (2), the diastolic thickness of the left ventricular posterior wall and cardiac weight at autopsy were increased significantly with age.

Differently to the early IOPD, clinically relevant cardiomyopathy is not frequent in LOPD (childhood, juvenile and adult onset disease). Few reports about echocardiography findings are available. Dilated cardiomyopathy is described in adult patients (4). Biopsy endomyocardial, in this setting, is necessary for diagnosis after other causes of dilated cardiomyopathies in patients have been excluded.

Conduction abnormalities are unusual. Mild and rather non-specific cardiac abnormalities, which can be detected by CMR only in a small proportion of patients with LOPD, seem to result from an interplay between the storage disease and other comorbidities and may not affect short-term to midterm prognosis in patients with LOPD (13).

Treatments

Drug therapy with Angiotensin Converting Enzyme inhibitors, calcium antagonists and beta-blockers can be indicated for treatment of cardiac manifestations of Pompe disease, but these treatments should be used with caution and only by a pediatric cardiologist experienced in treating pediatric patients with heart failure (14).

Thanks to progresses made in understanding the pathophysiology of lysosomal storage disorders, newly targeted therapeutic options have been validated also for Pompe disease (15).The availability of enzyme replacement therapy (ERT) in 2006 transformed the course of the disease with little side effects (16, 17). ERT effectively reverses cardiomyopathy, may improve motor development, and significantly prolongs survival (16). Decrease in ventricular hypertrophy and improvement in ventricular function and synchrony have been suggested as secondary efficacy endpoint of treatment (2, 18). BNP or pro-BNP are usually substantially elevated in the beginning and normalize quickly with start of ERT, reflecting the improvement of cardiac function (6, 14).

Mucopolysaccharidoses (MPSs)

Mucopolysaccharidoses (MPSs) represent a group of lysosomal storage disorders caused by deficiencies of the lysosomal enzymes required for the breakdown of glycosaminoglycans (GAGs) leading to substrate accumulation in various cells and tissues, and progressive multi-organ dysfunction. MPS are characterized by progressive, systemic

Table. I. *Lysosomal storage diseases causing heart involvement.*

DISEASE GROUP AND SUBTYPES	CARDIAC MANIFESTATIONS
Glycogen storage diseases	Hypertrophic or dilated cardiomyopathy
	Heart failure
Pompe disease	Conduction disorders
	Abnormal coronary perfusion
Mucopolysaccharidoses	
I H (Hurler)	Valve desease
I S (Scheie)	Cardiomyopathy
II (Hunter)	Disorders of the conduction system
III (Sanfilippo)	Coronary artery disease
IV (Morquio)	
VI (Maroteaux-Lamy)	
VII (Sly)	
IX (Natowicz)	
Glycosphingolipidoses	
	Cardiomyopathy
Anderson-Fabry disease	Arrhythmias and conduction abnormalities
	Valvular heart disease
Gaucher disease	Valve thickness and calcification
• Type I	Atrial and/or ventricledilatation
• Type II	Left ventricularhypertrophy.
• Type III	

clinical manifestations and a coarse phenotype (19) with a heterogeneous clinical presentation in terms of inheritance (autosomal recessive and X-linked), age of onset (infants, children, and adults) and severity of clinical phenotype. Seven distinct types of MPS disorders (I, II, III, IV, VI, VII, and IX) with 11 specific lysosomal enzyme deficiencies have been described (20). Despite their biochemical and genetic heterogeneity, different types share key clinical features in varying combinations, including joint and skeletal dysplasia with stiffness (except MPS IV where there is laxity) and pain, coarse facial features, corneal clouding, inguinal or abdominal hernias, recurrent upper respiratory tract infections, heart valve disease, carpal tunnel syndrome, and variable neurological involvement (21, 22).

MPS diagnosis is usually made through an initial clinical suspicion, followed by biochemical analysis, including urinary GAGs and enzymatic assays, and confirmed by molecular diagnosis. Thanks to progresses made in understanding the pathophysiology of lysosomal storage disorders, newly targeted therapeutic options have been validated also for MPS (15). ERT is currently available for five type of MPS (MPS I, MPS II, MPS IV A, MPS VI, MPS VII). It has been widely demonstrated that ERT, which is currently also practicable in-home setting for some MPS subtypes (23), is effective in reducing urine GAGs excretion, resolving hepatosplenomegaly and improving cardiopulmonary function (24, 25). Hematopoietic stem cell transplantation (HSCT) has been successfully applied almost exclusively in Hurler syndrome and rarely in MPS VI, but the best results were obtained when it was done before the age of two (26, 27). Gene therapy was also considered as a therapeutic option for MPS (28) but the application of its use in clinical practice is still far away.

Cardiac involvement in MPSs

One of the most important clinical aspects of MPS is cardiovascular pathology, which is frequent and with a wide spectrum of manifestations (29) The onset and severity of cardiovascular defects differ in each type of MPS, with the most described abnormalities being cardiac valve thickening, valvular regurgitation and stenosis, and cardiac hypertrophy. The valve defects and cardiomyopathy result from GAGs accumulation in the spongiosa part of cardiac valves, myointima of coronary arteries, and myocardium (21). Cardiac involvement, especially valve disease, may be seen more commonly in MPS I, II, and VI since the increased accumulation of dermatan sulphate in these subtypes. The myocardium can be hypertrophied, and coronary intimal sclerosis may be present. The left side of the heart is more severely affected than the right side, being the most frequent features mitral/aortic valve stenosis (60– 90% of patients), and cardiomyopathy, which are usually observed in adult age.

Mitral or aortic leaflet thickening and calcification may lead to stenosis or regurgitation, and deformities in cardiac structures resulting in cardiac dysfunction can significantly increase morbidity and mortality in patients with MPS (20). Disorders of the conduction system can be also found in all forms of MPS, due to the deposition of GAGs in the conduction tissue. Atrioventricular block of various degrees is the arrhythmia most frequently found in patients with MPS. Diffuse intimal proliferation from GAGs deposition within coronary arteries can occur.

Generally, the onset and the progression of the cardiologic symptoms are insidious, but some patients, have developed a rapidly progressive disease (30). Cardiologists play a fundamental role in the diagnosis and subsequent follow-up of patients with MPS. The Recommendations of the GICEM (Italian Group of Cardiologists and Metabolic Diseases Experts) indicate the electrocardiogram and the echocardiogram as instrumental level I examinations for evaluation of cardiac involvement in patients with MPS (29). The 24-hour Holter ECG is indicated in the presence of atrioventricular blockages to the basal ECG and/or in the presence of symptoms such as palpitations or syncope. Cardio-CT and cardio-MRI may be useful in symptomatic or paucisymptomatic patients with doubtful stress test, or in the presence of new onset of heart failure to rule out ischemic heart disease and to study myocardial structure and function.

Coronary angiography is indicated as a second level test in symptomatic patients with a positive

or doubtful provocative test and before valve replacement surgery. Right catheterization should be considered before intervention for mitral and / or aortic valve disease, when it is necessary to know the pulmonary pressure and pulmonary vascular resistance. For patients who do not present cardiac involvement at diagnosis, cardiological follow-up is expected every 1-2 years, while it must be more intensive and closer in subjects with heart disease from the beginning (29).

Treatments

Although various treatment strategies have been proposed for the treatment of MPS, to date, two therapeutic options are available and used in clinical practise ERT and hematopoietic stem cell transplantation (HSCT) (31). Evidence from case studies in patients with MPS suggests that HSCT has a positive effect on cardiac manifestations, (32) above all in preservation of cardiac function and regression of cardiac hypertrophy while cardiac valve pathology may increase, despite success fulen graftment after HSCT. None the less, given the high associated risks of HSCT, it is considered a valid option for only the most rapidly progressing forms of MPS I (33).

Currently ERT is available for MPS I (laronidase; Aldurazyme®), MPS II (idursulfase; Elaprase®), MPS VI (galsulfase; Naglazyme®), MPS IVA (elosulfase alfa; Vimizim®), and for the MPS VII (vestronidase alfa-vjbk; Mepsevii™) in the United States, Europe, and over 40 other countries. Similar approaches are also in progress for the MPS-III B by Bio Marin Pharmaceutical Company using recombinant human alpha-*N*-acetylglucosaminidase (NAGLU) fused with a peptide derived from insulin-like growth factor II (IGF-II) (19, 34).

Since MPS are progressive diseases, ERT seems to prevent significant deterioration of the pathology but is not able to reverse it except for LVH. Many authors reported positive or mildly positive effects of ERTon the cardiac diameters in MPS patients (35). Brands et al. (36) studied cardiac abnormalities observed in 24 patients with MPS I, II, and VI and the effect of ERT upon them. They described a significant reduction in the size of the interventricular septum thickness during diastole without significant effects on ejection

Table. II. *Signs and Symptoms of AFD stratified by age.*

Sign/ Symptom	Childhood	Adolescence	Adulthood
Acroparesthesias	+	+	+
Gastrointestinal symptoms (diarrhea, abdominal pain, vomiting, nausea, postprandial vomiting)	+	+	+
Cornea verticillata	+	+	+
Angiokeratoma	+/-	+	+
Hypo/anhidrosis	+/-	+	+
proteinuria	-	+/-	+
End-stage renal disease	-	-	-
Levt ventriculat Hypertrophy	+/-	+/-	+
Cardiomyopathy	-	-	+
Arrhythmias and conduction abnormalities	+/-	+/-	+
Heart valve disease	-	+/-	+
Strokes	-	-	+

+ present; - absent; -/+ occasionally present.

fraction, valve disease, or other cardiac parameters. These findings, together with other clinical reports (37, 38), suggest that ERT has limited effect on the cardiac valves in MPS patients which are probably only slightly accessible to ERT, whereas it has some effect on cardiac dimension (36).

Moreover, Braunlin et al studied the effects of ERT in patients with MPS VI and demonstrate that long-term ERT is effective in reducing intraventricular septal hypertrophy and preventing worsening of cardiac valve damage when administered to patients <12 years of age. (39) Subsequently Dafne et al. confirmed the beneficial effects of precocious ERT in patients with MPS VI. (40) All these evidences show that ERT can provide stabilization of damage, so it is best to initiate treatment before cardiac organic manifestations (41).

Despite these encouraging results, due to diagnostic delay, most patients still require specific drug therapy for heart disease. Given the high incidence of left ventricular hypertrophy, the most used drugs are ACE inhibitors and angiotensin II receptor antagonists (sartans). The use of beta-blockers must be cautious, especially in the presence of atrioventricular blockages and respiratory problems. Diuretics are indicated in patients with signs and symptoms of heart failure, while subjects with severe valvulopathy should carry out antibiotic prophylaxis for endocarditis.

In addition, surgical possibilities have been explored for patients with MPS. Open-heart operations in patients with mucopolysaccharidoses are extremely rare because of multiple issues such as poor life expectancy, multiple infiltrated organs (myocardial tissue included) and, especially, airway complications. There are few reports of surgical management of cardiac abnormalities in MPS patients (42).

Anderson-Fabry Disease

Anderson-Fabry disease (AFD) is an X-linked lysosomal storage disorder caused by deficiency of the lysosomal enzyme α-galactosidase A, leading to progressive accumulation of glycosphingolipids, predominant lyglobotriaosylceramide (Gb3), in lysosomes of various cell types throughout the body causing a multisystemic clinical presentation including neurological (43, 44), ocular, skin, gastrointestinal (45, 46), renal, and cardiac manifestations. Clinical manifestations of AFD first appear in childhood or adolescence (47) and include acroparesthesias, gastrointestinal symptoms, hypohidrosis, and mild proteinuria, although her signs of renal involvement cannot be excluded even in children (48). In adulthood, progressive renal, cardiac, and cerebral involvement (stroke) characterize clinical picture and could be the major causes of death related to AFD. An extreme intra and inter-familial variability of clinical presentation has been described although to date the causes that underlie this extreme variability are still not clear (49, 50).

The comprehension of the pathophysiological mechanisms that underlie AFD as well as that of other LSDs (15), allowed the realization of a specific cause-therapy for this disease. ERT, which became available in 2001, in two different enzyme preparations: agalsidase alfa and an alsidase beta. Clinical studies and emerging evidence suggest that ERT may slow disease progression and improve many of the features of AFD (51, 52), although response to therapy appears to depend heavily on the stage of the disease (51, 53) and on other variables not yet fully understood (54). Table. II shows signs and symptoms of AFD stratified by age.

Cardiac manifestations are frequent in AFD and are one of the most important causes for reduced life expectancy and disease-related mortality (55). Cardiac manifestations including hypertrophic cardiomyopathy, valvular abnormalities, arrhythmias and chronic heart failure occur frequently in patients with AFD (56, 57), and early signs of cardiac abnormalities can be found in pediatric age. With the aim of identifying a diagnostic tool or screening parameter for high-risk patients, as well as a tool for evaluating the response to ERT, several biomarkers as lyso-Gb3 (58,59), sphingosine-1 phosphate (S1P) (60), and high-sensitivity troponin (hs-TnT) (61), and their correlation with cardiac involvement have been studied, as it was done for the evaluation of other organs (62, 63).

ERT resulted in a significant reduction in left

ventricular mass, with improvement in regional myocardial function and in exercise capacity (53, 64, 65), although some studies didn't show significant changes in left ventricular mass and/or ventricular wall thickness after ERT (66, 67) and this poor response to ERT is highly related to the extent of myocardial fibrosis suggesting that an early start of ERT is always desirable. A more detailed description of cardiac involvement in AFD is treated elsewhere in this review.

Gaucher disease

Gaucher disease (GD) is a rare autosomal recessive lysosomal storage disease and the most common sphingolipid storage disorder (68). GD is caused by a functional deficiency of the lysosomal acid hydrolase, β-glucocerebrosidase (GBA), an enzyme that converts glucocerebroside to ceramide and glucose. The lack of the enzyme causes the accumulation and the storage of the substrate, glucosylceramide, in the reticuloendothelial cells

Table. III. *Clinical features and classification of GD subtypes.*

	GD1 **Non neuronopathic** **(adult)**	**GD2** **acute neuronopathic** **(infantile)**	**GD3** **chronic/subacute neuronopathic** **(juvenile)**
Age at onset	Young adults/adults	Infants	Children/young adults
Distinguishing Symptoms	Liver and spleen involvement - Hepatomegaly - Splenomegaly Bone involvement: - acute bone crisis, chronic bone pain, fractures, medullary infarction, osteonecrosis, osteopenia, pathological fractures, cyst-like or lytic lesions, skeletal deformities, growth failure Hematological complications: - Thrombocytopenia, leukopenia, anemia - coagulation or primary hemostasis disorders No neurological impairment	Early and severe neurological impairment: - hypotonia - progressively impaired cognition - impaired hearing - ocular involvement (impaired vision, ocular apraxia, strabismus, ophthalmo paresis - bulbar and pyramidal signs Severe visceral disease	Later onset of nervous system problems: - incoordination - mental deterioration - myoclonic seizures, progressive myoclonic epilepsy - oculomotor apraxia, supranuclear gaze palsy, saccadic eye movements. - dementia,ataxia Visceral disease GD3a: dominant neurological manifestations, mild visceral disease GD3b: massive visceral disease and skeletal manifestations, mild neurological signs GD3c: visceral disease and cardiac involvement.
Disease course	variable ranging from asymptomatic to mild or severe forms	Death in infancy (before the second year of life)	Slowly progressive becomes severe later in childhood

of the liver, spleen, bone marrow and lungs leading to visceral manifestations of GD. GD can be considered a multi-systemic chronic disorder with hematological, visceral and bone damages (68). The characteristic lipid-laden cells, called Gaucher cells, can be found in the spleen, liver, bone marrow and lung causing hepatosplenomegaly, pancytopenia, bone complications, lung involvement (68).

Traditionally, GD is subdivided in three major phenotypic variants based on, the age of onset, progression, and especially distinguished by the absence (type 1, GD1) or presence (types 2, GD2) and (type3 GD3), of neurologic involvement (68, 69). GD1 is the most common subtype and is characterized by the absence of central nervous system involvement. The main clinical manifestations include anemia, thrombocytopenia, skeletal complications (osteopenia, lytic lesions, pathological fractures, chronic bone pain, bone crisis and infarcts, osteonecrosis, skeletal deformities) and liver failure while lung involvement can be found in a small number of patients (69, 70, 68).

GD2 is the acute neuropathic form with severe prognosis and reduced life expectancy. It manifests in early childhood; usually neurological deterioration progresses quickly and death generally occurs before the age of 2 years. GD3 can be considered as an intermediate form between types 1 and 2, called subacute neuropathic form usually occurs later, in adolescence and shows a slower neurological involvement. Neurological symptoms include supranuclear gaze palsy, spasticity, myoclonus, ataxia, seizures and intellectual deterioration (71).

Patterson et al. proposed a subdivision of type 3 Gaucher's disease: patients with several neurological complications as myoclonus, ataxia, brainstem signs, and intellectual deterioration but with relatively mild visceral manifestations should be classified as type 3a while patients with supranuclear horizontal gaze palsy and with aggressive visceral involvement as type 3b (72). Type IIIC was recently descripted in patients with the homozygous missense variant (D409H) in *GBA* gene. They present cardiac valvular calcifications and neurological manifestations (73). In Table III, clinical features and classification of GD subtypes are described. Despite this traditional classification, several evidences demonstrate that many genetic and environmental factors have a role in the disease phenotype (74).

Cardiac manifestations in Gaucher disease

Although cardiac involvement was considered a very unusual manifestation of GD and very few cases have been described, a rare variant of type III, called type IIIc, has been described, characterized by severe valvular and aortic arch calcifications seen at a young age in patients homozygous for the D409H mutation (1342C) in the GBA gene (73, 75, 76).

Cardiac manifestations of GD include increase of mitral and/or aortic valve thickness due to the accumulation of calcium or infiltration with Gaucher cells (73, 77) sometimes even associated with calcification of the ascending aorta (76). Cardiac valves thickness and stenosis can be associated with various degree of valvular insufficiency or regurgitation (73, 77). In some cases, echocardiography may also show atrial and/or ventricle dilatation (73, 75) or left ventricular hypertrophy (75). Despite the Type IIIC is most frequently associated with cardiac anomalies, some authors have studied cardiac involvement also in GD1 (69, 78-80).

Solanich et al. described the case of a 62- year-old woman affected by GD1 on ERT with biatrial enlargement, dilated right ventricle, mitral valve leaflets were thickened and patchy intramyocardial hyper enhancement with multiple foci detected by CMR consistent with interstitial infiltrative fibrosis but in absence of cardiac symptoms (78). Peric et al. described a GD1 60-year-old female, not on ERT with history of aortic valve pathology since the age of 30 years, who developed heart failure in whom echocardiography documented a severe fibrotic aortic valve stenosis (79). The development of pulmonary artery hypertension (PAH) is a rare event in GD1 and the incidence is variably, up to 20%, defined by Doppler echocardiographic measurements (81). Generally, PAH improves on ERT, but in some patients, it may worsen (82).

Roghi et al. studied cardiac involvement in nine GD1 patients using cardiac magnetic resonance (CMR) including the evaluation of myocardial

infiltration and valve dysfunction. They found that three patients presented mild left atrial enlargement, one patient showed moderate aortic stenosis in bicuspid valve with mild aortic dilatation and one patient showed moderate mitral regurgitation. Tissue characterization with T1-weighted and T2-weighted short time inversion recovery images was normal. No evidence of myocardial enhancement was present after gadolinium contrast media. None of the enrolled patients presented pulmonary hypertension. Atrial dilatation has been described in GD patients with valvular diseases and in those with pulmonary hypertension. On the contrary, in this study this finding was unrelated to valvular diseases, pulmonary hypertension, left ventricular impairment, or myocardial fibrosis (69).

Lo Iudice et al. investigated the left ventricular geometry and function in 18 patients with GD1, compared to 18 normal controls and hypertensive patients. The study demonstrated an association between GD1 and subclinical left ventricular diastolic dysfunction, not correlated with the coexistence of arterial hypertension. The authors suggested the possible role of myocardial infiltrative damage in the pathogenesis of left ventricular diastolic dysfunction (80).

As described above, cardiological involvement plays an important role in Gaucher disease. Roghi et al. recommend a noninvasive cardiac evaluation using both echocardiography and CMR in any patient with GD to detect asymptomatic abnormalities and associated cardiovascular diseases, and to prevent ventricular dysfunction and heart failure (69). Moreover, the association between D409H mutation and cardiac calcification necessitates a complete cardiac evaluation and cardiac surveillance recommending annual echocardiogram for patients harboring the mutation D409H as a measure to detect the disease earlier (73).

Treatments

Many therapeutic options are today available for the treatment of Gaucher disease. The introduction of Enzyme Replacement Therapy (ERT) in 1991 was a real revolution in the management of GD patients. Imiglucerase (CerezymeTM, Sanofi/ Genzyme) replaced Alglucerase (CeredaseTM, Genzyme) in 90's and was the only ERT available for nearly 15 years. Later, two new ERTs have been developed: velaglucerase alfa (VPRIV TM, Shire) and taliglucerase alfa (ElelysoTM, Protalix/ Pfizer). Today, the availability of these 3 ERTs may differ between different countries. Recently imiglucerase biosimilar have developed and they are already available in some countries.

ERT is considered very safe for all groups of age and all GD types and, regarding efficacy, ERT has defined an important improvement in the natural history of the disease; many of the symptoms and signs of visceral GD, such as hepatosplenomegaly, as well as anemia, thrombocytopenia and often skeletal or lung involvement respond adequately to ERT (83). While ERT represents the main treatment for GD1, the iminosugar miglustat (Zavesca®; Actelion Pharmaceuticals Ltd.) is considered an alternative treatment strategy (substrate reduction therapy or SRT) (84) for adult patients with mild to moderate GD1, for whom ERT is 'unsuitable', although encouraging results have been obtained through combination therapies in patients with neuropathic subtypes (85). These therapies prevent the accumulation of the substrate, glucosylceramide, inhibiting its formation through a direct action on glucosylceramide synthetize. Miglustat is proven to be effective in reducing liver and spleen volume, increasing hemoglobin concentration and platelet count and improve bone mineral density (86).

More recently, a second SRT, eliglustat tartrate (Cerdelga TM, Sanofi/Genzyme), as first oral SRT for patients with GD1, is available in Europe from 2015. It is a ceramide analog that inhibits glucosylceramide synthase, reducing the synthesis of glucosylceramide and balance production with the impaired rate of degradation (87). To perform this therapy, a genotype investigation is required because not all patients with GD are eligible for treatment.

Pharmacological chaperone (PC) therapy is a new strategy to increase residual activity by stabilizing misfolded mutant proteins. PCs are typically small molecules capable of crossing the BBB, so they are considered a hope for patients with GD3, in which ERT was only effective for the non-neuropathic features. Some PC have already been tested and it

is hoped that one day some of them have been used to improve neurological symptoms (87). Stem cell therapy has also been proposed in the treatment of many metabolic disorders including Gaucher Disease (88).

Unfortunately, cardiac manifestations were fatal in most reported cases (73, 76). Some of the reported patients described in literature received ERT but did not seem to stop the calcification. Kör et al. conclude that even though ERT reduce glucocerebroside storage, relieving some symptoms, cannot resolve existing calcifications so a late diagnosis leads to disease progression and increased morbidity and mortality. In conclusion, therefore, early diagnosis would significantly reduce morbidity and mortality in such patients (76).

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Covid-19 and cardiac involvement in childhood: state of the art

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Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was first described in a cluster of patients in Wuhan, China, in December of 2019. Over the past few months, COVID-19 has rapidly spread worldwide becoming the first pandemic of the 21st century. COVID-19 results in mild symptoms in most infected children but can cause acute cardiac injury and death. In comparison to younger children, teenagers and infants are at higher risk for morbidity and mortality, with particular risk factors including pre-existing conditions like cardiovascular disease. Since this is an emerging infectious disease, there are limited data about the effects of this infection on patients especially in the pediatric population. We summarize here with the data on cardiovascular involvement in children and adolescents.

SARS-CoV-2 causes epidemics with variable clinical severity presenting with respiratory and extra-respiratory manifestations (1). Phylogenetic analysis of SARSCoV-2 suggest that SARS-CoV-2 uses ACE2 as a receptor to enter human cells, ACE2 is a membrane-bound protein that is expressed in different human cells, like vascular, endothelia, renal tissue, cardiovascular tissue, and small intestine epithelia (2) There appears to be two clinical stages to the disease. The first one consists in the replication of SARS-CoV-2, it lasts several days and the symptoms are relatively mild. The second stage consists in the immune response of the body. This leads to resolution of symptoms in most patients; unfortunately, some patients become critically ill and have a high risk of mortality.

The most common scenarios in these patients are acute respiratory distress syndrome (ARDS), multi-organ failure, secondary bacterial infections, viremia and acute cardiac injury (3, 4) The current data seems to indicate that the viral infection is capable of producing an excessive immune reaction in the host. In some cases, a 'cytokine storm' occurs of which the effect is extensive tissue damage. Interleukin 6 (IL-6) triggers this storm and acts on many cells and tissues. This cytokine storm may result in an acute systemic

Key words: Coronavirus disease 2019, COVID-19, SARS-CoV-2, acute cardiac injury, cardiovascular disease, children

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inflammatory syndrome characterized by fever and multiple organ dysfunction (5).

The pediatric population appears to be affected in much smaller proportions than adults, also COVID-19 results in mild symptoms in most infected children. This difference is due to having a more active innate immune response, healthier respiratory tracts and fewer underlying disorders. In addition, parents overprotect children, and if there is not any positive case in family, they have less chance of being infected decreases (6). The difference between adults and children in case of severity may be related to the ACE2 (the binding protein for SARS-CoV-2); in children, it is not as functional as it is in adults, and thus SARS-CoV-2 is less infectious (7).

According to the available data, fever, cough and shortness of breath are the most common reported symptoms in children (30). COVID-19 in children appears to be usually mild, but can cause acute cardiac injury, and death. In comparison to children, teenagers and infants are at higher risk for morbidity and mortality, with particular risk factors including pre-existing conditions like cardiovascular disease. A minority of children with COVID-19 require hospitalization. Subjects with certain serious underlying conditions and who are <1 year of age are at higher risk for severe diseases (8). With the increasing number of confirmed cases and the accumulating clinical data, in addition to the common clinical presentation of respiratory failure caused by COVID-19, the cardiovascular manifestations induced by this viral infection has generated considerable concern.

Diagnosis

Pediatric patients should be evaluated according to complaints, clinical findings, and exposure history (9) Firstly, it should be identified if a child has travelled or resided in an endemic area for COVID -19 or if he had contact with suspected or confirmed COVID-19 patient in the previous 14 days. This information helps to determinate the risk level. Afterward it should be identified if the patient has sign and symptoms of respiratory infection: fever, fatigue, anorexia, any symptom of respiratory disease like cough and shortness of breath, and any gastrointestinal symptoms like diarrhoea. Complete blood count should be tested. Common laboratory findings are lymphopenia, neutrophilia, elevated C reactive protein levels. Chest radiographs typically demonstrate bilateral air-space consolidation. Further examination is carried out for suspected patients. If a case is positive for nCoV-2019 in nasal/pharyngeal swap or blood samples by polymerase chain reaction (PCR) assay or if samples from the respiratory tract or blood samples are similar to nCoV-2019 genetically, then the case is defined as confirmed (8).

Transmission

According to the current data, SARS-CoV-2 is spread through droplets, contact, and entry through ocular tissue. Fecal shedding has been seen in up to 30% of patients, Respiratory droplets transmit the virus when patients cough, talk loudly or sneeze. Mother to infant transmission of SARS-CoV-2 is controversial. To date, there is minimal evidence of placental vertical transmission; probably most maternal to neonatal transmission occurs at birth or shortly after via droplet contamination (10, 11). Placental seeding and vertical transmission is unlikely. Transmission with breast milk is another matter of debate. It is not clear if the virus transmits through breast milk or via feeding the baby. Consensus guidelines for infants born to mothers with COVID-19 are not yet finalized, though are in process. Wang's report suggested that infants should not be fed with breast milk from mothers with neither confirmed nor suspected of 2019-nCoV. If breast milk of these mothers tested negative for 2019-nCoV, then infants may be fed with breast milk (1).

Cardiovascular involvement - acute myocardial injury

Acute myocardial involvement in coronavirus disease 2019 (COVID-19) is currently described as 'acute cardiac injury', defined as blood levels of cardiac biomarkers (high-sensitivity troponin I (hs-TnI)) above the 99th-percentile upper reference limit (12) It is described in more than 20% of adult patients and seems to be related to increased mortality (13, 14). The diagnosis is based upon clinical data, imaging and biomarkers of acute cardiac damage

(15, 16).

In adults, myocardial injury is associated with impairment of cardiac function, ventricular tachyarrhythmias and has a significant association with fatal outcomes of COVID-19, (17). There are two different ways by which COVID-19 may lead to myocardial injury: one way is indirect, due to the cytokine storm and the immune inflammatory response (18), second way may be due to SARS-CoV-2 direct viral invasion in cardiomyocytes but it has not yet been proven in pathology studies (19) The virus may cause acute respiratory damage (ARDS) with severe hypoxia, this may lead to oxidative stress, increased myocardial oxygen demand and myocardial injury. Another possible explication for cardiac injury is the expression of ACE2 in the heart, and the SARS-CoV-2 virus uses this enzyme as a receptor to enter inside the cell (20). While the mechanism of cardiac injury is not fully described, many studies documented COVID-19 and its effects on the cardiovascular system.

Kawasaki-like disease

Kawasaki disease is an acute self-limiting vasculitis with specific predilection for the coronary arteries that affects previously healthy young infants and children. In the past 20 years, viruses of the coronavirus family have been proposed as possibly implicated in the pathogenesis of Kawasaki disease. The coronavirus might represent one of the triggers of Kawasaki disease, SARS-CoV-2 being a particularly virulent strain able to elicit a powerful immune response in the host. An Italian study reports a high number of Kawasaki-like disease cases in the Bergamo province following the SARS-CoV-2 epidemic, with a monthly incidence that is at least 30 times greater than the monthly incidence of the previous 5 years. In this study, the clinical and biochemical features of patients with Kawasaki disease diagnosed during the COVID-19 pandemic appeared to differ from their historical cohort of patients; therefore, they have classified these patients as Kawasaki-like disease.

From a clinical perspective, these patients were older, had respiratory and gastrointestinal involvement, meningeal signs, had a higher rate of cardiovascular involvement and features of macrophage activation syndrome (MAS) compared to patients diagnosed with Kawasaki disease in era pre-Covid. From a biochemical perspective, they had leucopenia with marked lymphopenia, thrombocytopenia, and increased ferritin, as well as markers of myocarditis. Similar clinical features are shared by patients with COVID-19. The association between SARS-CoV-2 and Kawasaki-like disease should be taken into account when it comes to considering social reintegration policies for the paediatric population. However, the Kawasaki-like disease remains a rare condition (21).

Underlying cardiovascular disease (CVD)

Patients with underlying heart disease (both congenital and acquired) seem to have increased morbidity and mortality related to viral infections (22, 23). A previous study on adult population demonstrates that patients with underlying CVD and other comorbid conditions are more prone to experience myocardial injury during the course of COVID-19. For patients with underlying CVD, including hypertension, coronary heart disease, and cardiomyopathy, viral illness can further damage myocardial cells through several mechanisms including direct damage by the virus, systemic inflammatory responses, destabilized coronary plaque, and aggravated hypoxia.

Therefore, patients with CVD are more likely to experience myocardial injury after COVID-19 infection and have a higher risk of death (17) Children have mild symptoms in general, though we do not yet have data specifically about pediatric patients with_underlying CVD. There are some case series to suggest higher frequency of pediatric COVID-19 cases in infants <1 year of life with pre-existing conditions like cardiovascular disease compared with older children, but data is extremely limited (24). A very recent Italian report showed that patients with underlying CVD and other comorbid conditions were more prone to experience myocardial injury during COVID-19 (25).

Congenital heart disease

As far as we know, in the literature, there is only

one multi-centre, observational survey that describes patients with congenital heart disease (CHD) and COVID-19. The percentage of COVID-19, infected patients in Italy, was very high but the total number of these patients with CHD was relatively limited. The suggested reasons for this phenomenon were: the reduced chances of exposure of those patients, or the incomplete recognition due to mild or asymptomatic disease, rather than resistance to infection. In this Italian survey has been observed a high amount of myocardial involvement in CHD COVID-19-infected patients but no deaths were observed in the whole cohort of patients with CHD. They did not observe, in their cohorts of CHD patients, the same critical respiratory outcomes and the higher mortality risk previously described for cardiovascular diseases other than CHD. These finding suggested that the occurrence of cardiovascular risk factors, like older age, obesity, hypertension and diabetes (which is much lower in CHD patients than in general population) may play a major role in determining COVID-19 mortality, rather than cardiac disease "per se" (25).

Preventing getting sick

Since patients with underlying cardiovascular disease are at higher risk of morbidity and mortality, children with CVD should take great care in preventing infection. COVID-19 can be inactivated by lipid solvents, including ether (75%), ethanol, chlorine-containing disinfectant, peroxyacetic acid and chloroform except for chlorhexidine (26.5). Patients with congenital heart disease should be vaccinated against influenza and pneumococcal pneumonia (27) These patients should practise everyday preventive measures like: frequent handwashing, social distancing, proper and appropriate use of masks and other personal protective equipment, and cleaning and disinfecting commonly touched surfaces (26). If possible, regularly scheduled clinic visits should be converted to telehealth visits to minimize risk of acquiring an infection in the nosocomial setting (28).

There is only limited data detailing the effects of COVID-19 on the pediatric population but even if COVID-19 in children appears to be usually mild, it can cause acute cardiac injury, and even death. Pediatric patients with underlying cardiovascular comorbidities are at increased risk of morbidity and mortality from SARS-CoV-2 infection (29). While no studies on COVID-19 have included patients with congenital heart disease, it stands to reason that patients with congenital heart disease could be considered at higher risk for complications from COVID-19. The main point for COVID-19 infection is 'not to be infected.' Infection control precautions should be carried out carefully since the current pandemic of coronavirus might not be the last.

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Bicuspid aortic valve in children: importance of aortic shape, role of follow up and risk of aortic dilatation

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Patients with bicuspid aortic valve (BAV) have a higher risk of developing aortic disfunction and progressive proximal aorta dilatation. Some of these complications already appear in paediatric age, even if most children are symptomless. Partly, vascular involvement may be due to typical molecular alterations in association with valvular dysfunction. In paediatric patients, the correlation between dysfunction grade, aortic dilatation and aortic shape is not well documented but nowadays it is a matter of growing interest. We consider aortic shape as a possible predictor of the aortic dilatation's progression. Identifying the risk factor of aortic dilatation during childhood is useful to optimize a follow-up. This should be considered in prophylactic therapy.

Bicuspid aortic valve (BAV) is the most common congenital heart disease, affecting about 1% of population. BAV is commonly known not only as a disorder of valvulogenesis, but it also represents coexistent aspects of a genetic disorder of aorta and/or cardiac development. Even if many children are completely symptomless, they are predisposed to different complications regarding the valve itself (regurgitation, stenosis, endocarditis) and vessels (dilatation, aneurysm, dissection) (1). Studies have reported that approximately 50% of subjects with BAV show aortic root dilatation (2).

Some authors have suggested correlation between BAV and aorthopathies due to haemodynamic perturbations: the turbulent flow jet passing through the BAV into the aortic root increases sheer stress on the aortic wall, producing vessel enlargement (3). Another proposed mechanism considers inherited weakness of aortic wall that leads to progressive dilatation (4), with a fundamental role of genetic factors (5, 6). BAV seems to be inherited in an autosomal dominant pattern with increasing prevalence among first-degree relatives. The most common gene identified is NOTCH1 (5).

Pathogenesis

The typical histologic alteration is known as Erdheim medial necro-cystic degeneration. This alteration includes increased vascular smooth muscle cells apoptosis with a reduced production of extracellular matrix proteins, elastic

Key words: bicuspid aortic valve, BAV, children, aortic dilatation

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fragmentation and deficiency of fibrillin-1, which leads to matrix disruption. In addition, molecular analyses have detected an increased production of metalloproteinases (most of all MMP-2 and MMP-9) with altered equilibrium between these and their tissue inhibitors (TIMPs) (4). This characteristic plays an important role in generating aortic aneurysms. Another probably involved factor is TGF-β (7). Its hyperexpression increases elastic fibres fragmentation and reduces vascular smooth muscle cells connections.

Many currently published studies focus on the identification of risk factors of unfavourable outcomes of BAV during adulthood; but just few of them focus on paediatric population. The incidence of severe primary cardiac events in children with BAV in relatively low (8), however aortic dilatation already appears in paediatric age.

Different valvular morphologies seem correlated to different complications: bicuspid aortic valve with left-right coronary cusps fusion is associated with higher risk of aortic dilatation, otherwise non-coronary and left/right cusps fusion most frequently present valvular defects (9). In addition, valvular function plays an important role in determining progression rate of enlargement. Patients with severe aortic stenosis and moderate-severe insufficiency seem to have a faster progression rate than others (10). In our previous retrospective study, we purposed to correlate aortic shape with age, valvular dysfunction and general grade of dilatation.

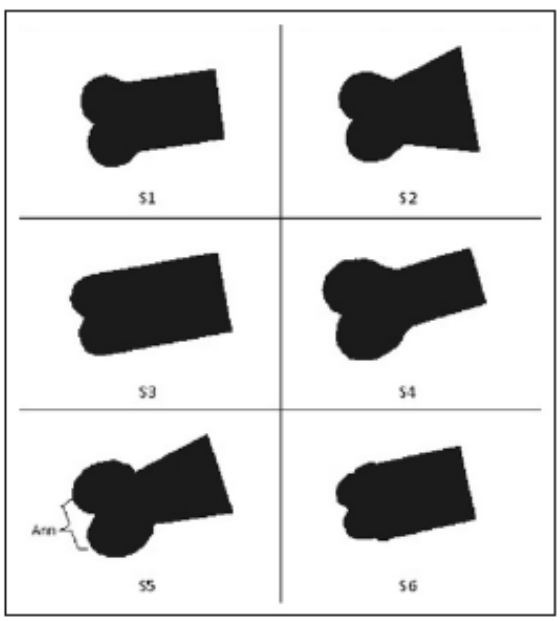

Fig. 1. ***S1*** *- normally shape aorta with single dilatation of annulus;* ***S2*** *- enlarged ascending aorta;* ***S3*** *- effacement of the sinotubular ridge;* ***S4*** *- Marfan-like with enlarged sinus;* ***S5*** *-enlarged sinus of Valsalva and ascending aorta;* ***S6*** *- normal annulus and enlarged sinus of Valsalva, sinotubular ridge and ascending aorta.*

Indeed, considering paediatric population, six patterns of dilated aorta were found (Fig. 1) (11). Studies on adults identified four aortic shapes (12-15), corresponding to the first four group in children, likely S5 and S6 shape progressively turn in another shape during years (11).

Patients with bicuspid aortic valve (BAV) should be considered as a risk population for developing aortic dysfunction and progressive proximal aorta dilatation during growth. Paediatric cardiologists are aware about risk of valvular complications in children, especially when a rapid progression is highlighted in the first years of life. Aortic enlargement in children with bicuspid aortic valve is not well described in literature. This situation does not occur in a homogenous manner (16) and today the focus on vessels' shape patterns appears interesting to understand the mechanisms of vascular dilation. Aortic valve dysfunction seems to be an independent factor from aortic shape, but it could contribute to aggravate aortic enlargement, probably because of a hemodynamic effect (17).

Similarly, the role of therapy is not well standardized. Even if heart failure therapy in severe valvular involvement is fundamental, the role for prophylactic strategy to avoid future complication is not clear. Focusing on therapy, Losartan was the most frequently used drug. This is because of its double role: it has an antihypertensive effect, but it also acts as TGF-β antagonist (18). Considering that it was noticed as a hyperexpression of this marker in patients with BAV and aortic aneurysm, Losartan could have an "etiopathogenetical" role, as already tapped in Marfan syndrome (18). Further studies will be necessary to validate aorticshape role in thefollow-up of patients with BAV and aortic dilatation to improve the accuracy of prophylactic therapy and timing of echocardiographic controls.

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Preterm Patent Ductus Arteriosus: Controversies Overview

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For over five decades healthcare providers have argued whether to treat the patent ductus arteriosus (PDA) in preterm infants or not and if so, when and how. On the one hand, physiology and statistical association with adverse outcomes suggest that it is pathological. On the other hand, clinical trials of treatment strategies have failed to show any long-term benefit. We provide a chronicle of the evolution of knowledge about the ductus arteriosus (DA) and the varying nature of controversies concerning the determination (and definition) of hemodynamic significance PDA (hsPDA), appropriate identification of infants for therapy, selection of treatment regimen, and the exact impact of PDA treatment on meaningful short- and long-term outcomes.

Historical highlights

In the history of human medicine, the DA occupies a most singular position. The first description of a fetal vascular conduit interconnecting the aorta and the pulmonary artery without understanding its function was described by Galen in the 2nd century A.D. in his "*De usu paritum*":

"Neither vessel (*Foramen ovale* and *Ductus arteriosus*) is small or the result of an accident; on the contrary, they are very broad and have notable lumens, which could not fail to be recognized not only by anyone having eyes but even by anyone having a sense of touch, provided that he wished to busy himself with dissecting" (1). "And so it is right to admire nature here too, because when the *viscus* (the lung) needed only to grow, she supplied it with pure blood, and when it was changed so that it moved, she made its flesh light as a feather in order that it might be easily dilated and compressed by the thorax. This is the very reason, why a passageway (the *Foramen ovale*) was made connecting the *Vena cava* and the venous artery (*V. pulmonalis*) in the fetus. In as much as this vessel (the *V. pulmonalis*), however, serves as a vein for the viscus, it was necessary, I suppose, for the other one (*A. pulmonalis*) to change into the usefulness of an artery, and therefore nature also connected this one to the great artery (the aorta), but in this case, because there was a space between the vessels, she created another, small, third vessel (the *Ductus arteriosus*) to join the two" (2).

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A more accurate description of the Galen's circulatory paradigm appeared only 13 centuries later when both Servetus (3) and Colombo (4) recognized the role of the pulmonary transit (Fig. 1): "Not just air, but blood mixed with air is sent from the lung to the arteria venosa (the pulmonary vein). Therefore, the mixture occurs in the lungs...Thus the vital spirit is transfused from the left ventricle into the arteries of the whole body".

In the same year, the Italian surgeon Leonardo Botallo pretended to have discovered the *Foramen ovale* ("*Vena arteriarum nutrix* to *nullo antea notate*") in an appendix to his monograph de catarrho (5). There is no mention of the ductus arteriosus, also not in the 1641 reedition of Botallo's de via sanguinis (6-7). Due to misinterpretation of a publisher footnote, Botallo became famous and made his way into the nomina anatomica at the Basel Conference (8) in 1895.

Embryology

In normal cardiovascular development, the proximal portions of the sixth pair of embryonic aortic arches persist as the proximal branch pulmonary arteries, and the distal portion of the left sixth arch persists as the DA, connecting the left pulmonary artery with the left dorsal aorta. Normally, the distal right sixth aortic arch loses its connection to the dorsal aorta and degenerates. This process is complete by the eighth week of fetal life and is vital for normal development of fetal circulation throughout gestation (Fig. 2).

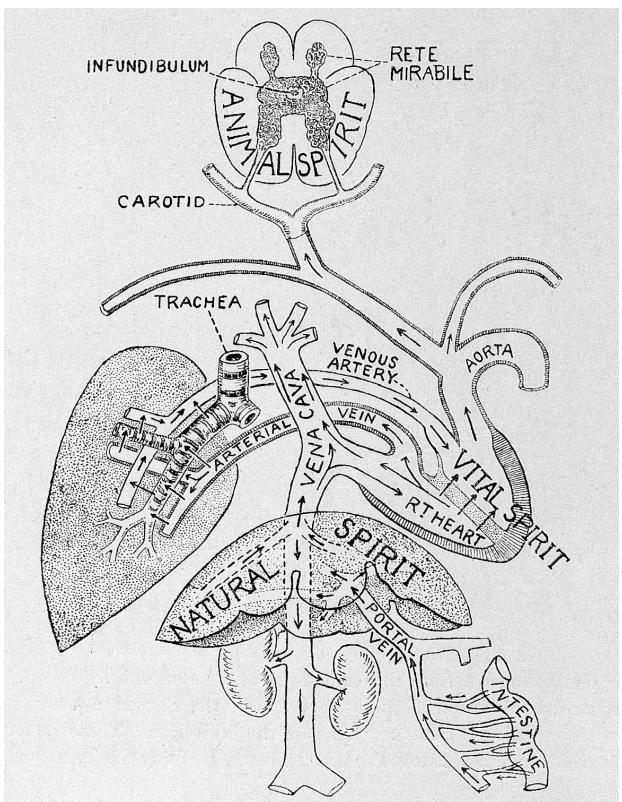

Fig. 1. *Diagram illustrating Galen's physiological scheme; with permission of the Wellcome Collections (Licensed Creative Commons Attribution CC BY 4.0).*

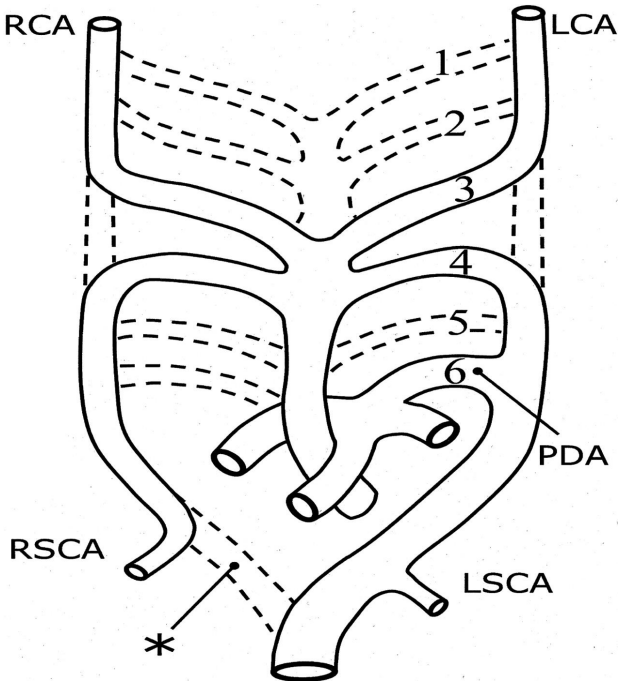

Fig. 2. *Embryonic aortic arch system. The six pairs of embryonic aortic arches are demonstrated (left-sided arches are numbered). The portions that normally involute are indicated by broken lines. The distal left sixth embryonic arch normally persists and becomes the patent ductus arteriosus (**PDA**) (*) eighth segment of the right dorsal aorta; **RCA** right carotid artery; **LCA** left carotid artery; **RSCA** right subclavian artery; **LSCA** left subclavian artery.*

Mechanisms of Normal Closure

Factors responsible for fetal patency of the DA include relatively low oxygen tension and arachidonic acid metabolites primarily prostaglandin (PGE_2), a potent relaxant of the vessel, and prostacyclin (PGI_2), (9) which are regulated by placental and

pulmonary metabolism. Postnatally, the process of DA closure occurs in two sequential steps-functional and structural. The first is due to overlapping of both physiological increase in blood oxygen tension and fall of PGE_2. Oxygen inhibits potassium and activates calcium channels, which ultimately leads to a rise in intracellular calcium concentration consequently inducing phosphorylation of the myosin light chain and thereby constriction of the spiral medial muscle layer with shortening and thickening of the ductus. Synergistically, withdrawal of PGE_2, a potent relaxant of the vessel and a prime determinant for ductal patency in utero, after elimination of the placenta contributes to its constriction.

The second, the structural phase, is a remodeling process leading to a permanent closure. It initiates antenatally and may encompass 4 distinct mechanisms: (i) development of intimal cushions; (ii) mechanical solicitation from turbulent blood flow along the narrowing lumen; (iii) intramural hypoxia due to the collapse of vasa vasorum in the constricting DA; (iv) interaction of platelets with the vessel wall (10).

Clinical epidemiology and natural history of PDA

Prompt closure of the DA after birth is essential for vascular transition to the mature circulation. In most term infants, the DA constricts in the first 72 hours after delivery (11). Failure of its closure, termed PDA, is primarily an affliction of prematurity, with the DA remaining open at 7 days of age in up to 2%, 65% and 87% of infants born respectively at 30 through 37, 25 through 28 and 24 week's gestation (12). While the DA remains open a left-to-right shunt will progressively increase as pulmonary vascular resistance declines over the first days of life. This "ductal steal" results in excessive blood flow through the lungs, predisposing to development of pulmonary congestion, pulmonary edema, and worsening respiratory failure. Similarly, diversion of blood flow from the systemic circulation may result in compromised perfusion of vital organs, including bowel, kidney, and brain. Although failure of early constriction is associated with several morbidities, i.e. bronchopulmonary dysplasia (BPD), pulmonary hemorrhage, necrotizing enterocolitis (NEC), impaired renal function, intraventricular hemorrhage (IVH), periventricular leukomalacia, and cerebral palsy, the degree to which these adverse outcomes are attributable to the hemodynamic consequences of ductal patency, has not been established (13).

Assessment of hemodynamic significance

Despite widespread use of the term, there is no consensus on the definition of hsPDA. Investigators have used ultrasound alone, or in combination with physical examination findings and biomarkers (14). Over the first days following birth, the transition from fetal to neonatal circulation induces a fall in pulmonary vascular resistance, which in turn leads to pulmonary over-circulation and systemic hypoperfusion due to systemic to pulmonary ductal shunting. The hemodynamic effects of a large left-to-right shunt associated with a PDA may be evident by physical examination, echocardiography, or measurement of serum biomarkers. Physical signs and symptoms of a PDA typically appear 3 to 4 days after delivery and are characterized by failure to wean ventilator pressures, a coarse systolic murmur at the left sternal border, increased precordial impulses, prominent peripheral pulses and either low systolic and diastolic blood pressure or low diastolic blood pressure with a widened pulse pressure (15). Often, the presence of a large ductal shunt is suspected only based on respiratory findings, such increasing requirements for supplemental oxygen, or inability to reduce mechanical ventilator support.

Echocardiography is currently the preferred tool for predicting or diagnosing ductal patency, assessing hemodynamic significance and monitoring treatment response. In an attempt to standardize the echocardiographic assessment of hemodynamic significance of a PDA, European Special Interest Group "Neonatologist-Performed Echocardiography", endorsed by the European Society for Paediatric Research and the European Board of Neonatology proposed that a comprehensive assessment of the PDA should include information on (a) PDA characteristics-diameter, flow direction, (ratio of) systolic and diastolic flow velocities; (b) Indices of pulmonary overcirculation-left ventricular output + one parameter of left-sided volume loading

OR left heart pressure loading; (c) Systemic shunt effect-Doppler flow pattern in the systemic circulation (16). The essential echocardiographic requirements for the assessment of hemodynamic significance of a PDA are summarized in Table I.

There is growing interest in exploring other biomarkers for diagnosing PDA, such as brain-type natriuretic peptide (BNP) and N-terminal pro-BNP (NTpBNP). The inactive precursor pro-BNP is released from ventricles in response to pressure or volume overload and then cleaved into the metabolically active BNP and inactive metabolite, NTpBNP (17-19) There is some evidence that in the presence of critically low diastolic arterial pressure, there is a negative effect on coronary arterial blood flow, which may lead to ischemia of the myocardium, resulting in elevated cardiac troponin T levels. Its predictive role as an exclusive biomarker in early diagnosis of hsPDA is limited and should be utilized as one component of an integrated hsPDA evaluation, which includes echocardiography and other biomarkers (20) Doppler flow measurements of the cerebral arteries are of limited value in the diagnosis of hsPDA, but in patients with low or negative end-diastolic flow, high pulsatility index or resistance indices, they may assist in earlier identification of hsPDA (21). Continuous measurement of cerebral oxygenation by near-infrared spectroscopy may be helpful in detecting a decrease in oxygenation as a sign of compromised brain perfusion due to a hsPDA (22).

Table I. *Essential echocardiographic requirements for the assessment of hsPDA.*

1) PDA characteristics of dimension and flow
a) diameter flow
b) flow direction (left to right, bidirectional with right to left ≤ or > 30% of the cardiac cycle, right to left)
c) velocity in systole and diastole (m/s) and gradient
2) Indices of pulmonary overcirculation
a) LVO (ml/kg/min)
b) Left heart volume loading : choose one parameter:
i) LA/Ao, LVEDD (mm)
ii) Pulmonary vein d wave velocity (m/s)
iii) LPA diastolic velocity (m/s)
c) Left side pressure loading : choose one parameter:
i) Mitral valve E:A
ii) IVRT (m/s)
3) Indices of Systemic shunt effect
a) Flow direction in one of the following post-ductal artery
i) descendant aorta or
ii) celiac trunk
iii) middle cerebral artery (forward, absent, reversed)
Legend :Ao aortic root, IVRT isovolumic relaxation time, LA left atrium, LPA left pulmonary artery, LVEDD left ventricular end-diastolic diameter, LVO left ventricular output

Taking into consideration the heterogeneity in clinical and echocardiography significance, similar in outline to the classifications used in NEC or hypoxic-ischemic encephalopathy, a "PDA disease staging" system that incorporates clinical condition and echocardiographic findings has been suggested, but its clinical usefulness has not been evaluated (23). As recommended in a recent leader, the inclusion of important antenatal and perinatal clinical factors should also be an important part in characterizing hemodynamic significance. Lower gestational age (GA), growth restriction, lack of antenatal steroids, and other adverse perinatal events may also play a role in exacerbating the detrimental effects of PDA shunting (24). Therefore, a definition of an hsPDA must not only include echocardiographic surrogate markers of shunt volume and a comprehensive assessment of left ventricle diastolic function but must also incorporate important clinical characteristics. Any such definition should also be prospectively evaluated to determine its ability to accurately predict ductal related morbidities facilitating a more accurate identification of high-risk infants who are likely to benefit from treatment.

Treatment Options for Closure of PDA

Generally, the treatment options for PDA have been grouped into active and conservative, with the first sub-divided into medical (indomethacin and ibuprofen) and surgical. The timing of active treatment may be prophylactic, symptomatic, or early targeted treatment. Prophylactic and early targeted treatments have the potential of closing the PDA before it is symptomatic. The prophylactic treatment, once popular, has now become controversial as it unnecessarily exposes a large proportion of preterm babies to the side effects of treatment who would have closed the PDA spontaneously. Persistence of PDA is, however, associated with increase in morbidity and mortality. Early targeted treatment

seems promising as it allows selection of babies who are less likely to close the PDA spontaneously and it has the benefits of a prophylactic approach. This approach is currently being tested in large randomized trials awaiting results (see detailed discussion below).

Since the recognition of the PDA as an important entity in the hemodynamic physiology of the premature neonate in the 1960s, a wide variety of diagnostic and treatment strategies have been implemented. From the 1970s to the mid-2000s the prophylactic approach to PDA management was widely accepted, whereby the aim was to close all DA in all low birth weight and gestation preemies as early as possible, either surgically or medically regardless of an assessment of the hemodynamic impact of the duct to the infant. In the 1980s, several small, single-center, randomized controlled trials (RCTs) suggested that prophylactic administration of indomethacin, within the first 24 hours after birth, reduced the incidence of severe grades of intracranial hemorrhage, symptomatic PDA, and surgical PDA ligation (25-30). This led to a widely publicized multicenter RCT, the Ment trial, (31) that confirmed the benefits previously observed in the smaller, single-center RCTs. The TIPP trial conducted a few years later, (32) although confirmed the beneficial effects of early intervention in reducing the incidence of severe intracranial hemorrhage and PDA in extremely low birth weight , failed to find a benefit in its primary outcome (improved survival/neurodevelopmental outcome) and may have discouraged, therefore, the prophylaxis use of indomethacin (33).

Early targeted treatment is an attractive potential option, as only those infants likely to develop a significant PDA are treated. The echocardiography in extreme preterm infants in the first 72hrs should use criteria that have a high sensitivity for diagnosing PDA that is unlikely to close spontaneously. It should take account of the transitional circulation and be able to identify babies who have impending or established pulmonary hypertension. There is little evidence using this approach, with few studies, all small sample sizes. The results of the Australian double-blind randomized controlled prematurely withheld (34) have shown in preemies treated with indomethacin a reduction in early pulmonary haemorrhage and later medical treatment but no effect on the primary outcome of death or abnormal cranial ultrasound.

Currently, there are ongoing large randomised trials including RCT-Baby OSCAR, the French TRIOCAPI, the Dutch BeNEDuCTUS and the Australian U-PDA aiming to study the impact of early-targeted active medical treatment and supportive care alone of large PDA in very preterm infants. With these limited data and the absence of long-term outcomes it is difficult to draw any meaningful conclusions. The ongoing substantial heterogeneity in clinical practice regarding PDA management was recently highlighted by the European Population-Based Cohort Study (EPICE) study, which reported that PDA treatment varied from 10% to 39% between regions, and that this difference could not be explained by differences in perinatal characteristics (35).

Both indomethacin and ibuprofen reduce prostaglandin-mediated vasodilatation by inhibition of the cyclo-oxygenase and peroxidase sites on prostaglandin H_2 synthetase (PGHS) inhibitors. Efficacy decreases postnatally as the balance of vasodilators changes (36). When used prophylactically, intravenous indomethacin and ibuprofen appear to have similar efficacy (37-38), but only the first significantly reduces the risk of IVH (39). A recent meta-analysis Cochrane review reports equivalent efficacy with indomethacin with reduced incidence of NEC, but this may be related to heterogeneity of gestation, dosage and administration route (40). Greater efficacy with higher ibuprofen doses (20, 10 and 10 mg/kg) has been reported at low GA (41). The optimal age-appropriate dosing schedule is still under consideration since the effects of ibuprofen on total and free serum bilirubin concentrations raise concerns about the safety of some of the higher dose options. Enteral ibuprofen may offer benefits, possibly because of its longer half-life, even at lower GA and birth weights (42-43).

Major concerns with indomethacin and ibuprofen are their detrimental effects on the gastrointestinal tract and kidneys. Spontaneous intestinal perforation has been associated with both indomethacin and

ibuprofen, particularly if given in the first week or with comorbid hypotension or glucocorticoids (44). The meta-analysis of RCTs that compared oral ibuprofen to indomethacin suggests that oral ibuprofen treatment may be associated with a lower incidence of NEC than indomethacin (40). Detrimental effects on renal function include oliguria and decreased creatinine clearance (45). Usually, these can be managed with anticipatory fluid restriction, but PGHS inhibitors should be avoided in patients with hypotension or renal impairment. Moreover, there is biological variability in response to all therapies; longer courses of treatment may be required in some infants, but exposure to PGHS can be minimized by echocardiographic evaluation of individual treatment response (46).

Paracetamol has been less extensively studied but appears to have similar efficacy without side effects (47-48). PDA closure is probably mediated by inhibition at the peroxidase site of PGHS (49). Paracetamol has been used for rescue therapy in extremely premature infants after failed response to indomethacin, resulting in 46% of infants having a smaller or closed DA (50). When used as primary treatment, the efficacy ranges from 70% to 81% (51-52). Efficacy appears to be affected by both gestational age and postnatal age, with improved efficacy noted when treatment was started within the first week (53). The paracetamol dose used in most case series and trials was 15 mg/kg dose every 6 hours for 3 days (52).

The appropriateness and optimal timing of surgical ligation is the subject of much debate and controversy. Broadly, surgical intervention is contemplated if medical treatment fails and the PDA remains hemodynamically significant, based on clinical and echocardiography markers. However, in some centers, although there is no evidence to support this approach, surgery is considered the first-line treatment in infants with NEC, IVH, pulmonary hemorrhage, thrombocytopenia, or severe oliguria (54). Ligation is typically performed with an open thoracic approach, and either using a metal clip or tying off the vessel.

Direct surgery-related complications include intraoperative bleeding, pneumothorax, vocal cord paralysis, chylothorax, scoliosis, and phrenic nerve injury. The collective incidence of these complications is usually low (55). Sudden changes in cardiopulmonary physiology after surgery may lead to a post-ligation cardiac syndrome (PLCS) in up to 50% of infants. Post-ligation cardiac syndrome, defined as hypotension requiring inotropic support and failure of oxygenation and ventilation, may occur 6-12 hours following ligation due to left ventricular systolic and diastolic failure, respectively. Implement of perioperative strategies aimed to reduce the frequency of post-ligation may improve outcomes for neonates in this vulnerable patient population (56).

Early PDA ligation is an independent risk factor for BPD (57) and worse neurodevelopment compared with ligation at a later age (58). Reexamined data from the Trial of Indomethacin Prophylaxis suggest that surgical PDA ligation may be associated with increased risks of BPD, severe retinopathy of prematurity and neurosensory impairment in extremely low birth weight infants (59). In retrospective cohort study of 754 preterm infants younger than 28 weeks gestational age, PDA ligation there were no differences in chronic lung disease, retinopathy of prematurity, or neurodevelopmental impairment among survivors. Therefore, previously reported poor outcomes after PDA ligation may be overestimated by trial design (60), suggesting that surgical approaches deserve full consideration for infants with refractory, symptomatic PDA. More recently, transcatheter PDA closure has been described as a viable option in very low weight infants. Over the last few years, interventional catheterization has emerged as an alternative to surgery for closure of a PDA in very low weight infants. To prevent complications, it requires a perfect selection of the device and an accurate positioning. However, further studies are needed to optimize the indications and timing of hsPDA closure and their consequences on clinical outcome (61).

The concept of a conservative approach has been fraught with much controversy and has led to confusion due to variability in its interpretation and application. The approach suggested by Benitz et al. is based on the premise that most PDAs eventually

close spontaneously in premature infants prior to hospitalization and may not require active medical or surgical closure management. Rather, modulation of trans-ductal flow by optimizing the determinants of Poiseuille's law, namely trans-ductal pressure or viscosity, may be preferable (62).

The strategies used to limit the consequences of a high-volume left-to right ductal shunting include fluid restriction, diuretics, modifications to neonatal ventilation support, permissive hypercapnia, targeting lower oxygen saturation ranges, tolerance of metabolic alkalosis, and maintaining a higher hematocrit (63-64). It is, however, prudent to recognize that the conservative approach does not imply "ignoring" the presence of a PDA, nor does it preclude active assessment and management of the PDA or permit that the known consequences of significant ductal patency on neonatal morbidity may be ignored (65).

As in other medical issues controversies depended upon the status of the cumulative contemporary medical knowledge, changing patient population characteristics, and mostly, the authoritative position of the persons offering opinions. We believe that understanding the natural history of the DA pathophysiology, relying on the combination of ultrasound findings and clinical markers, may be useful to clinicians in recognizing the selected PDA that should be closed to avoid adverse outcomes.

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