Research Square

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Global longitudinal strain by speckle-tracking outperforms conventional echocardiography across pediatric chronic kidney disease severity

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Abstract

Background:

Cardiovascular involvement is one of the causes of increased morbidity and mortality rates in patients with chronic kidney disease (CKD). Limited data on cardiovascular complications have been reported in children with CKD. Most children with CKD have well-preserved left ventricular function as assessed using conventional echocardiography. This study aimed to analyze the correlation between left ventricular function assessed using speckle-tracking echocardiography (STE) and estimated glomerular filtration rate (eGFR) in pediatric CKD.

Methods:

A cross-sectional analytic study from June to September 2025 of 38 children diagnosed with CKD were visited Dr Hasan Sadikin General Hospital, Bandung, Indonesia, for inpatient and outpatient care. Each subject underwent laboratory examinations for creatinine levels, conventional echocardiography, and STE using a Philips EPIQ machine performed by a pediatric cardiologist consultant on the same day. Stage of CKD determined by eGFR using the Schwartz formula from the creatinine results. Data were analyzed using unpaired t-test, ANOVA, and *Spearman's rho* correlation test.

Results:

Of the 38 children with CKD, 52.6% were male, with a median age of 14.04 years. Glomerular diseases, mostly focal segmental glomerulonephritis and lupus nephritis, were the most common cause in this study (89.4%). Variable CKD severity was observed in this study (39.5% of stage 1), followed by 28.9% (stage 5). A significant positive correlation was found between left ventricular function (ejection fraction/EF, fractional shortening/FS, and global longitudinal strain/GLS) measured by speckle-tracking echocardiography and eGFR ($r = 0.385$, $p = 0.017$; $r = 0.358$, $p = 0.027$; $r = 0.417$, $p = 0.009$, respectively).

Conclusion:

Left ventricular function (EF, FS, and GLS) assessed using STE positively correlated with eGFR. Speckle-tracking echocardiography can be used as a routine examination and modality to identify subclinical left ventricular dysfunction in pediatric CKD patients with a preserved ejection fraction.

Background

Children with chronic kidney disease (CKD) have a significantly increased risk of morbidity and mortality compared to healthy children of the same age. Chronic kidney disease (CKD) in children constitutes 1–3% globally, with a prevalence of 56–74.7 cases per million children, and data from European registries

show a prevalence of 56–96 cases per million children.[1,2] Based on *Kidney Disease: Improving Global Outcomes* (KDIGO) 2024, chronic kidney disease is defined as a progressive structural or functional kidney disorder lasting a minimum of 3 months with implications for health, such as a decrease in estimated glomerular filtration rate (eGFR) < 60 ml/minute/1.73 m^2, or the presence of kidney damage such as urine sedimentation, tubular disorder, or other abnormalities detected on imaging or laboratory or histological results of renal parenchyma.[3] Generally, children with early stage CKD are asymptomatic, which poses a challenge to clinicians. Data from *North American Pediatric Renal Collaborative Trials* (NAPRTCS) show progression of CKD from stage 2 to 4 to end-stage renal disease of 17% in one year and 39% in three years, with a median of 4.5 years.[1,4]

Cardiovascular involvement must be considered in all pediatric chronic kidney disease. Complications of the cardiovascular system are responsible for 28–32% of deaths, especially in dialyzed children.[5] In the United States, the mortality rate of pediatric CKD due to cardiovascular complications has reached 23% and 50% in Australia and New Zealand, respectively.[5,6] Only scant data on cardiovascular complications are available in children with CKD, and there are no reported data on the morbidity and mortality rates of pediatric CKD due to cardiovascular complications in Southeast Asia, particularly in Indonesia. The most common cardiovascular complications found in patients is left ventricular hypertrophy and left ventricular systolic and diastolic dysfunctions. This may be a result of several mechanisms, including anemia, uremia, hypertension, sodium and fluid retention, hyperparathyroidism, and calcium-phosphorus homeostasis disorders.[7,8].

Most children with CKD have well-preserved left ventricular function as assessed using conventional echocardiography. Conventional echocardiography cannot detect early cardiac dysfunction, while tissue Doppler imaging (TDI) is a method that can detect early cardiac dysfunction, but has limitations, such as being angle-dependent and load-dependent. However, the latest supporting examination, two-dimensional speckle-tracking echocardiography (STE), can be used to detect subclinical ventricular abnormalities in patients with early stage CKD. This modality assesses early changes in myocardial mechanics, especially in left ventricular function, before changes in ejection fraction (EF) or fractional shortening (FS) are detected on conventional echocardiography.[7–9] This will provide targeted intervention for pediatric CKD to prevent rising cardiovascular morbidity and mortality rates.

In the present study, we assessed left ventricular dysfunction in pediatric patients with CKD using STE and correlated it with CKD severity based on the estimated glomerular filtration rate (eGFR) calculated using the Schwartz formula.

Methods

This cross-sectional analytic study was conducted at the Department of Child Health, Dr. Hasan Sadikin General Hospital, Bandung, West Java, Indonesia, from June to September 2025. Pediatric patients aged 1 to < 18 years with a confirmed diagnosis of chronic kidney disease, underwent inpatient or outpatient

management were included in this study. Patients diagnosed with congenital heart disease, acquired heart disease, arrhythmia, massive pericardial effusion, any syndrome related to cardiovascular-like congenital rubella syndrome or Down syndrome, a hemoglobin level < 7 mg/dL, or a poor echo window were excluded. Poor echo window quality results from suboptimal image acquisition, primarily due to obesity or a history of pulmonary tuberculosis. Participants were selected using a consecutive sampling technique.

After meeting the inclusion criteria, the patients and their families were provided an explanation, written consent from parents and assent from subjects aged 12 - <18 years old were obtained. Characteristic data, such as sex, age, nutritional status using the 2006 and 2007 WHO growth charts, blood pressure, oxygen saturation, pulse rate, any cardiac manifestation, disease duration, disease etiology, history of kidney replacement therapy, biopsy, history of transfusion, hypertension, and immunosuppressive treatment, were collected. These data were obtained by history taking, physical examination, laboratory examination, and echocardiography results. Each subject underwent laboratory examinations for creatinine level, electrocardiography, conventional echocardiography, and speckle-tracking echocardiography using a Philips EPIQ machine, performed by a pediatric cardiologist consultant on the same day after inclusion. The stage of chronic kidney disease was determined by the estimated glomerular filtration rate (eGFR) using the Schwartz formula from creatinine results.

Echocardiography was performed using an ultrasound device (Philips Medical Systems, model EPIQ, Amsterdam, Netherlands) with an S5-1 MHz transducer. Conventional echocardiography measured ejection fraction (EF) and fractional shortening (FS) using M-mode as left ventricular systolic function. The E/A ratio was determined using mitral valve inflow Doppler flow to evaluate left ventricular diastolic function. The normal EF values ranged from 55% to 75%, and the normal FS values ranged from 25% to 45%. Mild diastolic dysfunction is characterized by a mitral E/A ratio of < 0.8.[10–12] Two-dimensional speckle-tracking echocardiography (STE) at four-, three-, and two-chamber apical images was recorded, and the global longitudinal strain (GLS) value was generated. Levy et al. conducted a meta-analysis and systematic review involving 2,325 children across 43 studies, establishing a mean of GLS in healthy children at -20.2%.[13]

Statistical Analysis

The collected data were entered into Microsoft Excel and analyzed using Statistical Package for Social Sciences (SPSS) (version 27.0; IBM Corp., Armonk, New York, USA) for Windows. Descriptive results for categorical variables are described as numbers and percentages, while continuous variables are described as mean ± SD or median (interquartile range) if the distribution of data was not normal. *Spearman's Rho* correlation was used to analyze the correlation between left ventricular function (EF, FS, E/A ratio, and GLS) and eGFR, determined using the Schwartz formula. One-way ANOVA and unpaired T test were used to analyze the GLS distribution based on the etiology of CKD, dialysis status, and history of antihypertension. A sample was sized using power and sample calculation with 90% power ($\alpha = 0.05$, 1-tailed) and magnitude of correlation coefficient 0.5 which is moderate correlation considered to be

significant because there were no previous similar studies results. A *p*-value of < 0.05 was considered statistically significant in all analyses.

Results

The baseline characteristics of the enrolled pediatric patients with CKD are shown in Table 1. Of the 42 subjects who met the inclusion criteria and underwent echocardiography, four subjects were excluded due to poor echo window results, and a total of 38 subjects were included in the final analysis. About 52.6% were male with a median age of 14.04 years (range 2.15–17.98). The patients with CKD in this study were predominantly adolescents (78.9%). The nutritional status was adequate (47.4%), and only 29% of the patients had short stature. CKD severity in this study was predominantly stage 1 (39.5%), stage 2 (13.2%), stage 3 (13.2%), stage 4 (5.3%), and stage 5 (28.9%). Glomerular disease was the most common etiology of CKD in the present study, which consisted of *focal segmental glomerulosclerosis* (FSGS) (52.6%) and lupus nephritis (39.5%), followed by non-glomerular diseases, such as congenital anomalies of the kidneys and urinary tract (CAKUT), most of which used immunosuppressive therapy (76.3%).

Table 1
Characteristics of Chronic Kidney Disease Patients

Variable	**N = 38**
Age (years), (Median, Min – Max)	14.04 (2.15–17.98)
Gender, n (%)	
Male	20 (52.6)
Female	18 (47.4)
Nutritional status, n (%)	
Severe malnutrition	3 (7.9)
Mild malnutrition	3 (7.9)
Normal	18 (47.4)
Overweight	11 (28.9)
Obese	3 (7.9)
Systolic blood pressure, mmHg (Median, Min – Max)	110 (90–155)
Diastolic blood pressure, mmHg (Median, Min – Max)	70 (60–100)
Pulse, (Median, Min – Max)	89 (72–140)
Oxygen saturation, (Median, Min – Max)	98 (96–99)
Severity of Chronic Kidney Disease, n (%)	
Stage 1	15 (39.5)
Stage 2	5 (13.2)
Stage 3	5 (13.2)
Stage 4	2 (5.3)
Stage 5	11 (28.9)
Creatinine serum, mg/dL (Median, Min – Max)	0.81 (0.35–8.44)
Duration of CKD, years (Median, Min – Max)	1.00 (0.25–12.00)
Renal Replacement Therapy, n (%)	
Yes	13 (34.2)
No	25 (65.8)

Description: n, frequency; %, percentage; IQR, interquartile range; SD, standard deviation; MMF, mycophenolate mofetil; MTX, methotrexate

Variable	N = 38
Etiology of CKD, n (%)	
Glomerular autoimmune	15 (39,5)
Glomerular non-autoimmune	20 (52,6)
Non-glomerular	3 (7,9)
History of imunosupressive therapy, n (%)	
Yes	29 (76.3)
No	9 (23.7)
Types of immunosuppressive, n	
Mecophenolate mofetil (MMF)	13
Cyclophosphamide	20
Cyclosporin	5
Methotrexate (MTX)	-
Rituximab	3
Azathioprine	1
History of antihypertension, n (%)	
Yes	26 (68.4)
No	12 (31.6)
History of transfusion, n (%)	
Yes	20 (52.6)
No	18 (47.4)
eGFR (ml/min/1.73 m^2), (Median, IQR)	74.91 (13.10–109.39)
EF (%), (Median, IQR)	67.7 (58.0–73.1)
FS (%), (Average ± SD)	35.8 ± 9.9
E/A ratio, (Average ± SD)	1.36 ± 0.35
GLS (%), (Average ± SD)	19.6 ± 4.5
Description: n, frequency; %, percentage; IQR, interquartile range; SD, standard deviation; MMF, mycophenolate mofetil; MTX, methotrexate	

Conventional echocardiography revealed that the average EF and FS were within the normal range, as was the E/A ratio on Doppler echocardiography. Meanwhile, GLS showed an average of 19.6 ± 4.5%, indicating a subclinical decline in myocardial function that correlated with the severity of kidney disease. A significant positive correlation between left ventricular function (EF, FS, and GLS) measured by speckle-tracking echocardiography and eGFR (r = 0.385, p = 0.017; r = 0.358, p = 0.027; r = 0.417, p = 0.009, respectively) is summarized in Table 2 and Fig. 1.

Table 2
Correlation of left ventricle function measured by speckle-tracking echocardiography with eGFR in pediatric CKD

Variable	**eGFR (ml/min/1,73 m^2)**	
	r coefficient	**P value**
EF (%)	0.385	0.017*
FS (%)	0.358	0.027*
E/A Ratio	0.215	0.195
GLS	0.417	0.009*
Description: r coefficient with Spearman's rho The * sign indicates *p*-value < 0.05 with meaning statistical significance.		

As shown in Table 3, the distribution of GLS in the CKD etiology group was similar (p = 0,797), as did the antihypertension history group (p = 0.418). A significant difference in GLS was observed based on dialysis status (p = 0.003).

Table 3
Global longitudinal strain distribution based on etiology of CKD, dialysis status, and history of antihypertension measured by speckle-tracking echocardiography in pediatric chronic kidney disease

Variable	GLS			*p* value
	n	Mean ± SD	95% CI	
Etiology of CKD				
Glomerular Autoimmune	15	20.0 ± 5.2	17.1–22.9	0.797
Glomerular non-autoimmune	20	19.5 ± 4.2	17.6–21.5	
Non-glomerular	3	18.1 ± 2.5	11.9–24.2	
Dialysis status				
Dialysis	13	16.7 ± 3.8	14.4–19.0	0.003*
Non-dialysis	25	21.1 ± 4.1	19.4–22.8	
History of antihypertension				
Yes	26	19.2 ± 4.4	17.4–21.0	0.418
No	12	20.5 ± 4.6	17.5–23.4	
Description: One-way ANOVA test and unpaired t-test; *p < 0,05 considered statistically significant				

Discussion

There was no predominance between the sexes in pediatric patients with chronic kidney disease in this study, with a median age of 14.04 years, and glomerular disease was the most common etiology. These results are consistent with other studies showing that age and sex do not determine disease progression, but that the predominance of adolescents in this study is related to the etiology of CKD.[4,7,14,15] The severity of CKD in this study varied, with the highest proportion in stages 1 (39.5%), 2 (13.2%), 3 (13.2%), 4 (5.3%), and 5 (28.9%), which is in line with Park's research, which showed that 45.6% of patients had stages 1 and 2 CKD.[16] Amanullah et al. reported different findings, with only 5.2% of children presenting with stage 1 CKD, whereas a higher proportion (72.5%) presented with stages 3–5 CKD. The symptoms of pediatric CKD in its early stages are nonspecific and difficult to diagnose. However, these research results indicate a promising condition because a large proportion of stage 1–4 pediatric CKD can be targeted for early detection and management as well as to observe complications in pediatric CKD, which may occur in the future.[2]

The duration of CKD in this study ranged from 3 to 144 months, with a median of 12 months; 34.2% of the patients had undergone kidney replacement therapy. The majority of etiologies were glomerular disease, both non-autoimmune diseases such as FSGS and autoimmune diseases such as lupus nephritis, and most of them (76.3%) received immunosuppressive therapy. Research on the outcomes of

pediatric FSGS in low- and middle-income countries showed that during a 13-year follow-up period, 58.9% of patients achieved complete remission, 2.7% achieved partial remission, and 38.3% did not. Immunosuppressive therapy with calcineurin inhibitors such as tacrolimus or cyclosporine resulted in complete or partial remission in 62% of the children, but 28.7% progressed to CKD stages 2–5 from the partial remission and no remission groups during the 12 months of follow-up.[17] The 10-year survival rate for children with FSGS in China was 62.58% and the 15-year survival rate was 34.66%.[18] The prognosis is poor if the chronic tubulointerstitial damage is ≥ 25%. Research in the Netherlands on lupus in children with a 20-year follow-up found that 60% had kidney involvement within less than 2 years of diagnosis, and 24% required kidney transplantation at the age of 24 years.[19]

In the present study, 68.4% of the subjects had a history of antihypertensive drug use and 52.6% had a history of blood transfusion. Anemia is a complication of pediatric CKD that causes various consequences in the future, including poor quality of life, decreased neurocognitive abilities, decreased physical activity capacity, and the development of cardiovascular risk factors, such as left ventricular hypertrophy. Data from the NAPRTCS showed that the prevalence of anemia in pediatric CKD patients was 73% in stage 3, 87% in stage 4, and > 93% in end-stage renal disease. Anemia in CKD is influenced by multifactorial causes, including decreased erythropoietin production and iron dysregulation. Other causes, such as autoimmune glomerular abnormalities in lupus, should be considered.[15,20,21] Unlike other complications, hypertension appears early in CKD and its prevalence increases as the glomerular filtration rate declines. The Chronic Kidney Disease in Children (CKiD) study group reported that hypertension already existed in 54% of subjects at baseline, and 48% of them remained abnormal despite receiving antihypertensive therapy. Masked hypertension is a risk factor for left ventricular hypertrophy.[15,22] There was no significant difference in the GLS parameters measured by speckle-tracking echocardiography between the hypertensive and non-hypertensive groups in the present study. Same result reported by Kizilka O et al in Turkey, which only the left ventricular mass index (LVMI) was found to be different.[7]

Cardiovascular complications in CKD are a major cause of increased morbidity and mortality. Cardiovascular abnormalities can appear at an early stage and develop as the CKD progresses. Left ventricular hypertrophy is an important indicator of cardiovascular risk in patients with CKD, and left ventricular geometric dysfunction is associated with a poor prognosis. Several factors influence myocardial remodeling adaptation, such as urea levels, anemia, Fibroblast Growth Factor 23 (FGF 23), phosphorus levels, hyperparathyroidism, fibrosis caused by oxidative stress, and the role of the renin-angiotensin-aldosterone system (RAAS).[14,23,24]

Echocardiography examination for assessing left ventricular function is still widely used in conventional echocardiography through EF and FS parameters; however, further examination using speckle-tracing echocardiography (STE) in the form of GLS values can detect early subclinical left ventricular dysfunction in pediatric CKD patients with preserved EF. In the present study, Spearman's Ro analysis showed a significant correlation between left ventricular function assessed using STE and eGFR

calculated using the Schwartz formula. Ejection Fraction (EF), FS, and GLS had a moderate positive correlation with eGFR (r = 0.385, p = 0.017; r = 0.358, p = 0.027; r = 0.417, p = 0.009), and GLS had the highest correlation among the analyzed parameters. Additionally, the results of the linear regression of EF, FS, and GLS parameters as dependent variables showed a positive correlation and were statistically significant. The R^2 value of the GLS parameter (0.235) was the highest compared to that of EF and FS, indicating that this parameter is more sensitive in detecting changes in left ventricular systolic function associated with renal function decline. These results support the notion that STE assessment based on GLS parameters can identify subclinical left ventricular dysfunction earlier than conventional echocardiography can. Unfortunately, the correlation results of the E/A ratio as a parameter of left ventricular diastolic function did not show a significant relationship with eGFR (r = 0.215; P = 0.195), indicating preserved diastolic function in the present study. This is consistent with research by Demetgul H, who examined EF, FS, E/A ratio, and GLS in 38 subjects and 37 controls. They found a significantly lower longitudinal strain in three 3-chamber and 2-chamber views compared to controls, where all subjects still had normal EF.[14]

Changes in diastolic function, as seen from the decrease in E/A ratio, were found in the CKD group compared to the control group. Diastolic function shows relaxation and compliance of the myocardium. This result indicates that changes in left ventricular diastolic function can occur early in the course of CKD and persist after dialysis and even up to 18 months after kidney transplantation, which is contrary to the present study.[25] Penachio et al. also showed decreased left atrial (LA) strain in the CKD group compared to that in the control group. An important sign of diastolic dysfunction is that changes in LA strain precede increases in the LA volume. However, the evaluation of diastolic dysfunction in children cannot be equated with that in adults because of the diversity of cardiomyopathy types in children[24], and the predominance of stage 1 CKD in this study may be one of the reasons for normal diastolic parameters and lack of correlation with eGFR.

A study by van Huis M on 36 pediatric patients with CKD (19 underwent dialysis, 17 post-kidney transplant) and 33 controls showed no significant differences in EF, SF, and E/A ratio parameters on conventional echocardiography and tissue Doppler imaging (TDI). Speckle-tracking echocardiography results showed a significant decrease in the mean longitudinal strain in CKD patients compared with the control group, which supports the use of STE examination to detect early myocardial dysfunction in pediatric CKD patients who do not show left ventricular systolic or diastolic dysfunction using conventional echocardiography and TDI.[26]

In the present study, there was a significant difference in GLS distribution between the dialysis groups. Early-stage pediatric patients with CKD who have not undergone renal replacement therapy have better left ventricular function. The same finding was reported by Rumman RK research in Canada, which found no significant difference in EF and FS parameters between controls, pediatric patients with CKD, dialyzed patients, and post-kidney transplantation patients. The longitudinal strain parameter results from STE were higher in the pediatric CKD group than in the control group. This value decreased in dialyzed

patients with CKD and improved in post kidney transplant patients.[25] This is in line with the study by Rakha et al., who found that GLS and EF parameters from speckle-tracking echocardiography were lower in the post-hemodialysis group. Hemodialysis aims to improve myocardial function by removing excess fluid and toxins; however, it can trigger cardiac stress, especially in pediatric patients, who are sensitive to acute changes in blood volume, arterial pressure, and electrolyte changes. The activation of injury during the chronic period of hemodynamic and metabolic changes caused by hemodialysis results in endothelial dysfunction, impaired neuronal response, and activation of the inflammatory system, which is known as hemodialysis-induced ischemia or myocardial stunning.[27]

This present study showed that speckle-tracking echocardiography can be used a modality in early detection of left ventricular dysfunction in pediatric chronic kidney disease. Annually program of screening using speckle-tracking echocardiography for children with chronic kidney disease is a targeted aimed in this study which can be minimize cardiac impairment in the future using early detection program.

Limitations

The limitations of this study include its cross-sectional design, which was conducted at a single point in time without follow-up of the cardiac function that may occur over the course of the disease. A single measurement may not reflect the long-term condition of patients or the dynamic changes experienced during the course of chronic kidney disease. This study did not analyze the association between hypertension, anemia, and hyperinflammation progression, which could be a factor in cardiovascular involvement. Other confounding factors such as patient compliance, renal replacement therapy, and medication adherence must be considered. This study was conducted at a single center, and further observational studies are required in the future.

Conclusions

In this study, global longitudinal strain was found to be positively correlated with CKD severity of chronic kidney disease based on the estimated glomerular filtration rate measured using the Schwartz formula. A better CKD condition had a more negative GLS value, which suggests that speckle-tracking echocardiography can be used as a routine examination and modality to identify subclinical left ventricular dysfunction in children with CKD and a preserved ejection fraction. Using this supporting examination, their availability and reliable reference values can be used as guidelines for periodic examination of patients with CKD.

Abbreviations

CAKUT Congenital anomalies of the kidneys and urinary tract

CKD Chronic kidney disease

eGFR estimated glomerular filtration rate

EF Ejection fraction

FGF23 Fibroblast growth factor 23

FS Fractional shortening

FSGS Focal segmental glomerulosclerosis

GLS Global longitudinal strain

KDIGO Kidney disease: improving global outcomes

LVMI Left ventricular mass index

NAPRTCS North American pediatric renal collaborative trials

RAAS Renin-angiotensin-aldosterone system

SD Standard deviation

SPSS Statistical package for social sciences

STE Speckle-tracking echocardiography

TDI Tissue doppler imaging

Declarations

Ethics approval and consent to participate

This study was approved by the Research Ethics Committee of Dr. Hasan Sadikin General Hospital, Faculty of Medicine, Universitas Padjadjaran, Bandung, West Java, Indonesia (Certificate of Approval No. DP.04.03/D.XIV.6.5/248/2025; dated June 11, 2025) and was conducted in accordance with the principles of the Helsinki Declaration. Written informed consent from parents and assent from patients aged 12 - <18 years old were obtained prior to the study being carried out.

Consent for publication

Not applicable.

Data availability declaration

Data supporting the findings of this study are available from the corresponding author (sri.endah@unpad.ac.id) upon request.

Competing interest

The authors declare that they have no competing interests.

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Authors' contribution

Conceptualization: JOT, SER, AW, RBK

Methodology: JOT, SER, AW, RBK, RG, DDLH

Data collection and analysis: JOT, SER, AW, RBK, RG, DDLH

Writing – original draft: JOT, SER, AW, RBK

Writing – review and editing: JOT, SER, AW, RBK, RG, DDLH

All authors read and approved the final manuscript.

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Figures

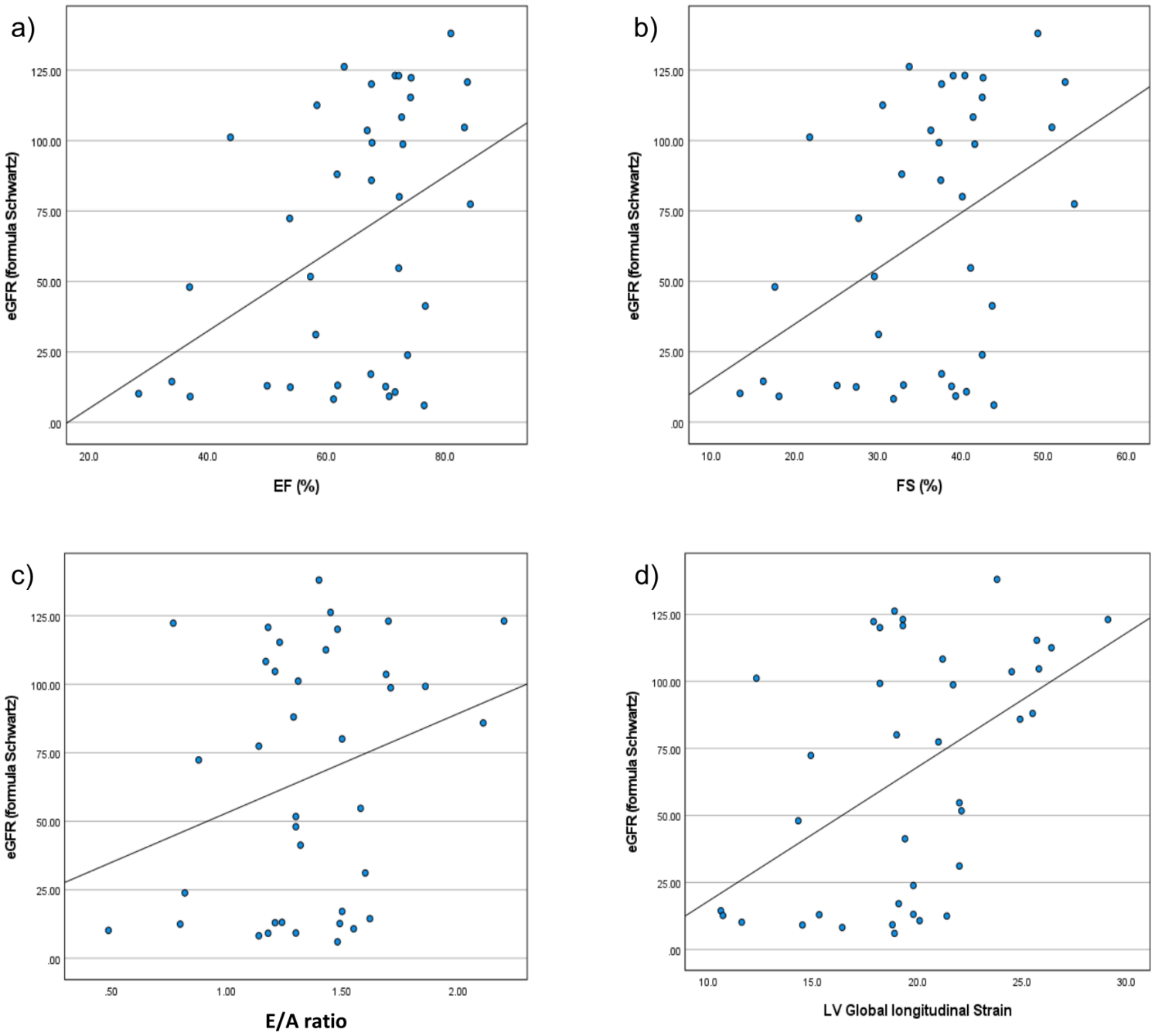


Figure 1

Scatterplot correlation left ventricle function measured by STE with eGFR in pediatric chronic kidney disease

Panel (a) shows the association between ejection fraction (EF) and eGFR, panel (1b) shows the association between fractional shortening (FS) with eGFR, panel (c) shows the association between E/A ratio with eGFR, and panel (d) shows the association between *Global Longitudinal Strain* (GLS) and eGFR. The lines in each panel represent the direction and strength of the association based on *Spearman's Rho* correlation analysis.