Serial Measurement of Integrated Ultrasonic Backscatter in Human Cardiac Allografts for the Recognition of Acute Rejection

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Cyclic variation of integrated ultrasonic backscatter (IB) was noninvasively measured in the septum and left ventricular posterior wall using a quantitative IB imaging system to assess the alterations in the acoustic properties of myocardium associated with acute cardiac allograft rejection. The study population consisted of 23 cardiac allograft recipients and 18 normal subjects. In each cardiac allograft recipient, one to eight (mean, four) IB studies were performed, each within 24 hours of right ventricular endomyocardial biopsy performed for rejection surveillance. The magnitude of the cyclic variation of IB in the posterior wall was 5.9±0.9 dB in normal subjects and 6.2±1.3 dB in the cardiac allograft recipients without previous or current histological evidence of acute rejection ($n=17$, $p=$NS vs. normal subjects). The magnitude of cyclic variation of IB in the septum was 4.8±1.1 dB in normal subjects and 3.8±2.0 dB in the cardiac allograft recipients ($n=15$, $p=$NS vs. normal subjects). A significant decrease in the septal IB measure was observed in cardiac allograft recipients with left ventricular hypertrophy (wall thickness of at least 13 mm) (2.6±1.7 dB, $n=8$, $p<0.05$ vs. normal subjects). IB studies were done before and during moderate acute rejection in 11 recipients (14 episodes). During moderate acute cardiac rejection, the magnitude of the cyclic variation in IB decreased from 6.7±1.3 to 5.1±1.4 dB in the posterior wall ($n=14$, $p<0.05$) and from 4.2±2.1 dB to 2.9±1.8 dB in the septum ($n=12$, $p<0.05$). These data suggest 1) the magnitude of the cyclic variation in IB of the septum is different in cardiac allografts with cardiac hypertrophy and normal subjects, possibly reflecting regionally depressed myocardial contractile performance and 2) acute cardiac rejection in humans is accompanied by an alteration in the acoustic properties of the myocardium. This change is detectable by serial measurement of the magnitude of the cyclic variation in IB, both in the septum and in the posterior wall. (*Circulation* 1990;81:829–839)

Improved immunosuppressive agents and endomyocardial biopsy surveillance for acute cardiac rejection have increased the 1-year and 5-year survival of cardiac allograft recipients to more than 80% and 60%, respectively.[1,2] Early detection of cardiac allograft rejection remains a challenge in patients treated with cyclosporine. Several noninvasive methods have been proposed for diagnosis of rejection and monitoring the efficacy of its treatment. They have included electrocardiography,[3,4] M-mode,[5–7] two-dimensional[8] and Doppler echocardiography,[9,10] magnetic resonance imaging,[11] or radioisotope-labeled lymphocytes.[12] Some of them are inadequate for the detection of acute rejection in individual patients, and others are still being evaluated. The endomyocardial biopsy has remained the primary means of rejection surveillance despite the recognized minor risk of complications, the major inconvenience for patients, and the small sampling error.

Integrated ultrasonic backscatter (IB) is a relatively new noninvasive measure of the acoustic properties of the myocardium.[13] Recent animal studies[14–18] have shown alteration in acoustic properties of the myocardium detected by the analysis of the cyclic variation of IB. The cyclic variation of IB can be noninvasively measured in humans, with alterations detected in myocardial ischemia, infarction, cardiomyopathy, and hypertrophy.[19–22] In this study, the magnitude of the cyclic variation of IB

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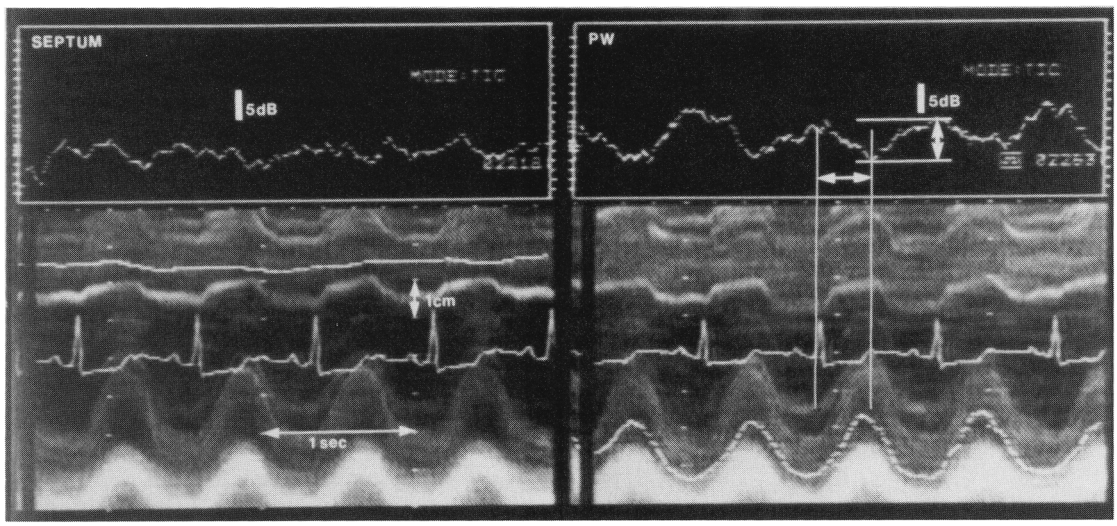

FIGURE 1. *Representative M-mode integrated backscatter images of a cardiac allograft without rejection (patient 6 in Table 3) with tracing of area-of-interest in septum* (left panel) *and in posterior wall* (right panel). *Area-of-interest was kept midway between endocardial and epicardial borders throughout cardiac cycle. Box above each image plots IB in decibels* (y *axis) versus time in area of myocardium designated by bright line overlaid on M-mode image. Measurements of magnitude of cyclic variation of IB and interval from onset of QRS wave of electrocardiogram to minimum IB value are shown in right panel. dB, decibels; IB, integrated backscatter; PW, posterior wall; sec, second.*

was measured in human cardiac allografts. The objectives of this study were 1) to compare the acoustic properties of the myocardium assessed with the IB measurement in normal subjects and cardiac allograft recipients without rejection, 2) to examine whether the IB measurement is affected by acute cardiac allograft rejection, and if so, 3) whether the IB measurement is useful for the detection of acute cardiac allograft rejection occurring with the initial posttransplantation period.

Methods

Ultrasonic Backscatter Instrumentation

IB was measured with a modified commercially available real-time two-dimensional imaging system (Hewlett-Packard 77020AC, Hewlett-Packard Co., Andover, Massachusetts) equipped with a 64-channel phased-array transducer of 3.5 MHz. In this system, either conventional two-dimensional and M-mode echo amplitude or IB images are obtained. In the IB imaging mode, the received ultrasound signal is amplified, mixed to an appropriate intermediate frequency, phased, and delayed. Different from a conventional imaging system, the signal is accessed with the IB processor to produce a continuous signal that is proportional to the logarithm of IB. In the calculation of the logarithm of IB, the integration time is 3.2 μsec, and the dynamic range of the IB processor is greater than 40 dB. Measurements of IB in absolute terms cannot be obtained with this system because calibration of the absolute time-averaged IB level is not available now. If IB is measured at two different instants (e.g., at diastole and systole) in the same area, however, the logarithm of the ratio of backscattered energies at these two instants is available quantitatively (in dB) by calculating the difference between the values of IB. Although the instantaneous value of IB is related to gain settings, the magnitude of cyclic *variation* of IB (in dB) is essentially independent of transmit power over the range of transmit power used in this study. The transmit power and time-gain compensation settings were adjusted so the myocardium was filled in with medium-to-low–level signal that showed the largest cyclic variation in the brightness visually, that is, maximum brightness in the late diastole and minimum brightness in the late systole. The gain controls were not adjusted equally throughout the sector field, and therefore, each depth had different gain emphasis. They were adjusted initially for each patient and kept constant, thereafter, throughout each study. Therefore, no attempt was made in this study to standardize instrument adjustment among the patients or subjects.

In our system, the value of IB (in dB) in an area-of-interest is obtained by placing a square-shaped area-of-interest of variable size anywhere on the frozen two-dimensional or M-mode IB images. Furthermore, in the frozen M-mode IB image, the serial time varying changes in the amplitude of IB on a relative scale are obtained by tracing the area-of-interest from left to right. This is displayed as a curve of IB versus time (Figure 1).

Patients

Twenty-eight consecutive adult patients with cardiac allografts were considered for this study. Five patients were excluded because of inadequate quality IB images, and therefore, the study population consisted of 23 patients with cardiac allografts. There

TABLE 1. Integrated Ultrasonic Backscatter Measures and Echocardiographic Parameters of Left Ventricle in Normal Subjects and in Cardiac Allograft Recipients Without Rejection

	Normal subjects	Recipients
n	18	17
Age (yr)	50±10	51±10
Gender	7F, 11M	5F, 12M
Magnitude (septum) (dB)	4.8±1.1	3.8±2.0 (*n*=15)
Magnitude (PW) (dB)	5.9±0.9	6.2±1.3
Time interval (septum) (msec)	329±56	344±83 (*n*=13)
Time interval (PW) (msec)	343±32	345±65
LVD-d (mm)	49±3	47±6
LVD-s (mm)	30±4	31±6
FS (%)	39±5	35±6
Th-d (septum) (mm)	9±1	12±2*
Th-s (septum) (mm)	13±1	16±2*
Th-d (PW) (mm)	9±1	11±2*
Th-s (PW) (mm)	16±2	19±3*
Th-index (septum)	0.18±0.03	0.26±0.06*
Th-index (PW)	0.19±0.03	0.24±0.05*
%Thickening (septum)	52±19	32±14*
%Thickening (PW)	69±15	67±16
HR (beats/min)	70±8	80±10*

Values are mean±SD. F, female; M, male; Magnitude, magnitude of cyclic variation of integrated ultrasonic backscatter; PW, posterior wall; Time interval, time interval from onset of QRS wave of electrocardiogram to minimum integrated ultrasonic backscatter value; LVD-d, left ventricular dimension at end diastole; LVD-s, left ventricular dimension at end systole; FS, fractional shortening; Th-d, wall thickness at end diastole; Th-s, wall thickness at end systole; Th-index, wall thickness index measured as wall thickness divided by left ventricular dimension at end diastole; %Thickening, percentage of systolic thickening; HR, heart rate.
*$p<0.05$ vs. normals.

were 17 men and six women, 19–58 years old (mean, 45 years). The patients received standard immunosuppressive therapy, which included prophylactic OKT3 for the initial two postoperation weeks, cyclosporine (3.5–11 mg/kg/day), azathioprine (1–2.5 mg/kg/day), and prednisone (0.2–0.8 mg/kg/day). All patients required maintenance antihypertensive therapy, that is, either prazosin (2–5 mg t.i.d.) or hydralazine (25–100 mg t.i.d.). Right ventricular endomyocardial biopsies were performed routinely according to our current protocol for rejection surveillance. Biopsies were performed on a weekly basis for the first month, starting on the sixth or seventh postoperative day. Thereafter, biopsies were performed during alternate weeks for the next 4–6 weeks, followed by monthly biopsies for the next 2–3 months. A minimum of four tissue samples were obtained to provide histological examination for assessment of cardiac rejection. The biopsies were graded according to previously published criteria[23] as follows: normal, absence of cellular infiltration or myocyte necrosis; mild, cellular infiltrate without myocyte necrosis; moderate, cellular infiltrate with myocyte necrosis; or severe, myocyte necrosis with hemorrhage. All biopsies were interpreted by an experienced senior pathologist. Episodes of moderate rejection were treated with either parenteral methylprednisolone (1,000 mg for 3 days) or with oral high-dose prednisone (100 mg daily for 2 days) and, then, rapidly tapering of the dose over 2 weeks.

The interval between IB measurements and biopsies was within 24 hours in all cases. The IB measurements were made without knowledge of patient status, endomyocardial biopsy findings, or Doppler echocardiographic results. In 17 of 23 patients, the first ultrasound study was performed before the first rejection episode, and in the other six patients, the first ultrasound study was performed after complete resolution of the first rejection episode (all of mild or moderate degree). In 19 of the 23 patients, the ultrasound study was repeated two to eight times within 4 months, and in 11 of the patients, the ultrasound study was performed before an acute rejection episode with myocyte necrosis (prerejection), during the acute rejection episode (rejection), and after complete resolution of the rejection episode (postrejection). In three patients, ultrasound studies were available before, during, and after a second episode of rejection. Eighteen normal subjects (11 men and seven women), in the age range of 35–65 years (mean, 50 years), served as controls. Before IB studies, all subjects were examined using a conventional two-dimensional and M-mode echocardiographic imaging system. The echocardiographic parameters of chamber size and wall thickness were measured in a standard manner from parasternal views[24] (Tables 1 and 2). Wall thickness index was

TABLE 2. Integrated Ultrasonic Backscatter Measures and Echocardiographic Parameters of Left Ventricle in Cardiac Allograft Recipients in Whom They Were Obtained in Rejection Period

	Prerejection	Rejection	Postrejection
n	14	14	13
Magnitude (septum) (dB)	4.2±2.1	2.9±1.8*	3.3±2.7
Magnitude (PW) (dB)	6.7±1.3	5.1±1.4*	5.8±1.9
Time interval (septum) (msec)	354±72	370±59	400±57
Time interval (PW) (msec)	331±58	352±55	353±49
LVD-d (mm)	45±7	44±6	43±7
LVD-s (mm)	29±6	30±6	30±6
FS (%)	35±6	32±6	34±8
Th-d (septum) (mm)	13±2	13±2	12±2
Th-s (septum) (mm)	16±2	16±3	16±3
Th-d (PW) (mm)	12±2	12±2	12±2
Th-s (PW) (mm)	19±2	19±3	19±2
Th-index (septum)	0.29±0.06	0.31±0.07	0.30±0.08
Th-index (PW)	0.27±0.06	0.29±0.08	0.29±0.07
%Thickening (septum)	30±14	24±14	30±14
%Thickening (PW)	60±17	57±21	55±21
HR (beats/min)	82±9	82±12	86±13

Values are mean±SD. Magnitude, magnitude of cyclic variation of integrated ultrasonic backscatter; PW, posterior wall; Time interval, time interval from onset of QRS wave of electrocardiogram to minimum integrated ultrasonic backscatter value; LVD-d, left ventricular dimension at end diastole; LVD-s, left ventricular dimension at end systole; FS, fractional shortening; Th-d, wall thickness at end diastole; Th-s, wall thickness at end systole; Th-index, wall thickness index measured as wall thickness divided by left ventricular dimension at end diastole; %Thickening, percentage of systolic thickening; HR, heart rate.

*$p<0.05$ vs. prerejection.

determined as the ratio of wall thickness at end diastole divided by left ventricular end-diastolic dimension.

All examinations were performed with the patient in a left lateral decubitus position. The transducer was placed at the parasternal border to image the long- or short-axis view of the left ventricle. The switch to provide the IB image was then toggled. The M-mode IB image was obtained by directing the selected ultrasound beam across the interventricular septum and left ventricular posterior wall, and M-mode IB images were frozen for the system's microprocessor analysis on screen.

Data Analysis

Temporal changes in the IB were measured by placing the area-of-interest between endocardial and epicardial borders and tracing midway between these borders along the M-mode frozen images. The size of the area-of-interest selected in this study was approximately 2.8 mm×80 msec. From the curve of time versus amplitude of IB, the magnitude of cyclic variation of IB was determined as a difference between minimum and maximum peaks. This IB measurement was obtained on screen using the software resident in the ultrasonic scanner. The time interval from the onset of the QRS wave of the electrocardiogram to the minimum IB value was also measured by hand on the hard copy of the line graph of time versus amplitude of IB. Averaged values over at least three beats were provided for quantitative analysis.

Statistical Analysis

Data are presented as mean±SD. Echocardiographic and IB parameters were compared between the groups and among the measurements using analysis of variance and Scheffe's *F* test for statistical significance. Relations between two variables were studied using a simple linear regression analysis. Sensitivity, specificity, and diagnostic accuracy were calculated in a standard manner.

Results

Comparison of Cyclic Variation of Integrated Ultrasonic Backscatter Between Normal Subjects and Cardiac Allograft Recipients Who Were Studied Before the First Rejection Episode

Cyclic variation of IB of more than 3 dB in magnitude was observed in the posterior wall of all normal subjects and in the septum in 13 of 16 normal subjects. In contrast, variation of IB of more than 3 dB was observed in the posterior wall in 16 of 17, and in the septum in eight of 17, cardiac allograft recipients. In two patients, surgically implanted intramyocardial markers were unavoidable in the ultrasonic image of the septum and were considered the reason for the absence of measurable cyclic variation of IB in the septum. Because these markers prevented technically good IB images of the septum throughout the cardiac cycle, data of the septum in these two patients were excluded from the following quantitative analyses. There was no difference in the variation

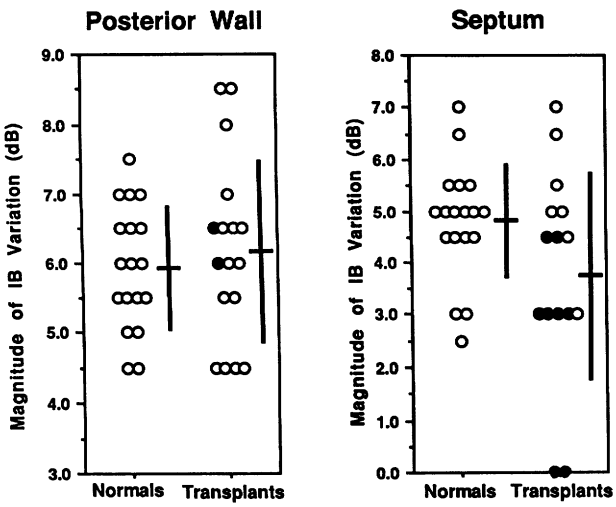

FIGURE 2. *Scatterplots showing comparison of magnitude of cyclic variation of IB for posterior wall* (left panel) *and septum* (right panel) *in normal subjects and cardiac allograft recipient without rejection. There was no significant difference in magnitude of cyclic variation of IB either for septum or for posterior wall between these two groups. If patients with wall thickness of at least 13 mm* (●) *were compared with normals, however, magnitude of cyclic variation of IB in septum was significantly smaller in these patients than in normal subjects (see text). Mean (horizontal bar) and standard deviation (vertical bar) are indicated. dB, decibels; IB, integrated backscatter.*

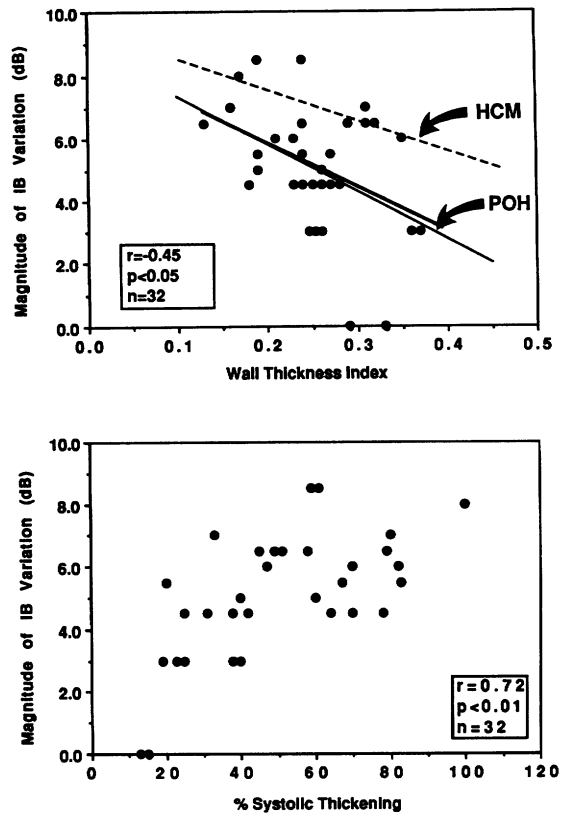

FIGURE 3. *Scatterplots showing magnitude of cyclic variation of integrated backscatter (IB) as plotted against wall thickness index* (top panel) *and percentage of systolic thickening of myocardium* (bottom panel) *in cardiac allografts without rejection. As wall thickness index increases, magnitude of variation of IB tended to decrease, and there was weak but statistically significant correlation between these two parameters* (r=*−0.45,* p<*0.05,* n=*32 for all data). Thick line indicates regression line of relation between these parameters in cardiac allograft recipients without rejection* (y=*−14.5*x+*8.6,* n=*32). Thin and broken lines indicate regression lines of relation between these parameters determined in patients with pressure-overload hypertrophy and patients with hypertrophic cardiomyopathy, respectively, in previous study.*[22] *It is noted that regression line obtained in cardiac allograft recipients without rejection is closer to that obtained in patients with pressure-overload hypertrophy than to that in patients with hypertrophic cardiomyopathy. Magnitude of variation of IB increases with percentage of systolic thickening of myocardium, and there was weak but significant correlation between these two parameters* (r=*0.55,* p<*0.01,* n=*32 for all data) using linear regression analysis. Polynominal regression analysis showed curvilinear relation of these data* (n=*0.72,* p<*0.01). dB, decibels; HCM, hypertrophic cardiomyopathy; POH, pressure-overload hypertrophy.*

of IB in the posterior wall between normal subjects and cardiac allograft recipients (5.9±0.9 vs. 6.2±1.3 dB, p=NS). The magnitude of the cyclic variation of IB in the septum was slightly but not significantly less in the cardiac allograft recipients than in the normal subjects (3.8±2.0 vs. 4.8±1.1 dB, p=NS) (Table 1 and Figure 2). In eight recipients with the septal wall thickness of at least 13 mm, the magnitude of the cyclic variation of IB in the septum was less as compared with normal subjects and the other seven recipients without septal hypertrophy (2.6±1.7 vs. 4.8±1.1 dB, $p<0.05$, and vs. 5.2±1.3 dB, $p<0.05$, respectively). These recipients with septal hypertrophy had normal left ventricular fractional shortening (35±6%) but the percentage of systolic thickening of the septal wall was significantly lower in the recipients with septal hypertrophy than in normal subjects (28±12% vs. 51±17%, $p<0.05$). Nine recipients showed paradoxical-to-flat septal motion, and the magnitude of the cyclic variation of IB in the septum was slightly but not significantly less in the nine recipients with paradoxical-to-flat septal motion as compared with normals and the other six recipients with normal septal motion (3.3±2.3 vs. 4.8±1.1 dB, p=NS, and vs. 4.6±1.4 dB, p=NS, respectively).

Relation Between the Magnitude of Cyclic Variation of Integrated Ultrasonic Backscatter and M-Mode Echocardiographic Parameters of the Left Ventricle

The magnitude of the cyclic variation of IB was compared with wall thickness index among the 17 patients (Figure 3). As the wall thickness increased, the magnitude of the variation of IB tended to decrease, and there was a weak but significant inverse correlation between absolute wall thickness and the magnitude of IB variation (r=0.52, $p<0.05$, n=32) for the septum and posterior wall and between wall thickness index and the magnitude of IB varia-

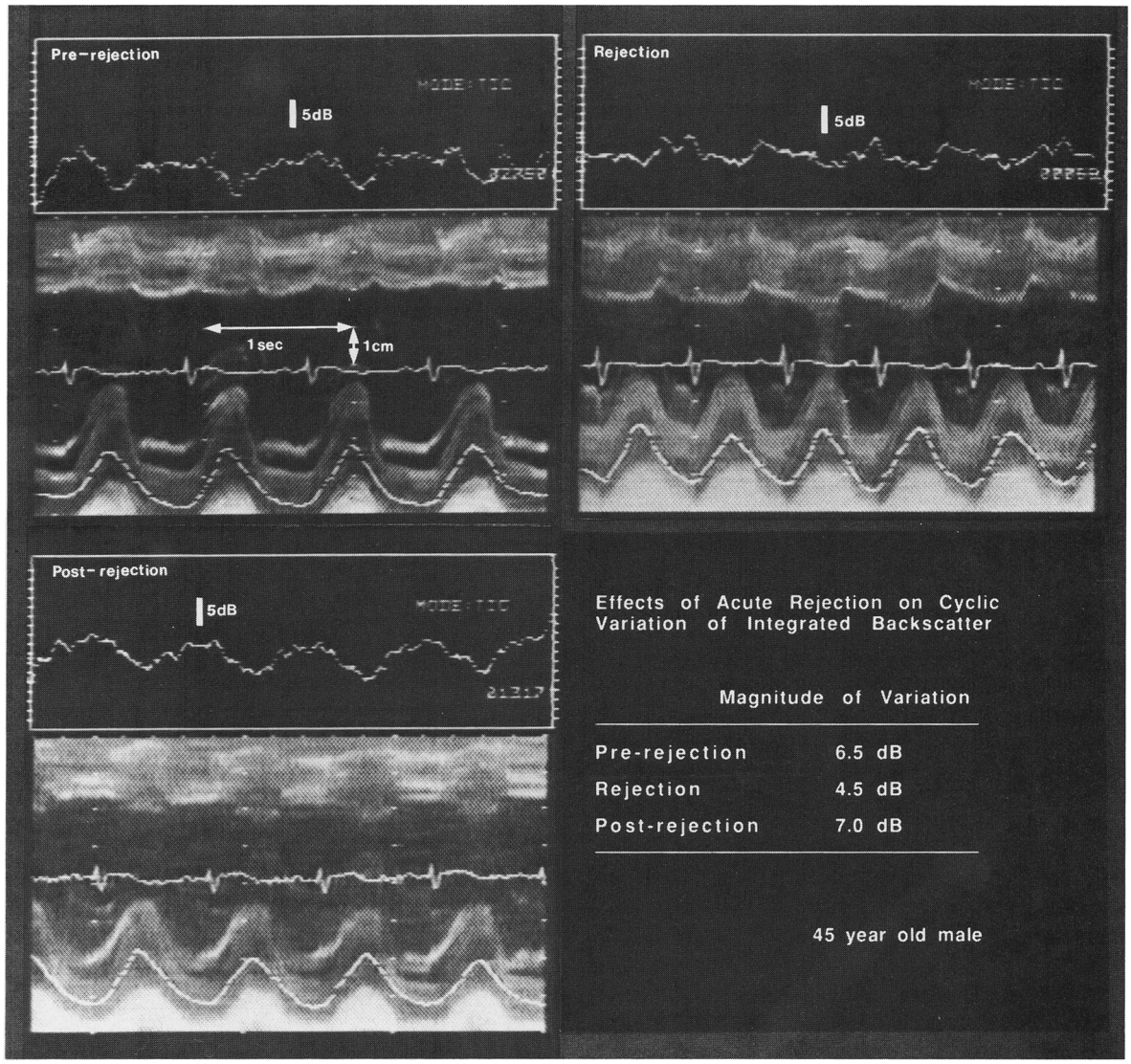

FIGURE 4. *Representative M-mode IB images with trace of area-of-interest and curve of IB versus time obtained from posterior wall of cardiac allograft at prerejection* (upper left panel), *during rejection* (upper right panel), *and at postrejection* (lower left panel) *periods. Magnitude of cyclic variation of IB decreased with rejection and almost fully recovered in postrejection study. dB, decibels; IB, integrated backscatter; sec, second.*

tion ($r=-0.45$, $p<0.05$, $n=32$) for the septum and posterior wall. The magnitude of the cyclic variation of IB also weakly but significantly correlated with the percentage of systolic thickening of the septal and posterior walls among the 17 patients ($r=0.55, p<0.01$, $n=32$). The relation seemed somewhat curvilinear; thus, when the second order polynominal regression analysis was applied to this relation, the correlation coefficient improved to r equaling 0.72 ($p<0.01$).

Effects of Acute Cardiac Allograft Rejection on Integrated Ultrasonic Backscatter Measures

The effects of acute cardiac allograft rejection on IB measures were studied in 11 patients (14 episodes) in whom IB measures were obtained before and during acute rejection with myocyte necrosis (Figure 4). There were no significant changes in heart rate or M-mode echocardiographic measures of left ventricular function with rejection (Table 2). The time from the onset of the electrocardiographic QRS wave to the minimum IB value did not significantly change with rejection. The magnitude of the cyclic variation of IB for the septum and posterior wall decreased with rejection from 4.2±2.1 to 2.9±1.8 dB ($n=12$, $p<0.05$) and from 6.7±1.3 to 5.1±1.4 dB ($n=14$, $p<0.05$), respectively. With resolution of rejection, the magnitude of the cyclic variation in IB for the septum and posterior wall tended to increase from 3.0±1.9 to 3.3±2.7 dB ($n=11$, $p=$NS) and from 5.0±1.4 to 5.8±1.9 dB ($n=13$, $p=$NS), respectively.

Reduced magnitude of the variation in IB in the posterior wall of 1.5 dB or more was observed in 12 of 14 episodes of acute rejection (Table 3), and if that level is taken as a criterion of rejection, then

TABLE 3. Magnitude of Cyclic Variation in Integrated Ultrasonic Backscatter of Left Ventricle in Cardiac Allograft Recipients in Whom They Were Obtained in Rejection Period

		Magnitude of IB variation (dB)					
		PW			Septum		
Patient	Age (yr)/sex	Pre	Rej	Post	Pre	Rej	Post
1	19/M	5.0	5.5	5.0	3.0	4.5	2.5
		5.0	2.5	2.5	2.5	1.5	2.0
2	19/M	5.5	3.5	6.5	NA	NA	NA
3	38/F	8.5	6.0	7.5	0	0	0
4	45/M	6.5	4.5	7.0	4.5	3.5	5.0
5	48/M	8.5	6.0	8.5	5.5	3.5	6.5
		8.5	6.0	8.0	6.5	5.5	5.0
6	53/F	6.0	4.5	2.5	3.0	2.0	2.0
7	54/F	6.5	5.0	6.5	7.0	5.0	6.0
		7.5	5.0	4.5	7.0	4.5	7.0
8	54/M	6.5	4.5	5.0	NA	NA	NA
9	55/M	5.5	4.0	5.5	3.0	0	0
10	56/M	6.5	8.0	7.0	5.0	3.0	0
11	56/M	8.0	6.5	...*	3.0	2.0	...*
Mean		6.7	5.1	5.8	4.2	2.9	3.3
SD		1.3	1.4	1.9	2.1	1.8	2.7

IB, integrated backscatter; PW, posterior wall; Pre, prerejection; Rej, rejection; Post, postrejection; M, male; NA, in these patients cyclic variation in integrated backscatter was not observed probably because of the effects of surgically implanted intramyocardial markers; F, female.

*Data in the postrejection period were not obtained due to sudden death.

sensitivity, specificity, and accuracy of the IB measure are 86%, 85%, and 85%, respectively (Table 4). In the septum, a reduced magnitude of the variation in IB of 1.0 dB or more was observed in 10 of 12 episodes of acute rejection, and if that level is taken as the criterion of rejection, then sensitivity, specificity, and accuracy of the IB measure are 83%, 71%, and 76%, respectively.

Discussion

In this study, acoustic properties of cardiac allografts with and without rejection were assessed by measurements of the cyclic variation in IB. The variation in IB, as opposed to measurement of absolute IB, is obtainable in humans with a current system because it does not require calibration of ultrasound signals and, thus, standardization for comparison among subjects. The magnitude of the cyclic variation of IB is considered to reflect alterations in the acoustic properties of the myocardium. This hypothesis is supported by several experimental animal studies[14–18] showing this parameter decreases with myocardial ischemia[15,18] and infarction.[17,18] Cyclic variation of IB also can be used to assess the acoustic properties of the myocardium in humans,[19–22] although the exact basis for cyclic variation in IB is still unknown.

Experimental studies[25–28] have indicated the magnitude of the cyclic variation of IB might be related to global and regional cardiac contractile performance. Animal, as well as clinical, studies have shown that regional myocardial contractile performance, assessed with the percentage of systolic thickening of the wall, correlated well with the magnitude of the variation of IB and that this relation is not linear.[16,22] These findings suggest regional myocardial contractile performance is one of the contributors to the variation of IB but is not the sole determinant. Other factors such

TABLE 4. Sensitivity, Specificity, and Accuracy of Change in Magnitude of Cyclic Variation of Integrated Ultrasonic Backscatter as an Index of Acute Cardiac Allograft

	Posterior wall (≥1.5 dB decrease in the magnitude of the cyclic variation in integrated ultrasonic backscatter)			Septum (≥1.0 dB decrease in the magnitude of the cyclic variation in integrated ultrasonic backscatter)		
	Positive	Negative	%	Positive	Negative	%
Biopsy positive	12	2	...	10	2	...
Biopsy negative	3	17	...	5	12	...
Sensitivity	...	...	86 (12/14)	...	...	83 (10/12)
Specificity	...	...	85 (17/20)	...	...	71 (12/17)
Accuracy	...	...	85 (29/34)	...	...	76 (22/29)

as fiber architecture, structure, or geometry of muscle fibers, elasticity or properties of the muscle, or any of these factors in combination, can also affect the variation of IB.[29]

Cyclic Variation in Integrated Ultrasonic Backscatter in Cardiac Allograft Recipients Without Rejection

There was no significant baseline difference in the magnitude of the variation of IB in the posterior wall between normal subjects and cardiac allograft recipients. In contrast, the variation of IB in the septum was significantly smaller in the allografts with cardiac hypertrophy than in normal subjects. A lower-than-normal variation of IB has previously been reported only in the septum of patients with pressure-overload hypertrophy.[22] This finding was considered to possibly reflect structural changes of an increase in fibrosis of the myocardium or depressed regional myocardial contractility. These two factors might also account for the decrease in the magnitude of the cyclic variation of IB in the septum of cardiac allograft recipients. Even without rejection, the cardiac allograft shows a fine diffuse interstitial fibrosis that might be related to cyclosporine or myocardial ischemia.[23,30] High vascular impedance, suddenly loaded on the donor heart, is a possible cause for initiation of hypertrophy. Additionally, most transplant patients treated with cyclosporine have systemic hypertension. Because there was a significant positive correlation between the percentage of systolic thickening of the walls and the magnitude of the variation of IB in cardiac allografts without rejection, the reduced magnitude of the cyclic variation of IB also might reflect depressed regional myocardial contractility. One might think that myocardial contractility is normal in cardiac allograft recipients because their left ventricular percentage fractional shortening at equivalent levels of end-systolic wall stress has been demonstrated to be normal.[31] We refer to "regional" rather than "average" or "global" myocardial contractility in this study, however, and these two might well be different. The left ventricular cavity is not truly a symmetrical ellipsoid, and therefore, significant regionality in the systolic wall stress as well as the myocardial contractile performance can occur. Regional myocardial contractile performance, however, might not necessarily be related to the wall motion in cardiac transplants. Nine of the 16 recipients showed the flat-to-paradoxical septal wall motion in this study; however, the magnitude of the cyclic variation of IB did not seem to be markedly affected by the abnormal septal wall motion. This finding is comparable with a recent preliminary report in which the magnitude of the cyclic variation was not distorted by altered wall motion per se in children with congenital heart lesions.[32]

It was previously found that the relation between wall thickness index and the magnitude of the variation of IB is different between secondary pressure-overload hypertrophy and the primary hypertrophy of hypertrophic cardiomyopathy.[22] The present findings suggest the hypertrophy of the cardiac allograft, without rejection, is closer to that produced as a consequence of physiological adaptation to increased systolic stress and less like the idiopathic form. The most probable causes for the hypertrophy in cardiac allograft recipients are now considered to be a compensatory or reactive hypertrophy produced by the ischemia during transportation of the donor hearts,[33] normal physiological response of the left ventricle to increased systemic vascular resistance (pressure overload), or both.[31] This postulation is consistent with the present findings.

Changes in Cyclic Variation in Integrated Ultrasonic Backscatter With Cardiac Allograft Rejection

During rejection, the variation in IB decreased, and with resolution of rejection, the variation in IB increased about halfway to prerejection values. These changes might be partially explained by associated changes in regional myocardial contractile performance as previously noted. Hansen et al[34] showed that acute rejection with myocyte necrosis depressed left ventricular systolic performance, using the assessment of torsional deformation of the left ventricular wall, which might be more sensitive to changes in left ventricular systolic performance than conventional ejection phase indexes such as stroke volume and ejection fraction. Data from this and other laboratories, however, suggest the cyclic variation of integrated backscatter is not solely associated with ejection-related indexes such as myocardial thickening.[16,22] It is well known that interstitial edema, hemorrhage, or both can occur in the presence of cardiac rejection.[23] Furthermore, a recent animal study[35] showed myocardial tissue blood flow decreases and myocardial water content increases with acute rejection. In patients, severe impairment of coronary reserve is observed during acute rejection.[36] The possible myocardial ischemia or alterations in water content can also partially account for the changes in the cyclic variation of IB associated with acute cardiac rejection.

The decreased magnitude of the cyclic variation of IB during rejection did not return to the prerejection values in all cases after resolution of the episode. It is known that scar or interstitial fibrosis formation follows myocyte necrosis,[23] and these irreversible changes in the myocardium can partially explain this finding. The mechanism of the changes in the variation of IB associated with rejection and its resolution is still unclear.

The magnitude of the cyclic variation of IB seems an accurate indicator of acute rejection with myocyte necrosis in this small series. Specificity and diagnostic value of this parameter are better in the posterior wall as compared with the septum. This can be partially explained by the variation of measurement of this parameter in the two areas. The reproducibility (intraobserver and interobserver variabilities) of measurements of this parameter has previously been assessed in our laboratory.[22,37] The reproducibility of

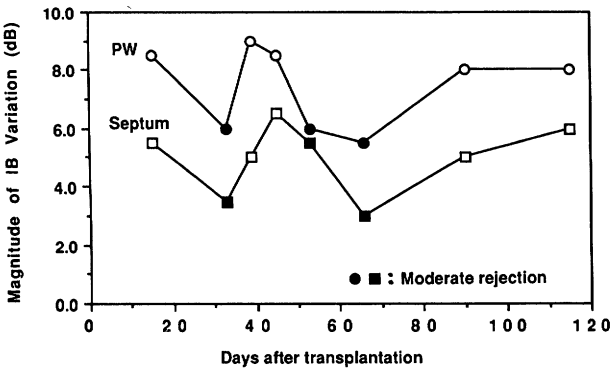

FIGURE 5. *Graphic plotting of postoperative course of cardiac allograft recipient (patient 5 in Table 3) during initial 120 days. Magnitude of variation of integrated backscatter (IB) (vertical axis) obtained in presence (closed symbols) or absence (open symbols) of rejection with myocyte necrosis by biopsy are plotted against number of days after transplantation (horizontal axis). Circles and squares stand for data measured in posterior wall and in septum, respectively. See text for explanation. dB, decibels; PW, posterior wall.*

measurements of this parameter in the posterior wall is within 1.0 dB and slightly more reproducible than in the septum, both in normal subjects and in patients with hypertrophied hearts, probably because of technical factors.[37] Day-to-day variation (assessed from the number of false positives in Table 4) was also smaller in the posterior wall as compared with the septum. Thus, the measurement in the posterior wall might provide a more reliable and accurate indicator of acute rejection with myocyte necrosis. It does not seem a reliable indicator of early rejection, without myocyte necrosis, however, even using group data as opposed to individual patient data (changes from prerejection to mild acute rejection, 5.8±0.8 to 5.4±0.9 dB [p=NS, n=6] for the posterior wall and 2.8±2.6 to 2.4±2.2 dB [p=NS, n=5] for the septum).

Figure 5 relates the changes in the magnitude of the cyclic variation of IB in a representative patient (patient 5 of Table 3) to myocardial histological findings. In this patient, the first episode of acute rejection with myocyte necrosis was detected by biopsy on postoperative day 32. This was associated with 2.5 and 2.0 dB decreases in the cyclic variation of IB from the prerejection value of 8.5 and 5.5 dB in the posterior wall and septum, respectively. Rejection was treated with 1 g methylprednisolone daily for 3 days. In 5 days (day 37), the magnitude of the cyclic variation of IB had increased from 6.0 to 9.0 dB in the posterior wall and from 3.5 to 5.0 dB in the septum, and the biopsy showed complete resolution of the rejection episode. Sixteen days later (day 53), the magnitude of the cyclic variation of IB decreased again from 8.5 to 6.0 dB in the posterior wall and from 6.5 to 5.5 dB in the septum, and the biopsy showed moderate rejection with myocyte necrosis. This rejection was also treated with standard methylprednisolone dosage but, 2 weeks later, further decreases in the magnitude of the cyclic variation of IB were observed both in the posterior wall and in the septum; biopsy and histological examination showed improvement but ongoing moderate rejection. Within a month (day 98), complete resolution of the rejection occurred, and the magnitude of the cyclic variation of IB increased from 6.0 to 8.0 dB in the posterior wall and from 3.0 to 5.0 dB in the septum.

Disagreement between IB measurements and biopsy data occurred in five of 34 and seven of 29 studies in the posterior wall and septum, respectively. Biopsy specimens are sampled from the right ventricular apex and septum, and IB measurements are performed in the left ventricular wall. The rejection process does not seem to affect left and right ventricles to the same extent, nor is the severity of rejection uniform throughout the ventricular wall.[38] Inhomogeneity of the rejection process and biopsy sampling error can partially explain the disagreement in some patients. Although Doppler echocardiography or magnetic resonance imaging might provide more global estimates of rejection, they were not used as standards for comparison in this study because they are not yet widely accepted.

Technical Limitations

Three technical limitations are noted. First, technically adequate IB images were not obtained in five (18%) of 28 patients. Second, all quantitative analysis was based on the IB values determined by hand tracing the area-of-interest on the IB image. There are inherent problems in this process, such as the effect of thick bright echo lines of the right or left septal endocardium, intramyocardial septal specular bright echoes, or both.[22,37] Finally, 15 of the 23 patients had implanted intramyocardial metallic markers for another research protocol.[34] These bright signals typically come into the ultrasound beam and are sampled only in systole or in diastole because of cardiac movement perpendicular to the ultrasound beam. Attempts to exclude such bright areas during data acquisition were not successful in three of the 23 patients, and they were excluded from the quantitative analysis.

Conclusions

This study has demonstrated that the magnitude of the cyclic variation of IB in the septum in cardiac allograft recipients with cardiac hypertrophy is different from normal subjects. The current equipment gives consistent results in the posterior wall of cardiac allografts without rejection. Patients with and without rejection cannot be separated from each other solely on the basis of a single measurement of the variation of IB because of the range of changes in this parameter with hypertrophy, infarction, and acute rejection. Using the patient as his own control, however, a decrease in the cyclic variation of IB was observed during cardiac allograft rejection in individuals. Thus, serial IB studies seem to be useful for detection of acute cardiac rejection in recipients,

although a single measurement of the variation of IB is not valid for this purpose. Although the technique used in this study can only detect rather gross changes in the acoustic properties of the myocardium, the ability to detect more subtle changes in the myocardium caused by cardiac allograft rejection might become a reality with further refinement of both the instrumentation and analysis methods.

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