Preclinical thalassemic cardiopathy: a study by acoustic densitometry

Roberto Di Paola, Giuseppe Di Guardo, Pietro Lo Giudice, Vincenzo Caruso*, Carmelo Magnano*, Salvatore Mangiameli

*Cardiology Unit, *Thalassemia Center, "Garibaldi" Hospital, Catania, Italy*

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Background. **The cardiac function in thalassemia major has never been studied at ultrasonic backscatter techniques. We assessed the utility of acoustic densitometry in thalassemic patients without clinical or echocardiographic signs of heart failure.**

Methods. **Three groups of subjects with comparable age, sex and body surface area were analyzed: 25 with beta-thalassemia major (group A), 14 with thalassemia intermedia (group B) and 10 healthy subjects (group C). All patients were asymptomatic and without conventional echocardiographic signs of systo-diastolic dysfunction. The left ventricular mass and volumes were echocardiographically evaluated. The ultrasonic myocardial integrated backscatter signal (IBS) was recorded and analyzed by means of acoustic densitometry in the parasternal long-axis view at the septum and posterior wall, both at the basal and intermediate levels. Both the average image intensity and the systo-diastolic variations of the IBS (cyclic variation index-CVIibs and peak-to-peak intensity-PPI), respectively related to the structure and contractility of the myocardium, were calculated. The serum ferritin and liver iron concentrations were also measured, as markers of tissue iron storage.**

Results. **The CVIibs was significantly lower in groups A and B than in group C at basal (22.7 ± 8.4 vs 22.1 ± 7.8 vs 31.8 ± 10.2%; p = 0.001) and intermediate septum (24.4 ± 7.6 vs 25.3 ± 8.1 vs 30 ± 9.8%; p = 0.03) and at basal (25.9 ± 7.6 vs 24.5 ± 6.1 vs 31.1 ± 10.6%; p = 0.02) and intermediate posterior wall (25.1 ± 5.1 vs 24.3 ± 6.2 vs 30.2 ± 6.6%; p = 0.02). The PPI was also significantly lower in groups A and B than in group C. Both CVIibs and PPI were comparable in groups A and B. The average image intensity and left ventricular mass and volumes were not significantly different in the three groups. No correlation was found between the densitometric findings and markers of tissue iron storage.**

Conclusions. **In asymptomatic patients with thalassemia major with normal conventional indexes of systo-diastolic cardiac function, acoustic densitometry may show a reduced cyclic variation of the IBS as a possible marker of initial myocardial contractile deficiency. On the contrary, neither structural alterations nor the extent of myocardial iron stores are detectable by this technique in this type of patients.**

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Address:

Dr. Roberto Di Paola

Via S. Citelli 4/B
95124 Catania
E-mail:
dipaolaroberto@tiscali.it

Introduction

The structural and functional alterations in thalassemic cardiopathy are not easily detectable in the early stages because clinical and echocardiographic signs manifest later[1,2]. It is also unclear whether, in the initial phases, cardiac involvement is dependent on hemodynamic factors (volume overload resulting from chronic anemia) or on myocardial iron storage related to blood transfusions. An earlier diagnosis and a correct pathophysiologic assessment might have positive implications on the therapeutic approach.

Acoustic densitometry (AD) is a new echocardiographic technique, based on ultrasonic backscattering, that permits myocardial tissue characterization[3,4]. In brief, the incidence of ultrasonic beam on ultrastructural myocardial constituents or scatterers (myocardial cells, myofibrils, small vessels, interstitial collagen) produces a signal (integrated backscatter signal-IBS) the characteristics of which are related to the myocardial structure and function. Namely the IBS intensity is sensitive to the myocardial histopathologic alterations[5,6] and its cyclic systo-diastolic variations are related to left ventricular contractile function[7,8].

Some authors analyzed the structural and functional manifestations in preclinical thalassemic cardiopathy[9-14], but none of them used AD.

The aim of this study was to verify whether AD can detect any structural and/or functional myocardial alterations in thalassemia major patients without clinical or conventional echocardiographic signs of cardiac dysfunction.

Methods

Three groups of subjects (age range 15-46 years) were analyzed: 25 with beta-thalassemia major (group A), 14 with thalassemia intermedia (group B) and 10 healthy subjects (group C). Basal characteristics of the patients are summarized in table I. All patients were asymptomatic and without conventional echocardiographic signs of systolic[15] or diastolic[16] dysfunction (Table II). In no case did the patients have a history of congenital cardiopathies, valve pathologies, rhythm disorders, arterial hypertension or diabetes mellitus.

Group A patients were receiving transfusion therapy, generally started within the first 2 years of life and then continued every 3 weeks to achieve a mean erythrocyte consumption equivalent to 120 ml/kg/year/patient[17]. They were also receiving chelating agents, consisting of deferoxamine subcutaneously infused by means of a microinfusor 5-6 days a week at a mean dosage of 40 mg/kg/day to maintain the serum ferritin (SF) levels < 1000 ng/ml. Group B patients only occasionally had received blood transfusions (i.e. during pregnancy). All patients were free of any medications (except deferoxamine in group A).

Table I. Basal characteristics of the three groups of patients.

	Group A	Group B	Group C
Age (years)	26.1 ± 10.2	25.3 ± 7.3	24.8 ± 6.6
Sex (M/F)	7/18	4/10	3/7
BSA (m^2)	1.47 ± 0.24	1.50 ± 0.31	1.51 ± 0.22
SH (g/dl)	9.6 ± 0.7	8.1 ± 1.1	14.4 ± 0.6*
SF (ng/ml)	1918 ± 1172	692 ± 418**	59 ± 22***
LIC (μg/g)	1432 ± 821	619 ± 389§	NE

BSA = body surface area; LIC = liver iron concentration; NE = not evaluated; SF = serum ferritin; SH = serum hemoglobin. * p = 0.001 groups A and B vs group C; ** p = 0.0009 group A vs group B; *** p = 0.0001 group A vs group C and p = 0.001 group B vs group C; § p = 0.001 group A vs group B.

Table II. Conventional echocardiographic and Doppler findings in the three groups of patients.

	Group A	Group B	Group C
EF (%)	64.2 ± 6.1	64 ± 7.4	62.8 ± 6.8
FS (%)	43.3 ± 5.8	39.6 ± 6	40.6 ± 6.4
IVRT (ms)	78.5 ± 15.4	83.7 ± 10.4	86.2 ± 14.3
E/A ratio	1.86 ± 0.5	1.56 ± 0.2	2.07 ± 0.5*
DTE (ms)	202 ± 35	193 ± 31	205 ± 44
IVRT§	2.83 ± 0.5	3.04 ± 0.4	2.91 ± 0.5
E/A ratio§	0.0671 ± 0.01	0.0569 ± 0.01	0.070 ± 0.01
DTE§	7.3 ± 1.3	7 ± 1.2	6.9 ± 1.4
LVM (g/m^2)	94 ± 22.9	90.6 ± 19.9	81.7 ± 19.5
LVEDV (ml/m^2)	58.8 ± 16.2	59.7 ± 10.7	52.1 ± 9.2
LVESV (ml/m^2)	21.9 ± 8	21.7 ± 4.8	19.3 ± 3.6

DTE = E-wave deceleration time; EF = ejection fraction; FS = fractional shortening; IVRT = isovolumic relaxation time; LVEDV = left ventricular end-diastolic volume; LVESV = left ventricular end-systolic volume; LVM = left ventricular mass index. * p = 0.006 group B vs group C; § heart rate corrected.

All subjects underwent two-dimensional color Doppler echocardiography performed using a Sonos 5500 instrument (Hewlett-Packard, Agilent Technologies, Andover, MA, USA) with a 3s transducer, during simultaneous electrocardiographic monitoring and according to the criteria and the recommendations previously described[18,19]. The ultrasonic myocardial IBS was analyzed using an AD module, according to the methods previously described[3,4]. In brief, we recorded the signal in the parasternal long-axis view with an elliptical region of interest placed in the mid myocardium at the septum and posterior wall, both at the basal and intermediate levels. Care was taken to exclude the specular echoes arising from the myocardial wall boundaries. The average intensity of the IBS (average image intensity) was measured. The systo-diastolic variations of IBS were also calculated and expressed both as the cyclic variation index (CVIibs) and as the peak-to-peak intensity (PPI). The diameters, wall thickness and percent fractional shortening of the left ventricle were measured in accordance with the recommendations of the American Society of Echocardiography[18]. Left ventricular mass was determined using Devereux equation[20]. Left ventricular end-diastolic and end-systolic volumes and ejection fraction were calculated using the Simpson's formula[21]. Left ventricular mass and volumes were calculated as the mean of five measurements taken during five consecutive cardiac cycles and normalized for the body surface area. The Doppler transmitral flow velocity profile was obtained from the apical 4-chamber view according to standard techniques[22,23] and the early peak of flow velocity (E-wave), late peak of flow velocity (A-wave), E/A ratio and E-wave deceleration time were assessed. The isovolumic relaxation time was also evaluated[24]. Each of the Doppler indexes was calculated as the mean of five measurements taken during five consecutive cardiac cycles and corrected for heart rate in accordance with Bazett's formula[25], in order to avoid the impact of heart rate on the assessment of Doppler indexes[26].

The SF and liver iron concentrations (LIC), commonly considered and used as markers of tissue iron storage[27-29], were also evaluated using the nephelometric method and magnetic biosusceptometry[27] respectively. Magnetic biosusceptometry was performed only in group A and group B patients using a biosusceptometer 5700 3-Channel (Tristan Technologies Inc., San Diego, CA, USA). SF (ng/ml) and LIC (μg/g) between 400 and 1000 and > 1000 were considered indicative of mild or significant tissue iron storage, respectively[27-29].

Statistical analysis. Data were expressed as mean ± SD. The Student's t test was used to compare the variables in the different groups; a p value of < 0.05 was

considered statistically significant. Linear regression analysis was performed to analyze the correlation between different parameters. A value of $r^2 > 0.200$ was considered statistically significant.

Results

Group A and B patients showed anomalous serum hemoglobin, SF and LIC values (Table I). The SF and LIC were significantly lower in group B than in group A, whereas the two groups of patients showed comparable values of serum hemoglobin. In healthy subjects (group C) both serum hemoglobin and ferritin were normal.

The CVIibs was significantly lower in groups A and B than in group C at basal (22.7 ± 8.4 vs 22.1 ± 7.8 vs 31.8 ± 10.2%; p = 0.001) and intermediate septum (24.4 ± 7.6 vs 25.3 ± 8.1 vs 30 ± 9.8%; p = 0.03) and at basal (25.9 ± 7.6 vs 24.5 ± 6.1 vs 31.1 ± 10.6%; p = 0.02) and intermediate posterior wall (25.1 ± 5.1 vs 24.3 ± 6.2 vs 30.2 ± 6.6%; p = 0.02). The mean value of CVIibs in the four examined myocardial segments was significantly lower in groups A and B than in group C (24.5 ± 7.1 vs 24 ± 7 vs 30.8 ± 9.3%; p = 0.02). The PPI was also significantly lower in groups A and B than in group C at basal (4.9 ± 0.34 vs 5 ± 0.39 vs 6.5 ± 0.42 dB; p = 0.01) and intermediate septum (5 ± 0.29 vs 5.1 ± 0.37 vs 6.2 ± 0.38 dB; p = 0.03) and at basal (5.1 ± 0.30 vs 4.8 ± 0.31 vs 6.4 ± 0.40 dB; p = 0.02) and intermediate posterior wall (5 ± 0.38 vs 4.9 ± 0.29 vs 6.3 ± 0.26 dB; p = 0.02). Both the CVIibs and PPI were comparable (p = NS) in groups A and B. The CVIibs and PPI showed no significant correlation with ejection fraction ($r^2 = 0.026$), left ventricular end-systolic volume ($r^2 = 0.0005$), isovolumic relaxation time ($r^2 = 0.003$) nor with E/A ratio ($r^2 = 0.022$).

The average image intensity was not significantly different in the three groups at basal (33 ± 7.6 vs 33.1 ± 5.8 vs 33.2 ± 6.8 dB; p = NS) and intermediate septum (28 ± 8.3 vs 29.8 ± 7.9 vs 28 ± 5.4 dB; p = NS) and at basal (30.7 ± 8.2 vs 30 ± 4.8 vs 28.9 ± 6.4 dB; p = NS) and intermediate posterior wall (26.8 ± 5.6 vs 28.3 ± 6.5 vs 26.2 ± 5.8 dB; p = NS).

Left ventricular mass (g/m^2) and volumes (ml/m^2), although higher in thalassemic patients than in healthy subjects, showed no significant difference between the three groups (Table II). No correlation was found between average image intensity and SF ($r^2 = 0.008$), between average image intensity and LIC ($r^2 = 0.008$), between cyclic variation indexes and SF ($r^2 = 0.016$) and between cyclic variation indexes and LIC ($r^2 = 0.032$).

With regard to group A, the average image intensity and CVIibs values showed no significant difference between patients with SF < and > 1000 ng/ml nor between those with LIC < and > 1000 μg/g (Table III).

Discussion

Cardiomyopathy is a frequent cause of morbidity and mortality in patients with beta-thalassemia major[1,2]. The cardiac involvement, easily detectable in advanced stages of the disease, is difficult to diagnose in the early phases when it is often clinically silent. In these latter stages, even the conventional echocardiographic indexes of both systolic[9-14] and diastolic[11,23] left ventricular functions are often within normal limits.

Abnormal wall kinetics and thickening[9], a reduced end-systolic pressure/end-systolic volume ratio[11] and a decrease in ejection fraction during dobutamine echocardiography[12] have been described in patients with beta-thalassemia major without heart failure and with a normal ejection fraction. In none of these experiences was the cardiac performance assessed by means of ultrasonic backscatter techniques. In only two studies[13,14] was thalassemic cardiomyopathy analyzed using ultrasonic tissue characterization methods, but in

Table III. Densitometric findings in the two different subgroups of thalassemia major patients.

	SF (ng/ml)		LIC (μg/g)	
	< 1000	> 1000	< 1000	> 1000
AII (dB)				
bIVS	33.9 ± 7.4	32.5 ± 6.5	33.2 ± 7.1	32.8 ± 6.8
mIVS	30.6 ± 6.9	27.5 ± 7.8	29.8 ± 8.5	28.2 ± 8.3
bPW	31.7 ± 7.8	29.6 ± 8.4	30.4 ± 6.9	30.8 ± 8.1
mPW	28.2 ± 6.1	26.3 ± 5.8	27.9 ± 6.2	26.8 ± 5.6
CVIibs (%)				
bIVS	21.9 ± 7.5	23.3 ± 7.8	22.8 ± 8.3	22.4 ± 8.4
mIVS	23.9 ± 7.6	25.1 ± 7.9	24.2 ± 6.8	24.8 ± 7.1
bPW	26.4 ± 7.4	25.8 ± 6.4	26.3 ± 6.9	25.9 ± 7.8
mPW	25.4 ± 5.6	24.8 ± 6.2	25.8 ± 4.9	25.2 ± 5.3

AII = average image intensity; bIVS = basal interventricular septum; bPW = basal posterior wall; CVIibs = cyclic variation index of integrated backscatter signal; dB = decibel; LIC = liver iron concentration; mIVS = intermediate interventricular septum; mPW = intermediate posterior wall; SF = serum ferritin.

none of them was the AD used or the cyclic variation of the ultrasonic backscatter signal evaluated.

Wickline et al.[7,8] documented a reverse correlation between myocardial contractility and cyclic variations of the ultrasonic backscatter signal. This correlation may be explained by the structural and functional properties of the myofibrils, the alterations of which may influence both myocardial contractility and (as scatterers) the characteristics of the ultrasonic backscatter signal[7,8]. According to these experimental observations, the AD is able to show anomalous IBS cyclic variations in many types of cardiopathy (ischemic, hypertensive, diabetic, primitive cardiomyopathies) with consequent abnormalities in the cardiac function[30-40].

In our experience the AD was used in subjects without clinical nor conventional echocardiographic signs of heart failure. In this kind of patients a correlation between IBS cyclic variations and myocardial contractility has not been clearly demonstrated. However, since cyclic variations of IBS were significantly lower in group A than in healthy subjects (group C), it is possible that in asymptomatic patients with thalassemia major an initial myocardial contractile defect is present, despite a normal systo-diastolic function. In agreement with this hypothesis, we found no correlation between cyclic variation indexes (related to myocardial contractility) and the echocardiographic parameters such as ejection fraction, left ventricular end-systolic volume, E/A ratio and isovolumic relaxation time (dependent on cardiac function).

The possible pathophysiologic mechanisms underlying the alterations in cyclic variation of IBS in our patients are the cardiac volume overload resulting from chronic anemia[41,42] and/or myocardial iron storage related to blood transfusions[1]. So we also used a control group (group B) of patients with chronic anemia (thalassemia intermedia) but without significant myocardial iron overload because only occasionally transfused. Of interest, these patients also showed significantly reduced cyclic variations of IBS. In keeping with this observation, we hypothesize that densitometric alterations, observed in our patients (groups A and B) as markers of possible early myocardial contractile dysfunction, are related to chronic anemia and to the consequent cardiac volume overload rather than to hemochromatosis. This hypothesis is also supported by the following data: 1) the lack of any correlation between cyclic variation indexes and the conventional indexes of tissue iron storage (SF and LIC); 2) no significant difference in CVIibs in group A between patients with mild (SF < 1000 ng/ml; LIC < 1000 μg/g) or moderate-severe (SF > 1000 ng/ml; LIC > 1000 μg/g) iron storage (Table III).

In this study the IBS intensity (expressed as average image intensity) in thalassemia major patients was not significantly different to that observed in healthy subjects. This parameter appeared therefore unreliable in identifying thalassemic cardiomyopathy in its preclinical stages. Left ventricular mass was also not significantly different in the three groups (Table II). These data are in contrast with those reported by Lattanzi et al.[13,14], who found an increased echoreflectivity and myocardial mass in patients with beta-thalassemia major. This discordance might be due to the different ultrasonic tissue characterization methods used by these authors. Moreover, it is possible that the patients analyzed in this study had more widespread myocardial fibrosis, which is a major determinant of the myocardial echoreflectivity[5,6] and is also one of the histopathologic abnormalities contributing to the increase in myocardial mass.

The quantitative assessment of myocardial iron stores in patients with thalassemia major is an unsolved problem, because there is no noninvasive and accurate method able to measure the iron load in the myocardium. SF and LIC are considered and used as indexes of tissue iron storage[27-29], but their linear relationship with the extent of myocardial iron deposits is inconstant. Furthermore, invasive procedures, such as endomyocardial biopsy, cannot be proposed as a routine technique for these patients who are generally asymptomatic and clinically stable. Our data do not support the hypothesis that the IBS intensity, detected at AD, is related to the extent of myocardial iron stores. This, owing to the following observations: 1) there was no significant difference in the average image intensity between patients with thalassemia major and other subjects (groups B and C) who, not being submitted to such numerous transfusions, did not present with myocardial hemochromatosis; 2) among group A patients, no significant difference in the average image intensity was observed between subjects with probably mild (SF < 1000 ng/ml; LIC < 1000 μg/g) and those with probably more severe (SF > 1000 ng/ml; LIC > 1000 μg/g) myocardial iron stores (Table III); 3) there was no correlation between the average image intensity values and the conventional indexes of tissue iron storage (SF and LIC).

A sufficient follow-up time will be necessary to assess the prognostic value of the densitometric parameters, in particular whether low IBS cyclic variations are able to identify those patients who are at a greater risk of developing a symptomatic thalassemic cardiomyopathy and related events. The identification of a CVIibs (or PPI) cut-off value, indicative of a reduced myocardial contractility and of a poor prognosis in this type of patients, should induce the clinical use of AD. In fact, this technique should allow us to identify those thalassemic patients requiring a close follow-up, an intensive transfusion and chelation therapy and an earlier use of drugs for heart failure.

In conclusion, in patients with beta-thalassemia major, even if asymptomatic and with normal conventional indexes of systo-diastolic cardiac function, AD is able to reveal reduced cyclic variations of the IBS as a possible marker of initial myocardial contractile defi-

ciency. On the contrary, in these patients AD does not seem to be useful for the detection and quantification of the myocardial iron load and related histopathologic alterations. The densitometric alterations in the early phases of thalassemic cardiomyopathy are likely to be dependent on the cardiac volume overload resulting from chronic anemia rather than from myocardial iron storage.

References

1. Engle MA. Cardiac involvement in Cooley's anemia. Ann NY Acad Sci 1969; 119: 694-702.
2. Bunn HF. Disorders of hemoglobin - Thalassemias. In: Wilson JD, Braunwald E, Isselbacher KJ, et al, eds. Harrison's Principles of Medicine. New York, NY: McGraw-Hill, 1991: 1542-52.
3. D'Sa A. White paper. Acoustic densitometry. Andover, MA: Hewlett-Packard, 1999.
4. Di Bello V, Pedrinelli R, Talini E, et al. Ultrasonic myocardial tissue characterization: a methodological review. Ital Heart J 2001; 2: 333-43.
5. O'Donnell M, Mimbs JW, Miller JG. Relationship between collagen and ultrasonic backscatter in myocardial tissue. J Acoust Soc Am 1981; 69: 580-8.
6. Maceira AM, Barba J, Varo N, Beloqui O, Diez J. Ultrasonic backscatter and serum marker of cardiac fibrosis in hypertensives. Hypertension 2002; 39: 923-8.
7. Wickline SA, Thomas LJ III, Miller JG, Sobel BE, Perez JE. A relationship between ultrasonic integrated backscatter and myocardial contractile function. J Clin Invest 1985; 76: 2151-60.
8. Wickline SA, Thomas LJ III, Miller JG, Sobel BE, Perez JE. The dependence of myocardial ultrasonic integrated backscatter on contractile performance. Circulation 1985; 72: 183-92.
9. Valdes-Cruz LM, Reinecke C, Rutkowski M, et al. Preclinical abnormal segmental cardiac manifestations of thalassemia major in children on transfusion-chelation therapy: echographic alterations of left ventricular posterior wall contraction and relaxation patterns. Am Heart J 1982; 103 (Part 1): 505-11.
10. Borow KM, Propper R, Bierman FZ, Grady S, Inati A. The left ventricular end-systolic pressure-dimension relation in patients with thalassemia major. A new noninvasive method for assessing contractile state. Circulation 1982; 66: 980-5.
11. Desideri A, Scattolin G, Gabellini A, et al. Left ventricular function in thalassemia major: protective effect of deferoxamine. Can J Cardiol 1994; 10: 93-6.
12. Mariotti E, Agostini A, Angelucci E, Lucarelli G, Sgarbi E, Picano E. Reduced left ventricular contractile reserve identified by low-dose dobutamine echocardiography as an early marker of cardiac involvement in asymptomatic patients with thalassemia major. Echocardiography 1996; 13: 463-72.
13. Lattanzi F, Bellotti P, Picano E, et al. Quantitative ultrasonic analysis of myocardium in patients with thalassemia major and iron overload. Circulation 1993; 87: 748-54.
14. Lattanzi F, Bellotti P, Picano E, et al. Quantitative texture analysis in two-dimensional echocardiography: application to the diagnosis of myocardial hemochromatosis. Echocardiography 1996; 13: 9-20.
15. Antonini-Canterin F, Pavan D, Nicolosi GL. Valutazione ecocardiografica dei volumi e della funzione sistolica globale del ventricolo sinistro. Ital Heart J Suppl 2000; 1: 1261-72.
16. European Study Group on Diastolic Heart Failure. How to diagnose diastolic heart failure. Eur Heart J 1998; 19: 990-1003.
17. Fosburg MT, Nathan DG. Treatment of Cooley's anemia. Blood 1990; 76: 435-44.
18. Sahn DJ, DeMaria A, Kisslo J, Weyman A. Recommendations regarding quantitation in M-mode echocardiography: results of a survey of echocardiographic measurements. Circulation 1978; 58: 1072-83.
19. Schiller NB, Shah PM, Crawford M, et al. Recommendations for quantitation of the left ventricle by two-dimensional echocardiography. American Society of Echocardiography Committee on Standards, Subcommittee on Quantitation of Two-Dimensional Echocardiograms. J Am Soc Echocardiogr 1989; 2: 358-67.
20. Devereux RB, Reichek N. Echocardiographic determination of left ventricular mass in man. Anatomic validation of the method. Circulation 1977; 55: 613-8.
21. Schiller NB, Acquatella H, Ports TA, et al. Left ventricular volume from paired biplane two-dimensional echocardiography. Circulation 1979; 60: 547-55.
22. Rokey R, Kuo LC, Zoghbi WA, Limacher MC, Quinones MA. Determination of parameters of left ventricular diastolic filling with pulsed Doppler echocardiography: comparison with cineangiography. Circulation 1985; 71: 543-50.
23. Kremastinos DT, Tsiapras DP, Tsetsos GA, Rentoukas EI, Vretou HP, Toutouzas PK. Left ventricular diastolic Doppler characteristics in beta-thalassemia major. Circulation 1993; 88: 1127-35.
24. Tseng KH, Walfish PG, Persaud JA, Gilbert BW. Concurrent aortic and mitral valve echocardiography permits measurement of systolic time intervals as an index of peripheral tissue thyroid functional status. J Clin Endocrinol Metab 1989; 69: 633-8.
25. Bazett HC. An analysis of the time relations of electrocardiograms. Heart 1918; 7: 353-70.
26. Galderisi M, Benjamin EJ, Evans JC, et al. Impact of heart rate and PR interval on Doppler indexes of left ventricular diastolic filling in an elderly cohort (the Framingham Heart Study). Am J Cardiol 1993; 72: 1183-7.
27. Brittenham GM, Farrell DE, Harris JW, et al. Magnetic-susceptibility measurement of human iron stores. N Engl J Med 1982; 307: 1671-5.
28. Angelucci E, Brittenham GM, McLaren CE, et al. Hepatic iron concentration and total body iron stores in thalassemia major. N Engl J Med 2000; 343: 327-31.
29. Telfer PT, Prestcott E, Holden S, Walker M, Hoffbrand AV, Wonke B. Hepatic iron concentration combined with long-term monitoring of serum ferritin to predict complications of iron overload in thalassemia major. Br J Haematol 2000; 110: 971-7.
30. Milunski MR, Mohr GA, Perez JE, et al. Ultrasonic tissue characterization with integrated backscatter. Acute myocardial ischemia, reperfusion, and stunned myocardium in patients. Circulation 1989; 80: 491-503.
31. Wickline SA, Verdonk ED, Wong AK, Shepard RK, Miller JG. Structural remodeling of human myocardial tissue after infarction. Quantification with ultrasonic backscatter. Circulation 1992; 85: 259-68.
32. Picano E, Faletra F, Marini C, et al. Increased echodensity of transiently asynergic myocardium in humans: a novel echocardiographic sign of myocardial ischemia. J Am Coll Cardiol 1993; 21: 199-207.
33. Bertini A, Di Bello V, Pedrinelli R, et al. Cyclic variation of the myocardial integrated backscatter signal and geometric remodeling in essential arterial hypertension. Am J Hypertens 2001; 14 (Suppl 1): A207-A208.

34. Di Bello V, Pedrinelli R, Bertini A, et al. Cyclic variation of the myocardial integrated backscatter signal in hypertensive cardiopathy: a preliminary study. Coron Artery Dis 2001; 12: 267-75.
35. Maceira AM, Barba J, Beloqui O, Diez J. Ultrasonic backscatter and diastolic function in hypertensive patients. Hypertension 2002; 40: 239-43.
36. Perez JE, McGill JB, Santiago JV, et al. Abnormal myocardial acoustic properties in diabetic patients and their correlation with the severity of disease. J Am Coll Cardiol 1992; 19: 1154-62.
37. Masuyama T, St Goar FG, Tye T, Oppenheim G, Schnittger I, Popp RL. Ultrasonic tissue characterization of human hypertrophied hearts in vivo with cardiac cycle-dependent variation in integrated backscatter. Circulation 1989; 80: 925-34.
38. Naito J, Masuyama T, Mano T, et al. Ultrasonic myocardial tissue characterization in patients with dilated cardiomyopathy: value in non-invasive assessment of myocardial fibrosis. Am Heart J 1996; 131: 115-21.
39. Pinamonti B, Picano E, Ferdeghini EM, et al. Quantitative texture analysis in two-dimensional echocardiography: application to the diagnosis of myocardial amyloidosis. J Am Coll Cardiol 1989; 14: 666-71.
40. Goens MB, Karr SS, Seibel N, Martin GR. Ultrasound myocardial tissue characterization by integrated backscatter in children treated with anthracyclines. Pediatr Cardiol 1999; 20: 264-70.
41. Duke M, Abelmann WH. The hemodynamic response to chronic anemia. Circulation 1969; 39: 503-15.
42. Varat MA, Adolph RJ, Fowler NO. Cardiovascular effects of anemia. Am Heart J 1972; 83: 415-26.