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Cardiovascular Function During Rest and Exercise in Patients With Sickle-Cell Anemia and Coexisting Alpha Thalassemia-2

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Cardiac function was measured at rest and during exercise in 9 patients with sickle-cell anemia (SS) and coexisting homozygous alpha thalassemia-2 (alpha thal-2). Results were compared with 18 sickle cell patients with normal alpha globin genes, who were matched to the study group by age, gender, and size, and to published normal values. SS alpha thal-2 patients were less anemic: 9.9 ± 1.0 vs 8.2 ± 1.2 gm/dl for SS alone ($P < .05$). Left ventricular dimensions were normal in SS alpha thal-2 (4.9 ± 0.7 cm), but increased in SS (5.4 ± 0.7, cm $P = .05$) (normal range, 3.7–5.6 cm). Left ventricular wall thickness was, however, dramatically increased in the SS alpha thal-2 patients (free wall, 1.8 ± 0.6 cm; septum, 1.6 ± 0.4 cm), though SS controls had normal wall thickness (free wall, 1.0 ± 0.2 cm; septum, 1.0 ± 0.2 cm, $P < .001$) (normal range, 0.6–1.1 cm). At rest, Doppler indices of systolic function were not significantly different between sickle groups and normal values. SS alpha thal-2 patients did have abnormal diastolic filling at rest, as evidenced by a reduced ratio of early/late diastolic filling, 1.4 ± 0.3 vs. 2.0 ± 0.5 for SS controls ($P < .01$), and 1.8 ± 0.4 for normals. An analysis of covariance suggested that this abnormality persisted after taking into account the previously demonstrated hypertrophy. During exercise, SS alpha thal-2 patients had higher heart rates and blood pressures than SS controls in spite of performing the same or less work. This resulted in a higher double product (an estimate of oxygen consumption) in SS alpha thal-2 patients (37,470 ± 2,310 mm Hg-BPM) than in SS controls (33,310 ± 1,490 mm Hg-BPM, $P < .01$). Work capacity, peak heart rate, and blood pressure were all abnormally decreased in both sickle-cell groups when compared to normal. Cardiac abnormalities noted at rest and during exercise in SS alpha thal-2 patients suggest a role of microvascular occlusion and a protective effect of decreased hemoglobin. © 1996 Wiley-Liss, Inc.

Key words: cardiac function, sickle-cell anemia, alpha thalassemia

INTRODUCTION

Patients with sickle-cell (SS) anemia have a dramatically diminished physical work capacity [1–4]. Transfusion studies have shown that this is related to the reduced oxygen carrying capacity associated with anemia [5–8]. Transfusion to a higher hemoglobin results in improved exercise performance [7]. Partial exchange transfusion of SS patients with normal donor blood to raise hemoglobin A concentrations without increasing the total hemoglobin level has also been shown to increase exercise capability [8], suggesting that sickle erythrocytes contribute to poor exercise capacity.

A significant percentage of patients with sickle-cell anemia have abnormalities of systolic and diastolic cardiac function which do not appear to be explained entirely on the basis of chronic severe anemia [2,3,9–18]. Vasoocclusive effects of sickle hemoglobin on the circulation, and possibly the heart, could contribute significantly to the reduced physical work capacity [3,16–18].

In order to discern the effects of severe chronic anemia from those due to the presence of sickle hemoglobin, a unique group of SS patients was identified, who were

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TABLE I. Anthropometric Data*

	Sickle cell (n = 18)		Sickle/thalassemia (n = 9)		
	m	SD	m	SD	*P*
Height (cm)	169.1	8.6	167.1	10.4	NS
Weight (kg)	59.1	8.6	57.8	7.0	NS
BSA (M^2)	1.68	0.15	1.64	0.15	NS
Age (years)	30.4	6.3	33.2	9.9	NS
RHR (bpm)	75.9	12.4	79.6	10.9	NS

*All values in this and subsequent tables are expressed as mean (m) + SD. BSA, body surface area; RHR, resting heart rate; M^2, square meters; bpm, beats per minute; NS, nonsignificant.

less anemic, but continued to have the vasoocclusive manifestations of SS disease. These patients are homozygous for coexisting alpha thalassemia-2 and have a deletion of one of the two alpha globin genes on each chromosome 16, responsible for alpha globin synthesis [19–21]. Homozygous alpha thalassemia occurs in 3–4% of patients with SS disease and should not be confused with S/beta^0 thalassemia, which is phenotypically similar to SS anemia. In this study, Doppler echocardiography and exercise testing were used to compare patients in this unique SS alpha thal-2 group with age-, gender-, and size-matched SS controls in order to determine which cardiac abnormalities persist in the absence of severe chronic anemia.

METHODS

Nine patients (age 33 ± 10 years) with SS disease and concomitant alpha-thal-2 were studied. Eighteen SS subjects, with a normal number of alpha chains matched by age, gender, and body size to the 9 study patients, were recruited from the patient population of the Comprehensive SS Center at the Medical College of Georgia (MCG) to serve as controls (Table I). DNA analyses were performed using the Southern blot technique [22,23].

All subjects were crisis-free for 2 weeks prior to their participation. None had been transfused in the preceding 3 months. This study was approved by the Committee on Human Assurance at MCG, and written informed consent was obtained from all participants.

A history and physical examination were performed to identify medical conditions that would preclude exercise testing. None of the patients or controls had evidence of valve disease, hypertension, or cardiac ischemia. Two study patients and 3 controls were excluded from exercise testing on the basis of hip discomfort or leg ulcers. Baseline data, including height, weight, heart rate, blood pressure, a complete blood count including percentage of fetal hemoglobin, an electrocardiogram, and an echocardiogram, were obtained immediately prior to exercise testing. None were excluded on the basis of an abnormal resting electrocardiogram.

Two-dimensional image-directed M-mode echocardiograms were obtained using standard techniques and commercially available ultrasound instruments. All measurements were made according to the recommendations of the American Society of Echocardiography [24]. The following dimensions were obtained: left ventricle end-diastole (LVED), left ventricle end-systole (LVES), posterior wall thickness (LVWD), interventricular septum thickness (IVSD), right ventricle end-diastole (RVED), aortic root (AO), and left atrium (LA). Tracings were obtained at a sweep speed of 50 mm/sec. Shortening fraction (SF) [(LVED-LVES)/LVED] × 100 was calculated.

Image-directed pulsed Doppler tracings of aortic valve outflow velocities and mitral valve inflow velocities were obtained for each subject. Aortic pulsed Doppler tracings were utilized to obtain the following systolic variables: aortic peak velocity (APV), and Doppler-derived LVPEP, LVET, and LVSTI. Mitral valve pulsed Doppler tracings were used to obtain peak early filling velocity (PFV) and peak atrial velocity (PAV). Diastolic time intervals included: time to peak filling (TPF) and time to peak filling after aortic ejection (TPFAE), area under the E-wave and area under the A-wave. Calculated measurements of diastolic left ventricular filling included: atrial filling fraction (AFF) (area under A/[area under E + area under A]) and PFV/PAV. All Doppler tracings were recorded with a simultaneous electrocardiogram. Two sets of measurements were obtained and averaged for each subject.

Exercise tests were performed in the postabsorptive state. All patients were fully ambulatory. Continuous graded maximal exercise was performed sitting on a mechanically braked cycle ergometer. Maximal workload was estimated upon the individual's height, according to James et al. [25]. Exercise began at 25% of maximal predicted exercise capacity, and the workload was increased to 50%, 75%, and 100% of maximal predicted work capacity at 3-min intervals. Patients exercised until exhausted (maximum voluntary effort). Heart rate, blood pressure, and three-lead electrocardiogram (leads AVF, V_2, and V_5) were recorded prior to and at each level of exercise. Exercise duration, physical working capacity indexed for body surface area (PWCI), percentage of work performed compared to that expected (percent expected), and double product (heart rate × blood pressure) at maximum exercise were calculated.

S-T segment morphology was assessed at each exercise stage.

Echo Doppler data from healthy normal controls were obtained from Feigenbaum [26], Gardin et al. [27], and Benjamin et al. [28]. Intergroup comparisons of SS patients with and without alpha thal-2 were performed, using one-factor analyses of variance. Analysis of covariance

TABLE II. Hematologic Variables

	Sickle cell (n = 18)		Sickle/thalassemia (n = 9)		
	m	SD	m	SD	*P*
Hgb (gm/dl)	8.18	1.21	9.88	0.93	<.05
Hct	23.9	3.4	29.1	3.6	<.05
HbF %	6.4	5.0	5.9	3.3	NS

Hgb, hemoglobin; Hct, hematocrit; HbF, fetal hemoglobin; gm/dl, grams/deciliter.

was employed in order to control for effects of hemoglobin, hematocrit, and ventricular wall thickness. The means of Doppler variables from both groups were compared to normal values using Z scores. Double products at peak exercise were compared between the two groups, utilizing a t-test.

RESULTS

Patient groups were well-matched for age, gender, and size (Table I). SS patients with alpha thal-2 had significantly greater hemoglobin (Hgb) and hematocrit (Hct) values than those subjects with SS anemia alone. The differences in percentages of fetal hemoglobin (HbF%) did not reach significance (Table II).

Rest

LVED and LVES were within normal range in the SS alpha thal-2 patients when compared to published normals. In contrast, LVED and LVES were increased in the group with SS disease alone and when compared to the SS alpha thal-2 group ($P < .05$). LVWD and IVSD were significantly increased in the alpha thal-2 patients, compared to patients with SS disease alone ($P < .001$) and published normal controls. Differences in LA and AO dimensions between groups did not reach significance (Table III). Normal values are from Feigenbaum [26].

Systolic Function

Differences in systolic function as assessed by aortic Doppler indices between groups were not significant.

APV was increased relative to normal values as described by Gardin et al. [27] for both groups (Table IV).

Diastolic Filling

Peak early filling velocity (PFV) was significantly lower in the SS alpha thal-2 group than in the group with SS disease alone ($P < .01$). By covariate analysis this occurred independent of differences in IVSD and LVWD ($P < .05$). Differences in peak atrial velocities (PAV) between groups were not significant. The ratio of PFV/PAV was significantly lower in the SS alpha thal-2 group than in the group with SS alone ($P < .01$). E-area was increased in the SS-alone group compared to published normal values, but the difference between the two SS groups did not achieve statistical significance. Differences between the two SS groups with regards to A-area, TPF, TPFAE, and AFF were not significant (Table V). A-area was increased in both groups, compared to published normal values [28].

Exercise

The SS alpha thal-2 group demonstrated higher heart rates just prior to exercise and in all phases of recovery than did those subjects with SS disease alone (Table VI). Maximum heart rate was lower than published normals [2] in both groups. The SS alpha thal-2 group also exhibited greater systolic blood pressures at maximal exercise and during all phases of recovery than the SS patients (Fig. 1). Patients with SS disease had lower blood pressures at maximum exercise than published normals [3], though this was less striking in the SS alpha thal-2 group. Resting systolic and diastolic blood pressures were normal, and differences between the two sickle-cell groups were not significant. Less consistent differences were seen in diastolic pressures, with significantly higher values in the SS alpha thal-2 subjects at maximal exercise and at minute 5 of recovery. Mean arterial pressures were higher throughout exercise in the SS alpha thal-2 subjects; however, these differences were significant only during the three phases of recovery.

Differences in percentage of expected work capacity in the two patient groups were not significant (Table VI), and both groups were reduced compared to published normal values [25]. SS alpha thal-2 subjects had significantly higher double products at peak exercise than patients with SS disease alone (37,470 ± 2,310 mm Hg-BPM vs. 33,310 ± 1,490 mm Hg-bpm; $P < .01$), suggesting a greater myocardial oxygen consumption. S-T depression was not seen in either group.

DISCUSSION

The interaction of alpha thal-2 with homozygous SS disease has been shown to affect both the hematologic indices as well as the clinical manifestations of SS disease [19–21,29]. SS patients with concomitant alpha thal-2 have significantly higher red-cell counts, Hgb, Hct, and Hgb A_2 [19–21]. They also exhibit lower mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume, reticulocyte counts, irreversibly-sickled cell counts, and serum total bilirubin level [21]. Because of the decrease in MCHC, they have a prolonged cell life span and a decrease in hemolytic anemia [19]. The decrease in hemolytic anemia is also mediated by a decreased intraerythrocyte hemoglobin S concentration [20]. However, the increased red-cell survival may actually lead to an increased frequency of microvascular oc-

TABLE III. M-Mode Measurements

	Sickle cell (n = 18)		Sickle/thalassemia (n = 9)			Normal	
	m	SD	m	SD	*P*	m	Range
LVED (cm)	5.43	0.68	4.87	0.66	.05	4.7	(3.7–5.6)
LVES (cm)	3.6	0.53	3.13	0.50	<.05		
LVWD (cm)	1.02	0.17	1.79	0.63	<.001	0.9	(0.6–1.1)
IVSD (cm)	1.03	0.17	1.63	0.39	<.001	0.9	(0.6–1.1)
AO (cm)	2.71	0.40	2.75	0.37	NS	2.7	(2.0–3.7)
LAD (cm)	3.53	0.45	3.69	0.65	NS	2.9	(1.9–4.0)
SF (%)	33.7	5.0	35.7	5.0	NS	36	(28–44)

SF, shortening fraction; LVED, left ventricular end-diastolic dimension; LVES, left ventricular end-systolic dimension; LVWD, left ventricular wall thickness in diastole; IVSD, interventricular septal thickness in diastole; AO, aortic root dimension; LAD, left atrial dimension.

TABLE IV. Doppler Indices of Systolic Function

	Sickle cell (n = 18)		Sickle/thalassemia (n = 9)			Normal	
	m	SD	m	SD	*P*	m	SD
APV (cm/sec)	113.3	22.8	110.3	33.0	NS	92	11
LVPEP (msec)	97.2	22.6	94.1	22.2	NS		
LVET (msec)	329.7	46.7	300.6	42.2	NS	294	19
LVSTI	0.30	0.10	0.32	0.09	NS		

APV, aortic peak velocity; LVPEP, left ventricular preejection period; LVET, left ventricular ejection time; LVSTI, left ventricular systolic time index.

TABLE V. Doppler Indices of Diastolic Function

	Sickle cell (n = 18)		Sickle/thalassemia (n = 9)			Normal	
	m	SD	m	SD	*P*	m	SD
PFV (cm/s)	80.0	14.5	64.5	10.2	<.01	66	14
PAV (cm/s)	41.5	10.0	46.3	9.1	NS	38	6
E-area (cm^2)	13.1	3.8	10.8	3.0	NS	8.5	1.8
A-area (cm^2)	5.3	1.2	5.2	1.5	NS	2.9	0.6
TPF (msec)	536.4	24.4	527.9	37.5	NS		
TPFAE (msec)	109.4	52.2	133.2	26.9	NS		
AFF	0.31	0.07	0.33	0.09	NS	0.25	0.05
PFV/PAV	2.01	0.51	1.44	0.31	<.01	1.75	0.4

PFV, peak filling velocity; PAV, peak atrial velocity; E-area, area under peak filling curve; A-area, area under atrial filling curve; TPF, time to peak filling; TPFAE, time to peak filling after ejection; AFF, atrial filling fraction.

clusive problems such as proliferative retinopathy, osteonecrosis, and visceral complications, as well as an increased incidence of painful crises due to the combination of poorly deformable cells without the protective viscosity-lowering effects of more severe anemia [21].

The SS alpha thal-2 group had near-normal left ventricular dimensions compared to those of the SS group, which were significantly increased. However, the alpha thal-2 group had significantly greater wall thicknesses. These differences may be related to differences in Hgb. A higher Hgb may have resulted in increased viscosity, which in turn increased systemic vascular resistance and left ventricular wall stress, resulting in hypertrophy. LA dimensions were in the normal range and differences between groups were not significant, possibly due to the opposing effects of anemia and ventricular compliance.

Differences between the two groups in systolic function at rest, assessed by M-mode and Doppler echocardiography, were not significant, and both groups were within previously-reported normal ranges [26,27].

Important differences in diastolic filling patterns between the two groups were observed in this study. PFV

TABLE VI. Response to Exercise

	Sickle cell (n = 15)		Sickle/thalassemia (n = 7)		
	m	SD	m	SD	*P*
Pre-ex heart rate (beats/min)	79.1	11.2	90.7	13.0	<.05
Max heart rate	150.7	20.2	167.7	15.7	.06
Rec 1	122.0	13.7	148.3	17.6	<.005
Rec 2	107.4	12.2	125.4	17.2	<.05
Rec 3	94.5	11.2	110.9	14.3	<.05
Duration (min)	6.3	2.6	5.1	2.0	NS
PWCI (KgM/m^2)	9.5	4.0	6.8	3.3	NS
% expected	51.7	22.1	39.3	13.4	NS
Double product (mm Hg · BPM)	33,310	1,490	37,470	2,310	<.01

Pre-ex, preexercise; Max, maximal exercise; Rec, recovery stage; Duration, duration of exercise; PWCI, physical work capacity index (kilograms-meters/meters^2); % expected, % of predicted work completed; Double product, heart rate × systolic blood pressure.

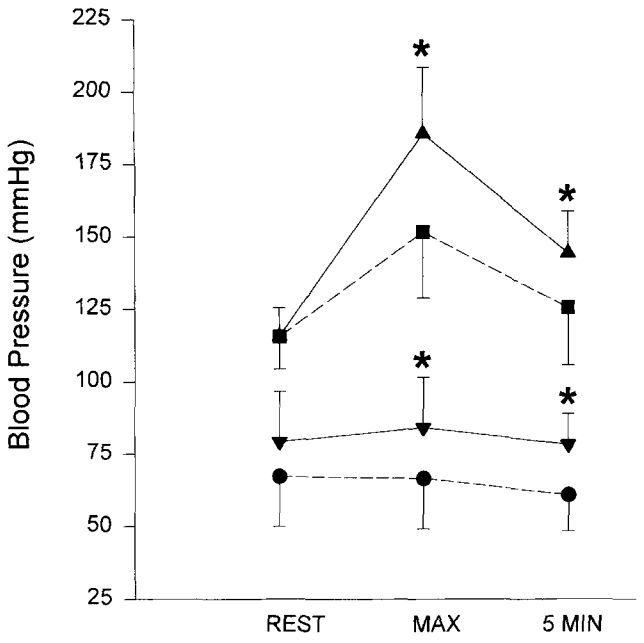

Fig. 1. Blood pressure response to exercise. Sickle-cell-thalassemia systolic (▲) and diastolic (▼), and sickle-cell systolic (■) and diastolic (●) blood pressures are plotted at rest, maximum exercise, and 5 min into recovery. Ranges indicate 1 SD. *Significant differences between groups.

was reduced in SS alpha thal-2 patients relative to other SS patients, and PAV was increased relative to normal values. PFV/PAV was reduced relative to SS patients and normals (Table V).

Abnormalities of early diastolic filling have been observed in several disease states, including adults with left ventricular hypertrophy associated with hypertension [30–31] or pressure overload lesions such as aortic stenosis [32], and in both adults and children with hypertrophic cardiomyopathy [33–35]. However, significant differences in peak or early diastolic filling seen in the SS alpha thal-2 subjects in this study persisted after differences in left ventricular wall thickness were accounted for statistically. This is similar to the findings of Snider et al. [30] in children with systemic hypertension in which diastolic abnormalities occurred in the absence of systolic abnormalities or left ventricular hypertrophy.

Evidence of altered left ventricular diastolic filling has been observed utilizing Doppler echocardiography, both in patients with acute myocardial ischemia as well as in patients with chronic coronary artery disease [36–38]. Fujii et al. [36] demonstrated the reversal of the typical early-to-late diastolic filling patterns in acute myocardial infarction. These abnormalities, as well as chest pain and S-T segment deviation on ECG [36–38], often precede Doppler evidence of systolic dysfunction.

Decreased early diastolic velocities and increased late diastolic velocities and areas in patients with coronary artery disease (CAD) suggest impaired early diastolic filling.

By decreasing preload with nitroglycerin, Choong et al. [39] demonstrated that PFV could be decreased. Therefore, the lesser left ventricular diastolic dimension in the SS alpha thal-2 group could possibly explain the diminished PFV, if not for the fact that PFV/PAV was also lower than published normals [28]. The diminished PFV/PAV in this group, therefore, is similar to that found in patients with CAD, strongly suggesting impaired coronary artery flow, possibly secondary to abnormal red-cell deformability and increased viscosity in the coronary microvasculature.

Several hemodynamic differences between the two groups were observed during exercise. SS alpha thal-2 patients had higher heart rates and arterial pressures during exercise and recovery, and a greater rate-pressure product at maximal exercise, than patients with SS disease alone (Table VI). The lack of improvement of duration of exercise in the less anemic SS alpha thal-2 patients

suggests that hypertrophy and increased systemic vascular resistance resulted in increased myocardial oxygen consumption at the same level of exercise. The less-than-anticipated exercise capacity could be due to abnormal diastolic filling or reduced blood flow to the myocardium, secondary to hypertrophy or microvascular effects of sickling.

REFERENCES

1. Alpert BS, Gilman PA, Strong WB, Ellison MF, Miller MD, McFarlane J, Hayashidera T: Hemodynamic and ECG responses to exercise in children with sickle cell anmeia. Am J Dis Child 135:362–366, 1981.
2. Covitz W, Eubig C, Balfour IC, Jerath R, Alpert BS, Strong WB, Durant RH: Exercise-induced cardiac dysfunction in sickle cell anemia: A radionuclide study. Am J Cardiol 51:570–575, 1983.
3. Balfour IC, Covitz W, Arensman FW, Eubig C, Garrido M, Jones C: Left ventricular filling in sickle cell anemia. Am J Cardiol 61:395–399, 1988.
4. Brown J, Geer M, Covitz W, Hellenbrand W, Leff S, Talner N, Robinson L: Electrocardiographic responses to exercise in sickle cell anemia. In Doyle EG, Engle MA, Gersony WM, Rashkind WJ, Talnea NS (eds.): "Pediatric Cardiology." New York: Springer-Verlag, 1986, pp 1111–1116.
5. Rodman T, Close HP, Cathcart R, Purcell MK: The oxyhemoglobin dissociation curve in the common hemoglobinopathies. Am J Med 24:558–566, 1959.
6. Varat MA, Adolph RJ, Fowler NO: Cardiovascular effects of anemia. Am Heart J 83:415–426, 1972.
7. Charache S, Bleecker ER, Bross DS: Effects of blood transfusion on exercise capacity in patients with sickle-cell anemia. Am J Med 74:757–764, 1983.
8. Miller DM, Winslow RM, Klein HG, Wilson KC, Brown FL, Statham NJ: Improved exercise performance after exchange transfusion in subjects with sickle cell anemia. Blood 56:1127–1131, 1980.
9. Gerry JL, Baird MG, Fortuin NJ: Evaluation of left ventricular function in patients with sickle cell anemia. Am J Med 60:968–972, 1976.
10. Rees AH, Stefadouras MA, Strong WB, Miller MD, Gilman P, Rigby JA, McFarlane J: Left ventricular performance in children with homozygous sickle cell anemia. Br Heart J 40:690–696, 1978.
11. Val-Mejias J, Lee WK, Weisse AB, Regan TJ: Left ventricular performance during and after sickle cell crisis. Am Heart J 97:585–591, 1979.
12. Denenberg BS, Criner G, Jones R, Spann J Jr: Cardiac function in sickle cell anemia. Am J Cardiol 51:1674–1678, 1983.
13. Balfour IC, Covitz W, Davis H, Rao PS, Strong WB, Alpert BS: Cardiac size and function in children with sickle cell anemia. Am Heart J 108:345–350, 1984.
14. Manno BV, Barka ER, Hakki AH, Manno CS, Iskandrian AS: Biventricular function in sickle cell anemia: Radionuclide angiographic and thallium-201 scintigraphic evaluation. Am J Cardiol 52:584–587, 1983.
15. Willens HJ, Lawrence C, Frishman WH, Strom JA: A non-invasive comparison of left ventricular performance in sickle cell anemia and chronic aortic regurgitation. Clin Cardiol 6:542–548, 1983.
16. Covitz W, Balfour IC, Alpert BS, Arensman FW: The heart as a target organ in sickle cell anemia. J Cardiovasc Ultrasonogr 5:177–181, 1986.
17. Arensman FW, Covitz W, Dicks G, Reyes L, Strong WB: Digitized echocardiographic assessment of left ventricular wall motion in children and adolescents with sickle cell anemia. J Cardiovasc Ultrasonogr 5:223–228, 1986.
18. Simmons BE, Santhanam V, Castaner A, Rao KRP, Sachdev N, Cooper R: Sickle cell heart disease: Two dimensional echo and doppler ultrasonic findings in the hearts of adult patients with sickle cell anemia. Arch Intern Med 148:1526–1528, 1988.
19. Embury SH, Dozy AM, Miller J, Davis JR, Kleman KM, Presiler H, Vichinsky E, Lande WN, Lubin BH, Kan YW, Mentzer WC: Concurrent sickle cell anemia and alpha-thalassemia: Effect on severity of anemia. N Engl J Med 306:270–274, 1982.
20. Higgs DR, Aldridge BE, Lamb J, Clegg JB, Weatherall DJ, Hayes RJ, Grandison Y, Lownie Y, Mason KP, Serjeant BE, Serjeant GR: The intervention of alpha-thalassemia and homozygous sickle cell disease. N Engl J Med 306:1441–1446, 1982.
21. Steinberg MH: The interactions of alpha-thalassemia with hemoglobinopathies. Hematol Oncol Clin North Am 5:453–473, 1991.
22. Southern EM: Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 98:503–511, 1975.
23. Orkin SH: The duplicated human alpha globin genes lie close together in cellular DNA. Proc Natl Acad Sci USA 75:5950–5954, 1978.
24. Sahn DJ, DeMaria A, Kisslo J, Weyman A: Recommendations regarding quantitation in M-mode echocardiography: Results of a survey of echocardiographic measurements. Circulation 58:1072–1083, 1978.
25. James FW, Kaplan S, Glueck GJ, Tsou J, Knight MJS, Sarwar CJ: Responses of normal children and young adults to controlled bicycle exercise. Circulation 61:902–912, 1980.
26. Feigenbaum H: Appendix: Echocardiographic measurements and normal values. In Feigenbaum H (ed): "Echocardiography," Ed 5. Philadelphia: Lea and Febiger, 1994, pp 658–683.
27. Gardin JM, Barn DS, Childs WJ, Henry WL: Evaluation of blood flow velocity in the ascending aorta and main pulmonary artery of normal subjects by Doppler echocardiography. Am Heart J 107:310–319, 1984.
28. Benjamin EJ, Levy D, Anderson KM, Wolf PA, Plehn JT, Evans JC, Comai K, Tuller DL, St. John Sutton M: Determinants of Doppler indexes of left ventricular diastolic function in normal subjects (The Framingham Heart Study). Am J Cardiol 70:508–515, 1992.
29. Milner PF, Barbutt GJ, Nolan-Davis LV, Jonah F, Wilson LP, Wilson JT: The effect of HbF and alpha-thalassemia on the red cell indices in sickle cell anemia. Am J Hematol 21:383–395, 1986.
30. Snider AR, Gidding SS, Rochini AP, Rosenthal A, Dick M III, Crowley DC, Peters J: Doppler evaluation of left ventricular diastolic filling in children with systemic hypertension. Am J Cardiol 56:921–926, 1985.
31. Phillips RA, Coplan NL, Krakoff LR, Yeager K, Ross RS, Gorlin R, Goldman ME: Doppler echocardiographic analysis of left ventricular filling in treated hypertensive patients. J Am Coll Cardiol 9:317–322, 1987.
32. Hanrath P, Matthey DG, Siegert R, Bleifeld W: Left ventricular relaxation and filling pattern in different forms of left ventricular hypertrophy: An echocardiographic study. Am J Cardiol 45:15–23, 1980.
33. Gidding SS, Snider AR, Rocchini AP, Peters J, Farnsworth R: Left ventricular diastolic filling in children with hypertrophic cardiomyopathy: Assessment with pulsed Doppler echocardiography. J Am Coll Cardiol 8:310–316, 1986.
34. Takenaka K, Dubestoni A, Gardin JM, Russell D, Clark S, Allfie A, Henry WL: Left ventricular filling in hypertrophic cardiomyopathy: A pulsed Doppler echocardiographic study. J Am Coll Cardiol 7:1263–1271, 1986.
35. Maron BJ, Spirito P, Green KJ, Wesley YE, Bonow RO, Arce J: Noninvasive assessment of left ventricular diastolic function by pulsed Doppler echocardiography in patients with hypertrophic cardiomyopathy. J Am Coll Cardiol 10:733–742, 1987.
36. Fujii J, Yazaki Y, Sawada H, Aizawa T, Watanabe H, Kato K: Noninvasive assessment of left and right ventricular filling in myocardial infarction with a two-dimensional Doppler echocardiographic method. J Am Coll Cardiol 5:1153–1166, 1985.
37. Wind BE, Snider AR, Buda AJ, O'Neill WW, Topol EJ, Dilworth

LR: Pulsed Doppler assessment of left ventricular diastolic filling in coronary artery disease before and immediately after coronary angioplasty. Am J Cardiol 59:1041–1046, 1987.

38. Masuyama T, Kodama K, Nakatani S, Nanto S, Kitabatake A, Kamada T: Effects of changes in coronary stenosis on left ventricular diastolic filling assessed with pulsed Doppler echocardiography. J Am Coll Cardiol 11:744–751, 1988.
39. Choong CY, Hewmann HC, Weyman AE, Fifer MA: Preload dependence of Doppler-derived indexes of left ventricular diastolic function in humans. J Am Coll Cardiol 10:800–808, 1987.