European Journal of Molecular & Clinical Medicine

ISSN 2515-8260 Volume 08, Issue 03, 2021

Assessment of Myocardial Abnormalities associated with Sickle Cell Disease Using Speckle Tracking Echocardiography

Mohamed Abd-Elmonem Ismail[1], SoadAbd-ElsalamShedeed[2], andAhmed AbdElsamadElhewala[3]

[1]Resident pediatric physician at Egyptian ministry of health;

[2] Professor of Pediatrics, Faculty of Medicine, Zagazig University.

[3]Assistant Professor of Pediatrics, Faculty of Medicine, Zagazig University.

Correspondingauthor: Mohamed Abd-Elmonem Ismail

Email: mohameda.e.monem@gmail.com

Abstract

Background:Sickle cell disease (SCD) is a major health problem that affects over 20 million people worldwide with over a billion dollars spent annually in the United States (US) itself to cover healthcare costs. Approximately 300,000 children are born annually with SCD at least 75% of them are in Africa. SCD affects about 1 in 500 African Americans and nearly 1 in 12 African Americans carry the autosomal recessive gene. It is estimated that there are almost 100,000 SCD patients in the United States. Speckle-tracking echocardiography (STE) is a new noninvasive ultrasound imaging technique that allows for an objective and quantitative evaluation of the global and regional function of atrial and ventricular chambers. Although in almost all patients with SCD, coronary angiography reveals normal coronary arteries, yet myocardial ischemia and infarction have been reported to occur in SCD. Some SCD patients have marked abnormalities in exercise capacity and cardiac filling abnormalities

Keywords:Sickle cell disease (SCD), Speckle Tracking Echocardiography, Myocardial Abnormalities.

Sickle Cell Disease (SCD):

Sickle cell disease (SCD) is a group of genetic disorders arising from hemoglobin mutation and the presence of hemoglobin S (HbS). It was first described in 1910 by Herrick and characterized by chronic hemolytic anemia and several acute and chronic complications, including pain episodes, stroke, and premature death **(1).**

Epidemiology:

SCD is present mainly in blacks and most common among the individuals of Sub-Saharan Africa, India, the Caribbean, the Middle East, and Mediterranean countries. Egypt was the first to document abnormal HbS and thalassemia in the Middle East Arab countries. Extensive studies about different hemoglobinopathies have been reported since then from nearly all Middle East countries **(2)**.

Approximately 300,000 children are born annually with SCD at least 75% of them are in Africa. SCD affects about 1 in 500 African Americans and nearly 1 in 12 African Americans carry the autosomal recessive gene. It is estimated that there are almost 100,000 SCD patients in the United States **(3).**

According to **Lippi and Mattiuzzi (4),**the worldwide incidence of SCD has increased by (+25.4%) since 1997 it was 0.61 million cases/year in 2017. The prevalence of SCD is about 3.14

million cases; 0.042% of the global population which increased by 52.4% in the last 20 years.

Morbidity and mortality:

SCD is the highest cause-specific disability-adjusted life years (DALYs) among all hemoglobinopathies (3.05 million). As concerns worldwide mortality, SCD causes (38,420 deaths; 0.069% of global deaths) which are fairly steady in the last 20 years **(4).**

Pathophysiology of sickle cell disease (Figure 1-2):

Sickle cell disease (SCD) is a group of genetic disorders, in which affected individuals inherit hemoglobin variants derived from single point mutations, leading to red blood cells (RBC) deformability, hemolysis, and vasoocclusion.Homozygous hemoglobin S (HbSS) and HbS β^0 thalassemia or sickle cell anemia (SCA) are the most common and clinically severe forms of the disease (**5**).

HbS is formed by mutation of GAG to GTG in the sixth codon of the β (beta) globin gene (HBB), where glutamic acid is replaced by valine (**6**). Under low oxygen tension, HbS forms long polymers due to the hydrophobic interaction of valine and phenylalanine.Therefore, RBCs of patients with SCD become less flexible since the polymers lead to biochemical and rheological changes and hence they impair the blood flow leading to vaso-occlusion (VO) **(7).**

The events of ischemia and reperfusion injuries lead to the generation of reactive oxygen species, activation of endothelial cells with increased endothelial adhesion molecule expression, and activation of monocytes, neutrophils, and platelets leading to a chronic inflammatory state. Additionally, intravascular hemolysis of sickle RBCs accelerates nitric oxide (NO) depletion by free Hb, contributing to endothelial dysfunction. Furthermore, the damage of the RBCs membrane by deoxy HbS polymerization through lipid peroxidation result in phosphatidylserine exposure and creating a hypercoagulable state. These mechanisms contribute to chronic tissue damage including sickle nephropathy, chronic lung disease, pulmonary hypertension, avascular necrosis of bone, and eventually to a shortened life expectancy **(8).**

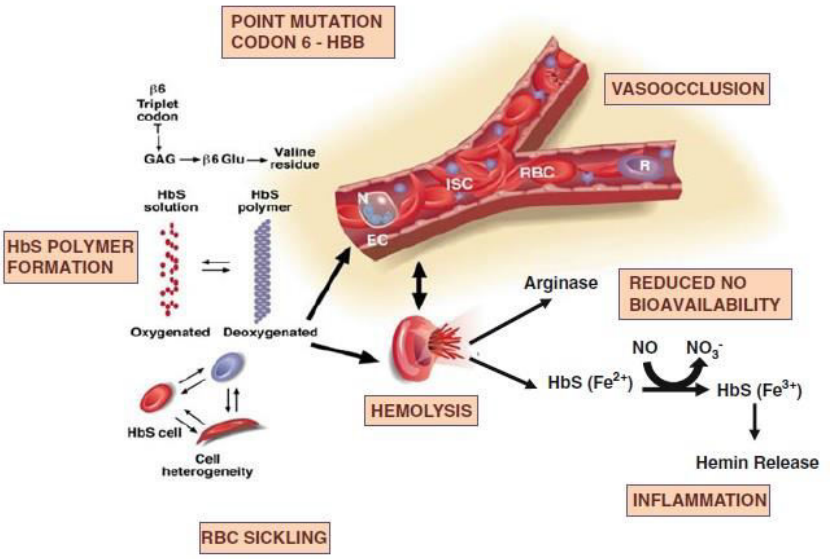

Figure (1): Picture of the pathophysiology of sickle cell disease **(9).**

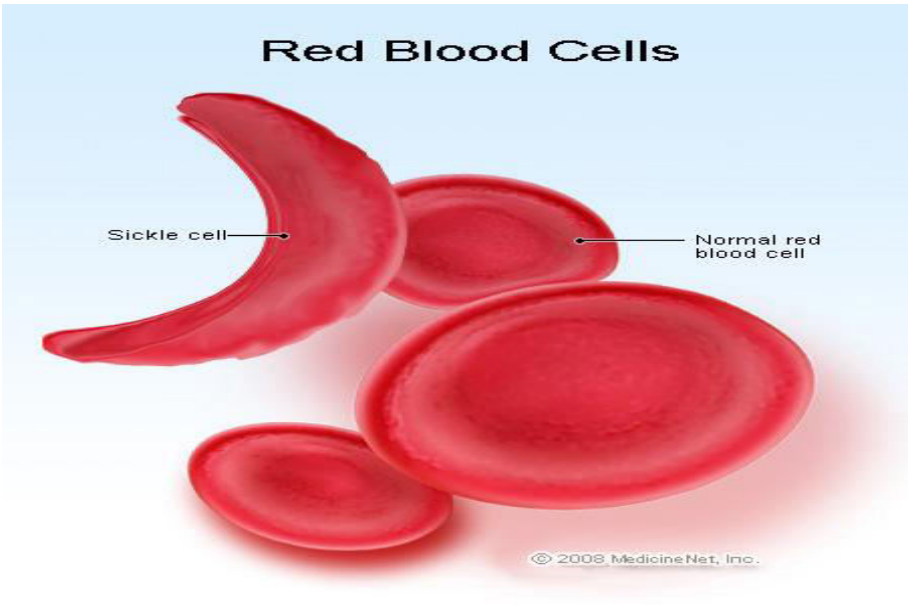

Figure (2): -Picture of Sickle Cell Red Blood Cell **(9).**

Clinical presentation:

History and clinical examination of a sickle cell patient vary widely from being asymptomatic in the first six months of age owing to the presence of fetal hemoglobin HbF that progressively decreases, and HbS begins to be prevalent. SCD presentation is dependable on the type of complication and which body part is affected **(10).**

Physical features:

According to **SA and BG, (11),** SCD patients may have one or more characteristic physical habitus, these include:

Craniofacial features as skull bossing of frontal, parietal, or occipital bones, flat nasal bridge, and gnathopathy. These are mainly due to bone marrow hyperplasia and expansion as compensation for chronic hemolysis resulting in protrusion of midface, maxilla, and or retraction of the mandible.

- Thin and long extremities.
- Short stature with reduced height and weight for age.
- Prominent abdomen by enlarged spleen or liver.
- Jaundice
- Pallor

Symptoms in early life.

Young infants until the age of 6 months are usually asymptomatic but a minor percentage may have neonatal jaundice with unclear explanation in the absence of jaundice known causes or sepsis. In addition, they may manifest progressive anemia after the age of three months triggered by the replacement of fetal hemoglobin HbF with sickle hemoglobin HbS**(11).**

Symptoms in late infancy up till the age of 2 years:

According to **SA and BG, (2020),** Vaso-occlusive crises and Pain is the most common

complaints, which may be acute and severe, or chronic, other symptoms include**:**

- Dactylitis
- Infections
- Anemic crisis
- Growth failure

Symptoms in older children:

Usually, the same as those of late infants and toddlers but dactylitis is not common after the age of two years the main presentation is VOC or anemic crisis which may latterly develop into a hyperhemolytic crisis, aplastic, megaloblastic, or sequestration crisis **(11).**

Myocardial infarction:

Although in almost all patients with SCD, coronary angiography reveals normal coronary arteries, yet myocardial ischemia and infarction have been reported to occur in SCD **(12)**. **Pavlu et al. (13)** revealed ECG abnormalities, defects in nuclear perfusion, MRI abnormalities, and high troponin levels that suggested acute myocardial infarction in SCD patients presenting with the acute crisis. These results were attributed to acute and chronic microvascular occlusion associated with chronic endothelial damage, procoagulant activity, and systemic vasculopathy. Exchange transfusion and aggressive supportive care for the ischemia were able to reverse the cardiac abnormalities.

Functional capacity:

Some SCD patients have marked abnormalities in exercise capacity and cardiac filling abnormalities. The suggested mechanisms for this limitation studied with cardiopulmonary testing include the state of anemia, vascular pulmonary disease, peripheral vascular disease, and/or myopathy. Assuredly, the 6-minute walk test can successfully measure the functional capacity in SCD patients, besides, the walk distance correlates indirectly with the severity of the PH and directly with peak oxygen consumption **(14)**.

In spite of mild increases in pulmonary pressures and pulmonary vascular resistance, **Barst et al. (15)** study on SCD patients showed that SCD patients had more severely reduced 6-minute walk distances in a comparison with patients with primary PAH.

In the Walk-Phasst study, the mean ± SD distance walked in 6-minutes was 409 ± 96 m, 438 ± 98 m, and 458 ± 91 m in patients with TRV values of ≥3.0 meters/sec, 2.7 to <3.0 meters/sec, and <2.7 meters/sec respectively. A reduction in 6-minute walk distance was independently correlated with echocardiographic measures of PH (TRV measuring) and with measures of diastolic dysfunction, which suggested two major independent predictors for cardiac dysfunction with exercise. The limitations in the 6-min walk distance test are known to predict morbidity and mortality in PAH, severe lung disease, and heart failure **(16)**.

Cardiac iron overload:

Myocardial iron deposition is one of the causes of cardiac abnormalities seen in SCD. Although autopsy studies have demonstrated myocardial iron deposition, **(17)**, a study using MRI T2* measurements suggested that myocardial iron deposition is rare even with significant transfusion history, systemic iron overload, or hepatic iron overload **(18)**.

Hepatic iron overload is lower for patients with SCD than for myelodysplastic syndromes or thalassemia major. It may be due to the intermittent course of the transfusions or the limitations for cellular iron accumulation provoked by the systemic inflammatory state. In patients with SCD, ferritin levels were found to be a univariate predictor of mortality. Although myocardial T2* measurements are associated with LV dysfunction and arrhythmias in thalassemia, this relationship has not been established in SCD which suggests that other etiologies for myocardial dysfunction may be more prevalent **(19)**.

Dysrhythmia:

Electrocardiographic abnormalities are not uncommon in SCD patients at rest including QT prolongation.**Liem et al. (20)** reported a prevalence of 38% of QT prolongation in children and young adults with SCD, but they found no correlation between the QT measurement and LV hypertrophy in their series.

Maisel et al. (21) studied 30 patients with SCD by electrocardiographic monitoring over 24-hours during the acute crisis and found that 80% of patients had significant arrhythmias and more than 50% of the patients had ventricular arrhythmias. A subgroup of these patients was included in gated nuclear studies and patients with ventricular dysfunction were found to have a trend towards more arrhythmias.

Sudden death:

Sudden death events are gradually more recognized and reported in aging SCD patients. This occurs during the setting of hospitalization for sepsis or multi-organ failure, recovery from a routine vaso-occlusive crisis, or at home. While this was historically assigned to narcotic "overdose", it is obvious that these incidents are a complication of pulmonary vascular and cardiac diseases **(22)**.

In a large autopsy series of 306 patients with SCD, **Manci et al. (23)** found that in 40% of patients death was sudden, unexpected, and was usually associated with acute events. Moreover, clinical examination of these patients revealed chronic organ injury in 25% of patients, a pathologic evidence of chronic organ injury in 75% of patients suggesting that the severity of the underlying disease is being underestimated.

Gladwin and Sachdev (24) have shown that the majority of deaths were related to cardiopulmonary problems, pulseless electrical activity, heart failure, myocardial infarction, pulmonary thromboembolism, and pulmonary hypertension. Cardiopulmonary problems were the most common findings at the time of death.

Screening of SCD pediatric patients for the risk of PH and cardiovascular morbidity:

Gordeuk et al. (25) included 310 SCD patients aged 3 to 20 years in a prospective longitudinal screening study. They found that TRV values are higher in children (compared with the adult cutoff of 2.5 m/s), a cutoff value of 2.6 m/, should be used for screening in children.

Elevated TRV was found in 11.0% of 310 children and was associated with an increased rate of hemolysis, low oxygen saturation, history of more transfusion settings, ACS, and stroke. In a longitudinal follow-up of 160 patients with Hb SS variant for a median time of 22 months, about

14% of patients had a high TRV (2.60 m/s). Importantly during follow-up, patients with both a high TRV and severe hemolytic anemia had an estimated leap of 4.4-fold in the odds of a 10% decline in the 6-minute walk distance test. This finding suggested that to identify a higher risk of future functional decline in pediatric patients a combination risk factor analysis might be needed **(26)**.

Klings et al. (27)to justify more intensive evaluations in children with SCD suggested that a combination of high TRV, associated clinical symptoms, or laboratory abnormalities might be required. The mortality rate is low in SCD children; while no long enough studies have followed them up to show a mortality risk relation with high TRV at a young age. Consequently, the recommendations for screening are not yet established. Screening should be reserved for SCD children presenting with additional risk factors suggesting early vasculopathy such as dyspnea, hypoxemia, right-sided heart failure, or laboratory evidence of high hemolysis levels or urine proteinuria.

Exchange transfusion therapy for SCD patients with severe PH or diastolic heart disease:
Erythrocytapheresis can rapidly improve cardiopulmonary functions and reduce the risk of death in adult SCD patients. From a physiological standpoint, PRBCs transfusion will increase Hb levels, improve oxygenation status, reduce cardiac wall stress, improve exercise capacity, and improve Po_2. Chronic exchange transfusion might control hemolytic anemia, reduce VOC episodes, and improve cardiopulmonary reserve **(28)**.

From a clinical standpoint, exchange transfusion also reduces VOC and the ACS events that are known to increase mortality in SCD patients. Prophylactic simple transfusion has been successful in reducing all SCD complications tested to date, including postsurgical ACS, VOC, and stroke, yet is complicated by iron overload. Therefore, exchange transfusion will restrict disease progression, improve exercise capacity, and prohibit interval episodes of VOC and ACS in SCD patients with baseline increased pulmonary pressures **(29)**.

The ATS guidelines for PH in SCD recommended that transfusion should be limited to patients with PH who did not tolerate or had suboptimal hydroxyurea response **(27)**. However, **Hassell et al. (30)**did not support this recommendation with prevarication on the need for screening for cardiovascular risk factors such as TRV and high NT-proBNP level as are no proven effective interventions.

Speckle tracking Echocardiography
Speckle-tracking echocardiography (STE) is a new noninvasive ultrasound imaging technique that allows for an objective and quantitative evaluation of the global and regional function of atrial and ventricular chambers, independently from the angle of insonation and cardiac translational motion **(31)**.

STE is based on spatial dislocation analysis (tracking) of speckles (spots that are generated by the interaction between the myocardial fibers and ultrasound beam) on 2-dimensional sonograms. Before the introduction of STE, the deformation components of myocardial dynamics could only be analyzed accurately by tagged magnetic resonance imaging (MRI) **(32)**.

In this area of study tagged MRI may be considered the reference standard; however, its routine use is limited by high cost, time-consuming image analysis, poor availability, and the relative complexity of acquisitions **(33)**.

By tracking the speckles displacement during the cardiac cycle, STE allows the semi-automated elaboration of myocardial deformation in three different spatial directions: longitudinal, radial, and circumferential. Additionally, STE offers an evaluation of the occurrence, direction, and velocity of left ventricle (LV) rotation. STE is more sensitive than conventional echocardiography regarding the detection of subclinical ventricular dysfunction in multiple clinical disorders **(32)**.

Although STE was introduced exclusively for the analysis of LV function, recently multiple studies have extended its applicability to other cardiac chambers, such as the left atrium (LA) **(34)**.

Main technical considerations:

The term speckle tracking indicates that this technique is principally based on speckles analysis during the cardiac cycle. Different speckles are merged in univocally identifiable functional units (kernels) given the unique disposition of the speckles. Subsequently, each kernel constitutes a design of ultrasound fingerprint that software can track during the entire cardiac cycle. Through the motion analysis of each kernel that composes a routine 2-dimensional grayscale image, the system, without using the Doppler signal, can calculate displacement, velocity (the rate of displacement), strain (deformation), strain rate (the rate of deformation of the selected myocardial segments), and LV rotation **(35).**

According to the indications derived from the literature and to reduce random noise, each sample for an STE analysis must be obtained by averaging at least 3 consecutive heart cycles, setting the frame rate of the routine 2-dimensional image acquisition between 60 and 110 frames per second **(35).**

Considering the close dependence of STE strain analysis, it is inconceivable to conduct a study in patients with non-sinus rhythms. STE-derived measurements have been verified against sonomicrometry and tagged MRI, showing high reproducibility and feasibility. Substantial potential limitations of STE are its strict dependence on the frame rate and high-quality 2-dimensional images, which are required to obtain an optimal definition of the endocardial border **(35).**

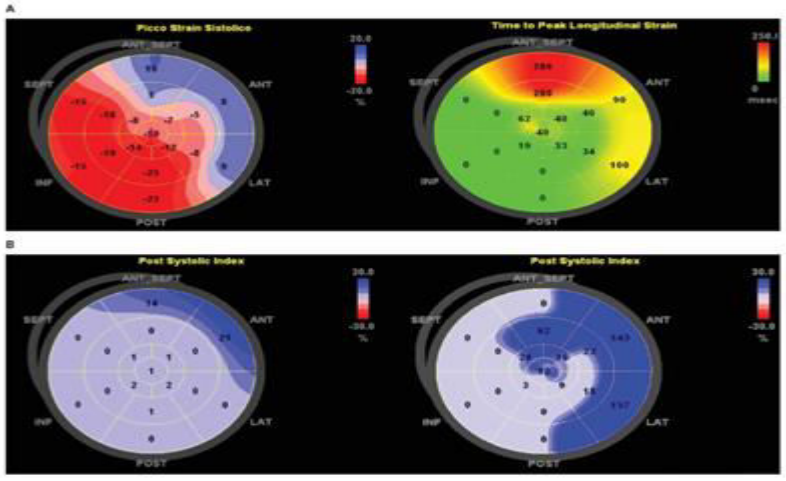

Figure (3): Topographic bull's-eye representing different strain measures

A, Represent longitudinal strain (**left**) and time-to-peak longitudinal strain (**right**) in a patient with

severe left anterior descendent artery stenosis. Note the chromatic individuation of the ischemic area (**left**), also, delayed contractions are shown as (red area with contraction delay of 286 milliseconds; **right**).

B, Postsystolic STE index measurement at baseline (**left**) and under physical stress (50 W; **right**) in a patient undergoing stress exercise echocardiography. Note the LV contraction delay getting worse in the anterolateral territory. Later coronary angiography showed left main and left anterior descendent artery disease. ANT indicates anterior; ANT_SEPT, anteroseptal; INF, inferior; LAT, lateral; and POST, posterior **(35).**

Speckle tracking echocardiography in SCD

Numerous studies have demonstrated the ability of echocardiography in the assessment of myocardial deformation accurately in variable diseases. Specifically, tissue Doppler imaging (DTI), the two-dimensional (2D), and speckle tracking echocardiography (STE). Echocardiography is used to detect any subtle changes in the LV systolic function with a preserved ejection fraction, and any changes in LV filling **(36)**

In the meantime, three-dimensional (3D) echocardiography (RT3DE) has developed into a useful modality for cardiac imaging. Real-time three-dimensional echocardiography can accurately estimate the LV volume throughout the cardiac cycle, including the ability to quantitatively evaluate LV filling. Furthermore, RT3DE imaging allows automated tracking and dynamic measurements of myocardial deformation **(37)**.

Park et al. (38) have used TDI and/or 2D-STE to assess myocardial strain in a variety of cardiac problems. The accuracy of these techniques is limited, since TDI is known to be angle-dependent, and 2D-STE can only track motion within the imaging plane. The through-plane motion affects tracking adversely when speckles move out of the imaging plane. On the other hand, the three-dimensional speckle tracking echocardiography (3D-STE) is mostly angle-independent and preferred for the assessment of myocardial deformation because moving speckles can be easily tracked from frame to frame since they remain in the imaging volume.

Maffessanti et al. (39)used a prototype software that reconstructs the beating endocardial surface by tracking speckles frame-by-frame in 3D space. Through this, LV volume is calculated over time, and myocardial strain is measured throughout the cardiac cycle.

Ahmad et al. (40) used 3D STE to assess any changes in the systolic and diastolic LV function in patients with SCD. They approved the ability of 3D-STE to confirm diastolic dysfunction. However, 3D-STE did not reveal any changes in LV systolic function.

ConflictofInterest: Noconflictofinterest.

References

1.**Snyder, A. B., Zhou, M., Theodore, R., Quarmyne, M. O., Eckman, J., Lane, P. A. (2019).** Improving an administrative case definition for longitudinal surveillance of sickle cell disease. Public Health Reports, 134(3), 274-281.

2.**El-Hazmi, M. A., Al-Hazmi, A. M., Warsy, A. S. (2011).**Sickle cell disease in Middle East Arab countries. The Indian journal of medical research, 134(5), 597.

3.**Ismail, A., Yusuf, A. A., Kuliya-Gwarzo, A., Ahmed, S. G., Tabari, A. M., Abubakar, S. A. (2019).** Correlating transcranial arterial Doppler velocities with haematologic parameters and haemolytic indices of Nigerian children with sickle cell anaemia. Ultrasound, 27(2), 101-110.

4.**Lippi, G., Mattiuzzi, C. (2020).** Updated worldwide epidemiology of inherited erythrocyte disorders. Acta haematologica, 143(3), 196-203.

5.**Weatherall**, **D. J. (2013).** The role of the inherited disorders of hemoglobin, the first "molecular diseases," in the future of human genetics. Annual review of genomics and human genetics, 14, 1-24.

6.**Serjeant, G. R., Vichinsky, E. (2018).**Variability of homozygous sickle cell disease: The role of alpha and beta globin chain variation and other factors. Blood Cells, Molecules, and Diseases, 70, 66-77.

7.**Kato, G. J., Piel, F. B., Reid, C. D., Gaston, M. H., Ohene-Frempong, K., Krishnamurti, L., Vichinsky, E. P. (2018).**Sickle cell disease. Nature Reviews Disease Primers, 4(1), 1-22.

8.**Hebbel, R. P., Hedlund, B. E. (2018).** Sickle hemoglobin oxygen affinity-shifting strategies have unequal cerebrovascular risks. American journal of hematology, 93(3), 321-325.

9.**Steinberg, M. H. (2016).** Overview of sickle cell anemia pathophysiology. In Sickle Cell Anemia (pp. 49-73). Springer, Cham.

10. **Sedrak A, Kondamudi NP. Sickle Cell Disease. [Updated 2021 Feb 26].**In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK482384/.

11. **SA, A., BG, O. (2020).** CLINICAL FEATURES OF SICKLE CELL DISEASE IN CHILDREN ADEGOKE SA AND OLOGUN BG. Sickle Cell Disease: From the Laboratory to Clinical Practice, 54.

12. **Pannu, R., Zhang, J., Andraws, R., Armani, A., Patel, P.,** Mancusi-Ungaro, P. (2008). Acute myocardial infarction in sickle cell disease: a systematic review. Critical pathways in cardiology, 7(2), 133-138.

13. **Pavlů, J., Ahmed, R. E., O'Regan, D. P., Partridge, J., Lefroy, D. C., Layton, D. M. (2007).** Myocardial infarction in sickle-cell disease. The Lancet, 369(9557), 246.

14. **Dang, N. C., Johnson, C., Eslami-Farsani, M., Haywood, L. J. (2005).**Myocardial injury or infarction associated with fat embolism in sickle cell disease: a report of three cases with survival. American journal of hematology, 80(2), 133-136.

15. **Barst, R. J., Langleben, D., Frost, A., Horn, E. M., Oudiz, R., Shapiro, S., Frumkin, L. R. (2004).**Sitaxsentan therapy for pulmonary arterial hypertension. American journal of respiratory and critical care medicine, 169(4), 441-447.

16. **ATS Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories. (2002).** ATS statement: guidelines for the six-minute walk test. Am J Respir Crit Care Med, 166, 111-117.

17. **Darbari, D. S., Kple-Faget, P., Kwagyan, J., Rana, S., Gordeuk, V. R., Castro, O. (2006).** Circumstances of death in adult sickle cell disease patients. American journal of hematology, 81(11), 858-863.

18. **Inati, A., Musallam, K. M., Wood, J. C., Sheikh-Taha, M., Daou, L., Taher, A. T. (2009).** Absence of cardiac siderosis by MRI T2* despite transfusion burden, hepatic and serum iron overload in Lebanese patients with sickle cell disease. European journal of haematology, 83(6), 565-571.

19. **Kirk, P., Roughton, M., Porter, J. B., Walker, J. M., Tanner, M. A., Patel, J., Pennel, D. J. (2009).** Cardiac T2* magnetic resonance for prediction of cardiac complications in thalassemia major. Journal of Cardiovascular Magnetic Resonance, 11(1), 1-316.

20. **Liem, R. I., Young, L. T., Thompson, A. A. (2009).** Prolonged QTc interval in children and young adults with sickle cell disease at steady state. Pediatric blood cancer, 52(7), 842-846.

21. **Maisel, A., Friedman, H., Flint, L., Koshy, M., Prabhu, R. (1983).** Continuous electrocardiographic monitoring in patients with sickle-cell anemia during pain crisis. Clinical cardiology, 6(7), 339-344.

22. **Graham, J. K., Mosunjac, M., Hanzlick, R. L., Mosunjac, M. (2007).** Sickle cell lung disease and sudden death: a retrospective/prospective study of 21 autopsy cases and literature review. The American journal of forensic medicine and pathology, 28(2), 168-172.

23. **Manci, E. A., Culberson, D. E., Yang, Y. M., Gardner, T. M., Powell, R., Haynes Jr, J., Investigators of the Cooperative Study of Sickle Cell Disease. (2003).** Causes of death in sickle cell disease: an autopsy study. British journal of haematology, 123(2), 359-365.

24. **Gladwin, M. T., Sachdev, V. (2012).** Cardiovascular abnormalities in sickle cell disease. Journal of the American College of Cardiology, 59(13), 1123-1133.

25. **Gordeuk, V. R., Minniti, C. P., Nouraie, M., Campbell, A. D., Rana, S. R., Luchtman-Jones, L., Castro, O. L. (2011).** Elevated tricuspid regurgitation velocity and decline in exercise capacity over 22 months of follow up in children and adolescents with sickle cell anemia. Haematologica, 96(1), 33.

26. Nouraie, M., Lee, J. S., Zhang, Y., Kanias, T., Zhao, X., Xiong, Z., Gladwin, M. T. (2013). The relationship between the severity of hemolysis, clinical manifestations and risk of death in 415 patients with sickle cell anemia in the US and Europe. Haematologica, 98(3), 464.

27. Klings, E. S., Machado, R. F., Barst, R. J., Morris, C. R., Mubarak, K. K., Gordeuk, V. R., Gladwin, M. T. (2014). An official American Thoracic Society clinical practice guideline: diagnosis, risk stratification, and management of pulmonary hypertension of sickle cell disease. American journal of respiratory and critical care medicine, 189(6), 727-740.

28. Detterich, J. A., Kato, R. M., Rabai, M., Meiselman, H. J., Coates, T. D., Wood, J. C. (2015). Chronic transfusion therapy improves but does not normalize systemic and pulmonary vasculopathy in sickle cell disease. Blood, 126(6), 703-710.

29. MekontsoDessap, A., Leon, R., Habibi, A., Nzouakou, R., Roudot-Thoraval, F., Adnot, S., Maitre, B. (2008). Pulmonary hypertension and corpulmonale during severe acute chest syndrome in sickle cell disease. American journal of respiratory and critical care medicine, 177(6), 646-653.

30. Hassell, K. L., Afenyi-Annan, A., Ballas, S. K., Buchanan, G. R., Eckman, J. R., Jordan, L., Ware, R. (2014). Practice guideline for pulmonary hypertension in sickle cell: direct evidence needed before universal adoption. American journal of respiratory and critical care medicine, 190(2), 237-238.

31. Cameli, M., Mandoli, G. E., Sciaccaluga, C., Mondillo, S. (2019). More than 10 years of speckle tracking echocardiography: still a novel technique or a definite tool for clinical practice? Echocardiography, 36(5), 958-970.

32. Kahil, A., Al-Habbaa, A., Allam, S. (2020). LEFT ATRIAL FUNCTION ASSESSMENT BY 2D-SPECKLE TRACKING ECHOCARDIOGRAPHY IN HEMODIALYSIS PATIENTS WITH NORMAL LEFT VENTRICULAR SYSTOLIC FUNCTION. Al-Azhar Medical Journal, 49(3), 1075-1092.

33. Shaw, S. M., Fox, D. J., Williams, S. G. (2008). The development of left ventricular torsion and its clinical relevance. International journal of cardiology, 130(3), 319-325.

34. van Dalen, B. M., Soliman, O. I., Vletter, W. B., Kauer, F., van der Zwaan, H. B., ten Cate, F. J., Geleijnse, M. L. (2009). Feasibility and reproducibility of left ventricular rotation parameters measured by speckle tracking echocardiography. European Journal of Echocardiography, 10(5), 669-676.

35. Mondillo, S., Galderisi, M., Mele, D., Cameli, M., Lomoriello, V. S., Zacà, V., Badano, L. (2011). Speckle-tracking echocardiography: a new technique for assessing myocardial function. Journal of Ultrasound in Medicine, 30(1), 71-83.

36. Dokainish, H., Sengupta, R., Pillai, M., Bobek, J., Lakkis, N. (2008). Usefulness of new

diastolic strain and strain rate indexes for the estimation of left ventricular filling pressure. The American journal of cardiology, 101(10), 1504-1509.

37. **Mor-Avi, V., Lang, R. M., Badano, L. P., Belohlavek, M., Cardim, N. M., Derumeaux, G., Zamorano, J. L. (2011).** Current and evolving echocardiographic techniques for the quantitative evaluation of cardiac mechanics: ASE/EAE consensus statement on methodology and indications endorsed by the Japanese Society of Echocardiography. European Journal of Echocardiography, 12(3), 167-205.

38. **Park, S. M., Miyazaki, C., Prasad, A., Bruce, C. J., Chandrasekaran, K., Rihal, C., Oh, J. K. (2009).** Feasibility of prediction of myocardial viability with Doppler tissue imaging following percutaneous coronary intervention for ST elevation anterior myocardial infarction. Journal of the American Society of Echocardiography, 22(2), 183-189.

39. **Maffessanti, F., Nesser, H. J., Weinert, L., Steringer-Mascherbauer, R., Niel, J., Gorissen, W., Mor-Avi, V. (2009).**Quantitative evaluation of regional left ventricular function using three-dimensional speckle tracking echocardiography in patients with and without heart disease. The American journal of cardiology, 104(12), 1755-1762.

40. **Ahmad, H., Gayat, E., Yodwut, C., Abduch, M. C., Patel, A. R., Weinert, L., Mor-Avi, V. (2012).** Evaluation of myocardial deformation in patients with sickle cell disease and preserved ejection fraction using three-dimensional speckle tracking echocardiography. Echocardiography, 29(8), 962-969.