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

A. Nicoletti[1], M. Vatrano[2], S. Sestito[1], R. Apa[1], S. Patroniti[3], G. Ceravolo[3], M.P. Calabrò[4], F. Parisi[1], K. Roppa[1], R. De Sarro[1], E. Gitto[5], L. Pensabene[1] and D. Concolino[1]

[1]Pediatric Unit, Department of Science of Health, University Magna Graecia of Catanzaro, Catanzaro, Italy; [2]UTIC and Cardiology, Hospital "Pugliese-Ciaccio", Catanzaro, Italy; [3]Department of Human Pathology in Adult and Developmental Age "Gaetano Barresi", Unit of Emergency Pediatrics, University of Messina, , "G. Martino" Policlinic, Italy; [4]Department of Human Pathology in Adult and Developmental Age "Gaetano Barresi", Unit of Pediatric Cardiology, University of Messina, "G. Martino" Policlinic, Italy; [5]Department of Human Pathology in Adult and Developmental Age "Gaetano Barresi", University of Messina, Messina, Italy, Neonatal and Pediatric Intensive Care Unit

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

Corresponding author:
Dr Daniela Concolino
Pediatric Unit, Department of Science of Health,
University Magna Graecia of Catanzaro, Catanzaro, Italy
Tel.: 0961883462 - Fax: 0961883489
e-mail: dconcolino@unicz.it

0393-974X (2020)

(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.

REFERENCES

1. Morris JK, Alberman E. Trends in Down'ssyndrome live births and antenataldiagnoses in England and Wales from 1989 to 2008: analysis of data from the National Down Syndrome Cytogenetic Register. BMJ 2009; 339: b3794.
2. Cuppari C, Colavita L, Miraglia Del Giudice M, et al. Recurrent respiratory infections between immunity and atopy. Pediatr Allergy Immunol. 2020; 31(S24):19-21.
3. D'Angelo G, Marseglia L, Manti S, et al. Atopy and autoimmune thyroid diseases: melatonin can be useful? Ital J Pediatr. 2016; 42(1):95.
4. Manti S, Cutrupi MC, Cuppari C, et al. Inflammatory biomarkers and intellectual disability in patients with Down syndrome. J Intellect Disabil Res. 2018; 62(5):382-90.
5. Manti S, Cuppari C, Tardino L, et al. HMGB1 as a new biomarker of celiac disease in children: A multicenter study. Nutrition 2017; 37: 18-21.
6. Cuppari C, Manti S, Salpietro A, et al. HMGB1 levels in children with atopic eczema/dermatitis syndrome (AEDS). Pediatr Allergy Immunol 2016; 27(1): 99-102.
7. Cuppari C, Manti S, Chirico V, et al. Sputum high mobility group box-1 in asthmatic children: A noninvasive sensitive biomarker reflecting disease status. Ann Allergy Asthma Immunol 2015; 115(2):103-7.
8. Manti, S, Cuppari C, Parisi GF. et al. An Overview of HMGB1 and its Potential Role as a Biomarker for RSV Infection. Current Respiratory Medicine Reviews, 2019, 15(3)205-9.
9. Marseglia L, Manti S, D'Angelo G, et al; Obesity and breastfeeding: The strength of association, Women and Birth 28, 2015; 81-6.
10. Gitto E, Pellegrino S, Aversa S, et al Oxidative stress and persistent pulmonary hypertension of the newborn treated with inhaled nitric oxide and different oxygen concentrations. The Journal of Maternal-Fetal and Neonatal Medicine, 2012; 25(9):1723-6.
11. Sharma M, Khera S, Sondhi V, et al, A study to determine the prevalence of pulmonaryarterialhypertension in children with Down syndrome and congenital heart disease. Med J Armed Forces India. 2013; 69(3):241-5.
12. Galiè N, Humbert M, Vachieryc JL et al, 2015 ESC/ ERS Guidelines for the diagnosis and treatment of pulmonary hypertension. The Joint Task Force for the Diagnosis and Treatment of Pulmonary Hypertension of the European Society of Cardiology (ESC) and the European Respiratory Society (ERS) Eur Heart J. 2015.
13. Schiller NB, Shah PM, Crawford M, et al. Recommendations for quantitation of the left ventricle by two-dimensional echocardiography. American Society of Echocardiography Committee on standards, subcommittee on quantitation of two-dimensional echocardiograms. J Am SocEchocardiogr 1989; 2:358-67.
14. Lang RM, Bierig M, Devereux RB, et al, Recommendations for chamber quantification. Eur J Echocardiogr 2006; 7:79-108.
15. Von Elm E, Altman DG, Egger M, et al. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. Lancet 2007; 370:1453e7.
16. Cappelli-Bigazzi M, Santoro G, Battaglia C, et al. Endothelial cell function in patients with Down'ssyndrome. Am J Cardiol 2004; 94:392-5.
17. Wood P. The Eisenmenger syndrome or pulmonaryhypertension with reversedcentral shunt. Br Med J 1958; 2:701–9.

18. Rabinovitch M. Pathobiology of pulmonary hypertension. Annu Rev Pathol 2007; 2: 369–99.
19. Rabinovitch M, Keane J, Norwood W. Vascularstructure in lung tissue obtained at biopsy correlated with pulmonary haemodynamic findings after repair of congenital heart defects. Circulation 1984; 69:655-67.
20. Pinto F.F., Nunes L., Ferraz F., et al. Down's syndrome: different distribution of congenital heart diseases between the sexes.Int J Cardiol. 1990; 27(2):175-8.
21. Greenwood RD, Nadas AS. The clinical course of cardiac disease in Down's syndrome. Pediatrics 1976; 58: 893-7.
22. Geggel RL, O'Brien JE, Feingold M. Development of valve dysfunction in adolescents and young adults with Down syndrome and no known congenital heart disease. J Pediatr 1993; 122:821-3.
23. De Miguel-Diez J, Villa-Asensi JR, Alvarez-Sala JL, Prevalence of sleep-disordered breathing in children with Down syndrome: polygraphic findings in 108 children. Sleep. 2003; 26(8):1006-9.
24. Weijerman ME1, van Furth AM, van derMooren MD, et al. Prevalence of congenital heart defects and persistent pulmonary hypertension of the neonate with Down syndrome. Eur J Pediatr. 2010; 169(10):1195-9.
25. Bossone E, D'Andrea A, D'Alto M, et al. Echocardiography in pulmonary arterial hypertension: from diagnosis to prognosis. J Am Soc Echocardiogr. 2013;26(1):1-14.
26. Janda S, Shahidi N, Gin K, et al, Diagnostic accuracy of echocardiography for pulmonary hyper- tension: a systematic review and meta-analysis. Heart. 2011; 97(8):612–22.