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Speckle tracking echocardiography in healthy children: comparison between the QLAB by Philips and the EchoPAC by General Electric

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Abstract

Speckle tracking echocardiography (STE) has become a useful tool in cardiology but remains scarcely developed in pediatrics. We aimed to evaluate the feasibility of STE analyses in healthy children and compare reliability of STE for left and right ventricles (LV, RV) between the EchoPAC (GE Healthcare) and the QLAB (Philips Healthcare) software systems. Healthy children were screened for this prospective cross-sectional study. Analyses were performed upon five levels of variability: intra/inter-ultrasound system, intra/inter-sonographer and intra/inter-analyzer. The feasibility was measured, and the tracking quality informed. The study included 156 healthy children. Mean age was 7.6 ± 5 years [1 month–16.8 years]. Conventional echocardiography variables were similar in both ultrasound systems. For both software brands, the tracking quality was excellent in the LV longitudinal and circumferential displacements, but more limited in the RV free wall longitudinal strain. Inter-ultrasound system correlation was poor for global longitudinal and circumferential LV strain (ICC of 0.34 [IC95% 0.06–0.57]) and 0.12 [IC95% –0.18 to –0.40], respectively). We observed poor inter-sonographer reliability for both global LV longitudinal strain and global LV circumferential strain with the two software systems. Inter-analyzer variability was good especially for the global LV circumferential strain using Philips software (ICC of 0.78 [IC95% 0.52–0.91]). In pediatrics, the Philips/GE inter-vendor level of variability in STE analysis is mainly due to inter ultrasound systems and inter sonographers' differences. These results need to be taken into account when using STE analysis in the follow-up of cardiac children. *Clinicaltrials.gov*: NCT02056925.

Keywords 2D strain · Inter-vendor · Variability · Pediatrics · Pediatric cardiology

Introduction

Among the new echocardiography techniques, speckle tracking echocardiography (STE) or “two-dimensional (2D) strain” evaluates the myocardial function with a dynamic regionalized analysis of the overall ventricular contraction. Using software for the spatial and temporal processing of the image, with short acquisition time, this technique measures localized myocardial movements of natural acoustic markers, also called “speckles” [1].

STE supposedly allows a rapid, precise and objective assessment of segmental and global myocardial function [2]. Three advantages are usually pointed out: (1) the STE technique is not dependent on the Doppler angle shot, as opposed to tissue Doppler imaging (TDI) [3], (2) STE is inexpensive and easily accessible, unlike the tagged-magnetic resonance imaging (MRI) and (3) STE is easy to perform from the 2D

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echocardiography gray-scale, with a possible *a posteriori* data analysis.

In adult cardiology, left ventricle (LV) 2D strain is now part of the practice for the analysis of myocardial function in dilated, hypertrophic or ischemic cardiomyopathies, and for the study of myocardial desynchronization [4–6]. In pediatric cardiology, some interest has also been shown for this non-invasive functional biomarker of myocardial function, but STE remains mostly used in clinical research but not extensively in the routine follow-up [7]. Yet, speckle tracking standards are available in healthy children [8–10], and STE anomalies have been reported in many situations involving myocardial dysfunction in children [11–15]. Nevertheless, unlike in the adult population, the feasibility of STE analysis in pediatrics has not been clearly studied, from birth to teenage [16, 17].

When focusing on the right ventricle (RV), STE analysis has also recently emerged as an interesting non-invasive tool [18]. Indeed, many congenital heart diseases (CHD) affect the RV and non-invasive biomarkers of the RV function are necessary in these patients. Indeed, medical and surgical advances in the past decades have increased the prevalence of heart diseases involving the RV [19]. The care of these children includes complex surgical reconstruction of the right outflow tract, balloon or stenting in the pulmonary arteries and advance pharmaceutical therapies for pulmonary arterial hypertension. As a result, the echocardiographic assessment of the RV function has become essential in pediatric cardiology. However, the vendors have not specifically developed the STE analysis for the RV and sonographers use the LV STE software function, which they apply to the RV, without any validation. Yet, some authors have associated RV strain values with cardiac clinical outcomes in the CHD population [20–24]. In the most complex CHD, such as systemic right ventricles (D-looped transposition of the great arteries after atrial switch procedure and congenitally corrected TGA [25]), RV function assessment with STE analysis correlates with MRI and exercise capacity [26–28].

A potential limitation of the STE technique in children may result from the existence, in the pediatric cardiology departments, of different software developed by most medical imaging companies [29]. Indeed, a weak correlation between inter-vendors specific software has been recently pointed out in adult cardiology, despite some improvement since the task force standardization initiatives for deformation imaging led by the European Association of Cardiovascular Imaging (EACVI), the American Society of Echocardiography (ASE) and the industry [30–33].

Therefore, we aimed to evaluate the reliability of STE analysis in the routine clinical practice of a pediatric cardiology consultation, among a large prospective cohort of healthy children. We thus planned a robust methodology to evaluate five levels of reproducibility of STE analysis

for both ventricles, according to the sonographer and two important ultrasound software vendors, namely the QLAB from Philips Healthcare, and the EchoPAC from General Electric Healthcare.

Materials and methods

Study design and patients

This prospective cross-sectional study was carried out from December 2013 to September 2015 in two tertiary care pediatric cardiology regional reference centers (Montpellier University Hospital and Saint-Pierre Institute, France). We screened all children aged 0 to 18 years referred to the outpatient pediatric cardiology consultation for innocent murmur, chest pain, palpitations or sports certificate. Only those with normal physical examination, electrocardiogram and echocardiogram were eligible for the study. Children with any chronic disease, under any treatment or requiring any further investigation, were not included.

The study was conducted in compliance with the Good Clinical Practices protocol and Declaration of Helsinki principles. It was approved by South Mediterranean IV Ethics Committee (2013-A00579-36) and registered on ClinicalTrials.gov (NCT02056925). Informed consent was obtained from all parents or legal guardians.

Echocardiographic measurements

Echocardiographic examinations were performed using the IE-33 ultrasound system (Philips Healthcare, Amsterdam, Netherlands) and the Vivid E9 ultrasound system (General Electric Healthcare (GE), Little Chalfont, United Kingdom). Five senior pediatric cardiologists, namely the “sonographers”, with experience in STE performed the echocardiography examinations, and following the international guidelines [34]. Image acquisition procedures were harmonized before the study started. The same setting was used for each ultrasound system: global gain, lateral gain, contrast, 60 to 80 frame rates to optimize myocardial deformation analysis, harmonic imaging, image colorizing, and probes adapted to the height and weight of the child (12 MHz, 8 MHz or 5 MHz). We systematically recorded three cardiac-cycle loops in the following views: apical LV view (4, 3, and 2 chambers), LV short axis view focused on papillary muscles, RV free wall on intercostal apical and sub-costal four-chamber views. The following conventional LV function variables were measured: LV ejection fraction (LVEF) and LV shortening fraction by the Teichholz method, LVEF by the Simpson biplane method, LV diastolic diameter, E/E' ratio measured with tissue Doppler imaging (TDI), and LV wall stress, determined by the pressure in the ventricle using cuff

blood pressure at the time of echocardiogram, the internal radius of the ventricle by M-mode, and the thickness of the wall [35]. The following conventional RV function variables were measured: tricuspid annular plane systolic excursion (TAPSE), and tricuspid S-wave with TDI.

The five-level reproducibility analysis

The first echocardiogram was performed with one of the two randomly selected ultrasound systems. As illustrated in the flow chart (Fig. 1), a second echocardiogram was performed upon three designs to evaluate different levels of reproducibility: (1) same ultrasound system and same sonographer (intra sonographer, intra ultrasound system reproducibility), (2) same ultrasound system and different sonographer (inter sonographer, intra ultrasound system reproducibility), and

(3) different ultrasound system and same sonographer (intra sonographer, inter ultrasound system reproducibility).

Post-data speckle tracking analyses were performed by a unique investigator for each software system from General Electric (EchoPAC version 112) and Philips (QLAB version 10). Two days (48 h) after the first STE analysis, a second one was performed by the same investigator (intra analyzer reproducibility) and by a second investigator (inter analyzer reproducibility), with no access to the results of first analysis. We did not use Automated Functional Imaging (AFI), only available for the EchoPAC, in order to harmonize analyses between both software systems. The investigators manually traced the endocardium in end-diastole. The software detected the movement of the entire myocardial wall (from the endocardium to the epicardium) and therefore defined the areas of interest, for which the quality was considered as acceptable or not. In poorly detected segments,

* Patients were randomly assigned to groups of analysis and ultrasound system.

Fig. 1 Flow chart. STE speckle tracking echocardiography

the sonographer readjusted the endocardial contour until a better detection was obtained. Whenever that was not possible, the segments in question were excluded from the analysis and were reported as “poor tracking quality” data in the “Results” section.

We recorded the following global and regional peak 2D strain and systolic strain rate data: LV longitudinal, radial (only available for the EchoPAC software) and circumferential strain; RV longitudinal free wall strain in intercostal apical and sub-costal four-chamber views.

Statistical analyses

In accordance with recommendations published in Shrout and Fleiss [36], we evaluated the reproducibility of the performed measurements by calculating intra class correlation coefficients (ICC) of different types, according to the situation encountered. We used the ICC of type (1, 1) to assess inter sonographer and intra sonographer reliability since five different pediatric cardiologists (i.e. the sonographers) could produce the measures. ICC of type (2, 1) was used to assess inter and intra analyzer reliability, since only one investigator was responsible for the double STE analysis to assess the intra analyzer reliability, and a single pair of investigators produced the two analyses to assess the inter analyzer reliability. Then, ICC of type (3, 1) was used to assess the inter ultrasound system reliability, as we evaluated the reproducibility between two ultrasound systems.

As defined by Koo et al. ICC values under 0.5, between 0.5 and 0.75, between 0.75 and 0.9, and above 0.90 were indicative of poor, moderate, good, and excellent reliability, respectively [37].

The reproducibility was also analyzed using the Bland and Altman method producing the graph of the differences between the two evaluations for the same child and the 95% limits of agreement, which are an estimate of the interval in which 95% of the differences are expected.

The statistical significance was set at 0.05, and analyses were performed using SAS version 9.2 (SAS Institute, Cary, NC, USA).

Results

Population

During the study period, 163 children were screened and 156 children were included. For 6 of them, the second echocardiogram could not be performed due to a poor compliance of the child ($n=2$) or technical problems ($n=4$). Therefore, complete analyses could be performed in 150 children (44% female). Mean age was 7.6 ± 5 years, with a range from

1 month to 16.8 years old. The flow chart was presented in Fig. 1.

Conventional echocardiography

The first echocardiogram was performed with the Philips IE33 ultrasound for 71 patients and with the General Electric (GE) Vivid E9 ultrasound for 79 patients. There was no significant difference between both ultrasound systems in terms of conventional echocardiography variables (Table 1). Children included in the study did not require any sedation.

Speckle tracking analysis

Overall, the global longitudinal and circumferential LV 2D strain measures were obtained in almost all patients, with only 0.7% and 6.7% poor tracking quality data, respectively. The feasibility of radial LV strain analysis by GE was also correct (missing data for only 3 children). Philips did not develop the LV global radial strain on the QLAB software; therefore these data were not available. We found no significant difference on the feasibility of global longitudinal and circumferential LV strain between Philips and GE. We observed a significant level of poor tracking quality for the RV, up to nearly 37% in the intercostal apical four-chamber view. The RV tracking quality was better in the subcostal four-chamber view, especially with the EchoPAC/GE (12.7% missing data) (Table 2).

Reliability and agreement of STE measures for RV and LV were reported in Tables 3, 4 and Figs. 2, 3. The inter ultrasound systems' correlation was low for both longitudinal and circumferential global LV 2D strain, with an ICC of 0.34 and 0.12, respectively (Tables 3, 4). We also observed a poor inter sonographer reliability in both longitudinal and circumferential LV 2D strain with the two software (ICC between 0.1 and 0.3). Intra sonographer correlation was good only for the longitudinal LV 2D strain with the EchoPAC/GE (ICC=0.77). The inter analyzer reliability was good, especially for the circumferential global LV 2D strain using the QLAB/Philips software (ICC=0.78) (Table 4; Fig. 3). The intra analyzer reliability was correct with the EchoPAC for both longitudinal and circumferential 2D strain (ICC $\approx$ 0.5), and good with the QLAB/Philips (ICC $\approx$ 0.6–0.7).

Correlation analyses for the RV STE parameters were not possible, as a result of poor tracking quality.

Discussion

This study provided some consistent speckle tracking data from a cohort of 156 healthy children, with relevant comparisons between the two main software systems, namely the

Table 1 Conventional echocardiography variables (first ultrasound)

Conventional echocardiography variables	Vivid E9 EchoPAC (GE) N = 79	IE33 QLAB (Philips) N = 71	P value
LVEF [Teichholz] (%)			
Mean ± SD	68.9 ± 5.6	69.5 ± 6.2	0.49
Median [Q1; Q3]	68.0 [66.0; 73.0]	69.0 [65.5; 72.5]	
LVEF [Simpson biplane] (%)			
Mean ± SD	64.9 ± 5.7	63.9 ± 7.0	0.45
Median [Q1; Q3]	65.0 [61.0; 68.0]	62.5 [59.0; 70.0]	
LV shortening fraction (%)			
Mean ± SD	38.1 ± 4.7	38.4 ± 5.2	0.72
Median [Q1; Q3]	38.0 [35.0; 41.0]	38.0 [35.0; 40.0]	
LV diastolic diameter (mm)			
Mean ± SD	37.5 ± 8.3	35.4 ± 9.1	0.15
Median [Q1; Q3]	38.5 [32.0; 43.0]	37.0 [29.5; 41.0]	
E/E' ratio [DTI]			
Mean ± SD	5.9 ± 1.3	5.8 ± 1.7	0.58
Median [Q1; Q3]	5.7 [4.8; 6.9]	5.7 [4.6; 6.9]	
LV wall stress (g/cm ²)			
Mean ± SD	40.2 ± 15.2	38.4 ± 10.4	0.89
Median [Q1; Q3]	37.3 [30.2; 49.3]	36.3 [32.2; 44.0]	
TAPSE (mm)			
Mean ± SD	19.57 ± 4.74	18.9 ± 5.4	0.41
Median [Q1; Q3]	19.0 [17.0; 22.0]	19.0 [15.0; 22.0]	
Tricuspid S wave [DTI] (cm/s)			
Mean ± SD	13.3 ± 2.6	12.9 ± 3.1	0.46
Median [Q1; Q3]	13.0 [12.0; 15.0]	13.0 [11.0; 15.0]	

Values are expressed as mean ± SD on the first line and as median [*Q1* first quartile, *Q3* third quartile] on the second line

DTI Doppler tissue imaging, *LV* left ventricle, *LVEF* left ventricle ejection fraction, *TAPSE* tricuspid annular plane systolic excursion

Table 2 Feasibility analysis: tracking quality on first ultrasound

2D strain analysis	Tracking quality	Vivid E9 EchoPAC (GE) N (%)	IE33 QLAB (Philips) N (%)	P value
LV				
Longitudinal global 2D strain	Poor	1 (1.2)	0 (0)	1
	Correct	78 (98.7)	71 (100)	
Radial global 2D strain	Poor	3 (3.8)	N/A	N/A
	Correct	76 (96.2)	N/A	
Circumferential global 2D strain	Poor	3 (3.8)	7 (9.9)	0.14
	Correct	76 (96.2)	64 (90.1)	
RV				
Longitudinal free wall global 2D strain [intercostal apical view]	Poor	29 (36.7)	25 (35.2)	0.75
	Correct	50 (63.3)	46 (64.8)	
Longitudinal free wall global 2D strain [subcostal 4-chamber view]	Poor	10 (12.7)	18 (25.4)	0.04
	Correct	69 (87.3)	53 (74.7)	

N/A not applicable

Table 3 Left ventricle longitudinal global 2D strain

	Vivid E9 EchoPAC (GE)			IE 33 QLAB (Philips)		
	N	ICC	95% CI	N	ICC	95% CI
Inter sonographer, intra ultrasound system reproducibility	29	0.24	0.10; 0.43	22	0.27	0.11; 0.49
Intra sonographer, intra ultrasound system reproducibility	26	0.77	0.55; 0.89	23	0.24	− 0.17; 0.58
Inter analyzer reproducibility	24	0.51	0.16; 0.75	25	0.35	0.02; 0.64
Intra analyzer reproducibility	25	0.52	0.18; 0.75	25	0.68	0.41; 0.84
			N			
Intra sonographer, inter ultrasound system reproducibility			47	0.34		
				95% CI		
				0.06; 0.57		

ICC intra class correlation coefficient; values less than 0.5, between 0.5 and 0.75, between 0.75 and 0.9, and greater than 0.90 were indicative of poor, moderate, good, and excellent reliability, respectively [37]; 95% CI 95% confidence interval of the ICC

Table 4 Left ventricle circumferential global 2D strain

	Vivid E9 EchoPAC (GE)			IE 33 QLAB (Philips)		
	N	ICC	95% CI	N	ICC	95% CI
Inter sonographer, intra ultrasound system reproducibility	26	0.22	0.08; 0.41	17	0.09	− 0.04; 0.32
Intra sonographer, intra ultrasound system reproducibility	26	0.46	0.10; 0.72	20	0.54	0.14; 0.78
Inter analyzer reproducibility	24	0.50	0.13–0.75	24	0.78	0.52; 0.91
Intra analyzer reproducibility	24	0.50	0.13–0.75	24	0.62	0.30; 0.81
			N			
Intra sonographer, inter ultrasound system reproducibility			44	0.12		
				95% CI		
				− 0.18 ; 0.40		

ICC intra class correlation coefficient; values less than 0.5, between 0.5 and 0.75, between 0.75 and 0.9, and greater than 0.90 were indicative of poor, moderate, good, and excellent reliability, respectively [37]; 95% CI 95% confidence interval of the ICC

EchoPAC from General Electric Healthcare and the QLAB from Philips Healthcare. Following a five-level reproducibility analysis (inter and intra ultrasound system, inter and intra sonographer, inter and intra analyzer), our results highlighted some strengths and weaknesses to consider when using STE analysis with these two software leaders in pediatric cardiology.

In this study, when the same sonographer performed twice the same measure, the EchoPAC from GE was more reliable; and when two different analyzers worked on the same STE software, the QLAB from Philips was more accurate. However, for both ultrasound systems, the lack of reliability did not come from the STE software itself. Indeed, we found a rather low variability between two measures made by the same or by a different analyzer on both the QLAB and the EchoPAC. As in many echocardiography validation studies, the highest degree of variability mainly came from the ultrasound examination itself. We can easily hypothesize that this level of variability is even more important in the pediatric population, as recently pointed out by Anwar *et al.* in their comparative

vendor-independent and vendor-specific STE software study, involving both adult and pediatric subjects [7].

In terms of feasibility, the tracking quality was excellent in the left ventricle longitudinal and circumferential displacements, for both software brands. Indeed, the left ventricle tracking quality was correct for all three displacements (longitudinal, radial and circumferential) and similar between GE and Philips when comparison was possible (longitudinal and circumferential displacements). However, our study brought out some concerns about the level of variability in STE analysis, mainly due to inter ultrasound systems and inter sonographers' differences. We did not find these differences for the conventional ultrasound variables and all sonographers were senior pediatric cardiologists, therefore this variability predominantly came from STE analysis itself. Thus, those two levels of variability should be taken into account when using speckle tracking in the routine follow-up of cardiac children. Indeed, different sonographers using different ultrasound systems, is a very common situation in clinical practice.

Inter sonographer,
intra ultrasound
system reproducibility

Intra sonographer,
intra ultrasound
system reproducibility

Inter analyzer
reproducibility

Intra analyzer
reproducibility

Intra sonographer,
inter ultrasound system
reproducibility

Fig. 2 Left ventricle longitudinal global 2D strain: Bland and Altman graph for EchoPAC/GE and QLAB/Philips software systems

Inter sonographer,
intra ultrasound
system reproducibility

Intra sonographer,
intra ultrasound
system reproducibility

Inter analyzer
reproducibility

Intra analyzer
reproducibility

Intra sonographer,
inter ultrasound system
reproducibility

Fig. 3 Left ventricle circumferential global 2D strain: Bland and Altman graph for EchoPAC/GE and QLAB/Philips software systems

On the opposite, our study showed that left ventricle STE analysis was reliable when the same ultrasound system and the same software were used. Therefore, from a clinical research point of view, STE analysis in children may represent an interesting non-invasive outcome. Indeed, several pediatric studies have pointed out the ability of left ventricle STE analysis to detect preclinical myocardial dysfunction, such as after anthracyclin chemotherapy [11, 38], abnormal left coronary artery from the pulmonary artery repair [13], or heart transplantation [15]. STE has also been recently considered in early detection of cardiomyopathy in Duchenne muscular dystrophy [39]. Recently, a randomized drug trial in children with Duchenne muscular dystrophy demonstrated an attenuation of the decline in LV function by eplerenone, measured by circumferential 2D strain [40]. In those clinical research studies, a unique ultrasound system equipped with its specific STE software was used, which provided such reliable results.

In our study, the right ventricle STE analysis was limited by a significant level of poor tracking quality, with both ultrasound and software systems (Philips and GE). Indeed, STE analysis could not be performed in up to 37% RV measures on the apical intercostal four-chamber view. However, in the subcostal view, the RV free wall tracking quality was acceptable, especially with the EchoPAC software from GE, which was significantly better than with the QLAB from Philips. In children, the sub-costal view usually provides a large acoustic window and should probably be preferred in RV STE analysis. Nevertheless, implementation of RV 2D strain in clinical follow-up has been hampered by the limited reference values and the lack of uniformity on the different software systems [41, 42]. In their recent review from the literature on STE in adult heart failure, Tadic et al. concluded that further investigations were necessary to establish RV strain as a standard variable for decision-making [43]. Yet, echocardiographic assessment of RV function remains essential in pediatric cardiology. As a result, despite these limitations in both feasibility and validation, many studies tried to correlate RV strain values to clinical outcomes. For instance, Okumura *et al.* stated that the RV global longitudinal strain in children with idiopathic pulmonary arterial hypertension was significantly reduced in those who died or underwent transplant, with a value superior to 14% predicting transplantation-free survival [20]. Similarly, several studies in patients with a systemic right ventricle or Tetralogy of Fallot showed that a low RV global longitudinal strain was associated with an impaired functional status [21–24]. Nevertheless, some pediatric studies are in line with our results and minimized the application of RV STE analysis in clinical follow-up. A recent systematic pediatric review (226 children from 10 studies) pointed out the inconsistency and heterogeneity of RV STE analysis with the EchoPAC ultrasound software, with a large range of global longitudinal

RV strain from -21% to -34% [18]. Even with a vendor-independent analysis (Tomtec 2D cardiac performance analysis[®]), the RV STE poorly correlated to catheter-derived measurements in children [44].

Study limitations

The normal pediatric population recruited in our study was screened from the outpatient pediatric cardiology consultation; however we applied very strict criteria to select a population as healthy as possible.

We purposely only included normal patients, for methodological reasons, as no other STE study with a similar design in healthy children had been previously reported. This typically resulted in a relatively narrow range in the results and therefore small differences between the measurements might have influenced the ICCs. Therefore, further works are necessary to demonstrate that STE analysis in children with cardiac conditions *versus* control subjects may measure significant differences and less inter-vendor variability.

We selected the two main software systems from Philips and GE, but did not analyze the data from other vendors or with vendor-independent software, such as the Tomtec 2D cardiac performance analysis[®]. Again, we aimed to provide relevant inter-vendor STE data for the pediatric cardiologists using in their daily practice these ultrasound leaders.

Conclusion

This study from 156 healthy children provided some important speckle tracking data with relevant comparisons between the two main vendors, namely the EchoPAC from General Electric Healthcare and the QLAB from Philips Healthcare. For both software brands, the tracking quality was excellent in the LV longitudinal and circumferential displacements, but more limited in the RV free wall longitudinal strain.

Following a five-level reproducibility analysis (inter and intra ultrasound system, inter and intra sonographer, inter and intra analyzer), our study found that the Philips/GE inter-vendor strain variability in healthy children was mainly due to inter ultrasound systems' differences and inter sonographers' differences. These results need to be taken into account when using STE analysis in the routine follow-up of cardiac children, as different sonographers using different ultrasound systems is a very common situation in "real life".

Therefore, the EACVI/ASE/Industry Task Force Standardization Initiatives for Deformation Imaging must pursue their efforts towards harmonization of STE software, including in the pediatric population.

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Compliance with ethical standards

Conflict of interest Authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was approved by South Mediterranean IV Ethics Committee (2013-A00579-36).

Informed consent Informed consent was obtained from all parents or legal guardians of children included in the study.

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