Ultrasonic myocardial tissue characterization: a methodological review

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Ultrasonic myocardial tissue characterization represents a relatively new diagnostic tool which allows integration of the conventional echocardiographic evaluation, in order to obtain specific textural parameters which reflect the myocardial ultrastructural texture. In particular, through this approach it is possible to obtain two different types of information: the first is static and consists of the absolute myocardial echo intensity that reflects the ultrastructural myocardial changes in different diseases; the second is dynamic and is related to the variations of echo intensity during the cardiac cycle which seem to be linked, even though not linearly, to the intrinsic myocardial contractility. Our research group has extensively applied this methodological approach to different pathophysiological models, in particular to essential hypertension. In the present review the technological evolution of the method and comparison with other research groups' experience with the specific pathophysiological models, are shown and discussed.

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Introduction

Different approaches and measurement techniques for myocardial tissue characterization have been developed in an attempt to quantify the ultrastructural myocardial texture. At first, methods based on radiofrequency, data analysis including integrated backscatter (IBS) imaging[1,2] and methods that quantify the echocardiographic gray level (videodensitometry)[3,4] have been used. More recently, real-time two-dimensional backscatter imaging has been integrated in digitized echographs, thus increasing the possibility of performing ultrasonic tissue characterization analysis[5]. Our research group has developed several studies in many pathophysiological models using all these ultrasonic tissue characterization methods, so acquiring a specific experience in this research field, which appears very promising especially for the early detection of the myocardial alterations induced by pathological conditions.

Methodology of ultrasonic characterization of myocardial tissue

Among the methods which have been developed in the attempt to identify structural myocardial alterations with both qualitative and quantitative approaches, the most utilized and promising are, in our opinion, those listed below.

Radiofrequency data analysis (integrated backscatter). This approach had represented an important field of research in tissue characterization; through this method the unprocessed radiofrequency signal returning from the myocardium is analyzed. This method has been used to quantify the degree of ultrasonic attenuation in transmission studies and the extent of ultrasonic backscatter in pulse echo studies; but its main application was the measurement of the backscattered signal reflected from a selected segment of myocardium[6]. An on-line radiofrequency analysis is performed to obtain quantitative operator-independent measurements of the IBS signal of the interventricular septum and of the posterior wall. These regions are visualized in the parasternal long-axis view. The acquisition of the backscattered signal is performed both at end-diastole and at end-systole considering the systematic variation in backscatter amplitude which occurs during the cardiac cycle[7,8].

The "native" (raw) radiofrequency signal is sampled before the processing chain of the

two-dimensional instrument. Briefly, the radiofrequency signal undergoes pre-amplification, bypassing the receiving circuits of the ultrasonic equipment. The analog signal is fed to an amplifier, and the gain sweep of the amplifier (from 2 to 60 dB) is accomplished in 30 steps. This operation allows the full utilization of the input dynamic range of the analog to digital converter (Fig. 1)[2]. The digitized signal is then analyzed in real time by a hardware prototype developed in the CNR electronics laboratory. The acquisition of the two-dimensional gate is visualized on the two-dimensional image in order to ensure its proper positioning perpendicularly on the selected beam. For analysis of the myocardium, the gate width is kept at 3 μs, which corresponds to 2.35 mm (for 128 points), since the velocity of ultrasound in biological tissues is 1.57 mm/μs. This allows sampling of the radiofrequency signal in the middle layers of the myocardium, thus excluding epi- and endocardial specular reflections. The acquisition gate is kept immediately behind the specular echo of the endocardium (left endocardium for the septum) in order to minimize the transmural variations in backscatter that are influenced by the position within the wall from which the signal is acquired (Fig. 2). For the evaluation of the pericardial echo, a 1.5 μs gate length is used (which corresponds to 1.2 mm, for about 64 points). The acquisition gate is centered on the strongest pericardial reflections just behind the mitral leaflets. Hardware analysis involves the measurement of the integrated amplitude of the rectified radiofrequency signal corresponding to the two-dimensional area selected from the echocardiographic image. The system provides a simultaneous display of conventional information together with tissue characterization parameters (the two-dimensional IBS alphanumeric index and the lateral displacement profile averaged over the selected depth). The integrated values of the radiofrequency from the myocardial wall are normalized for those from the pericardial interface and are expressed as percentages (IBS index). Two main parameters can be derived from backscatter analysis: indices measuring the cyclic (systolic-to-diastolic) variations, which are mainly linked to the intramural function and to intrinsic contractility, although in a complex and incompletely understood manner; and indices measuring the absolute echodensity value, which are mainly linked to the structural, histological component of the myocardial tissue, such as the collagen content. The reproducibility of this method is good: in our laboratories the coefficient of correlation (r) between intraobserver measurements was 0.92 and 0.88 for interobserver measurements.

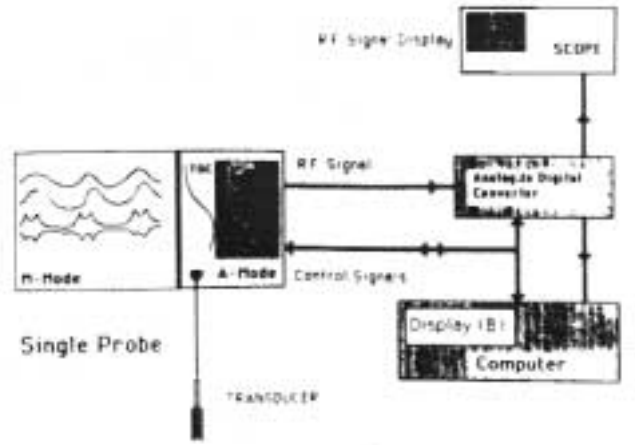

Figure 1. *Experimental system employed for the acquisition of myocardial structures* in vivo. *From Lattanzi et al.[2], with permission.*

Videodensitometric images digitization. Another myocardial tissue characterization method is represented by the conversion of analogic conventional ultrasonic im-

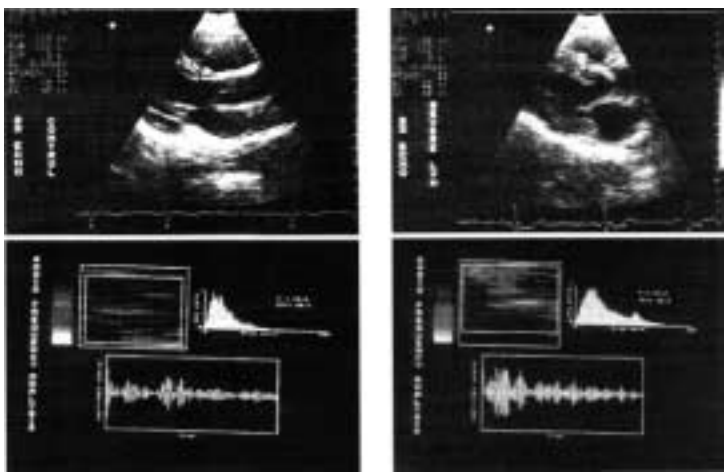

Figure 2. Top: *two-dimensional echo image showing a left ventricular long-axis view, with the sample volume positioned on the septum strictly perpendicular to the ultrasound beam in a control* (left) *and in a hypertensive patient* (right) *with severe left ventricular hypertrophy.* Bottom: *relative backscattered radiofrequency signal corresponding to the septum area. There is a frequency gray level histogram (*on the x-axis *the gray level value using a zero to 255 scale;* on the y-axis *the frequency of occurrence); the display of the radiofrequency signal in the time domain (*on the ordinate, *the height of the signal in millivolts;* on the abscissa, *time in milliseconds). In a hypertensive patient with severe left ventricular hypertrophy* (on the right) *the echo-reflectivity pattern is increased in comparison with that of the control* (on the left).

ages into a digitized form, which allows the quantitative analysis of the ultrasonic myocardial texture (videodensitometry)[9,10]. The videodensitometric approach to the ultrasonic analysis of the myocardium surely allows a more objective analysis in comparison with previous qualitative approaches which employed digitized images. However, even this analytical approach has some limitations, especially if some methodological aspects are not taken into account. With regard to this, when using the analogic images, the gain settings and compensation profiles are adjusted for all study subjects to obtain apparently uniform myocardial brightness throughout the echocardiogram and thus achieve a reproducible sampling of textural parameters (Fig. 3)[5]. The gray scale transfer function has to be adjusted to be linear for the entire video signal range and no reject, enhancement or dynamic range can be used with a 25-30 dB amplification at a depth of 18 cm. The optimal echocardiographic images are then directly transferred from the echocardiograph to a calibrated video digitization system, thus converting them into the digitized form. Particular care has to be taken to make sure that the sonic beam's angle of incidence is at approximately 90° to the area of the interventricular septum or to the left ventricular posterior wall when scanning the parasternal left ventricular long-axis view. The regions of interest (ROI) (chosen by consensus of two observers, who are strictly blinded versus the results of conventional echocardiography), always the same size, are placed in the same location in the septum (mid-septum) and in the posterior wall (mid-posterior) both at the end-systolic and the end-diastolic frames; this, taking into account that there is a displacement of the heart during the cardiac cycle, which is clearly visible in the sequence of frames as they appear on the computer screen. Only the myocardium must be included, whereas the endocardial and epicardial specular echoes must be excluded to avoid areas of echo dropouts and obvious artifacts[11]. The mean gray level of each cavity region (background signal) is subtracted from the absolute mean gray level obtained for each ROI (mean gray level, background corrected - MGL). A histogram of the echocardiographic gray level distribution is generated for each ROI with the gray level values on the abscissa and the frequency of the occurrence on the ordinate. A quantitative analysis of the shape of each distribution is also performed using Skewness and Kurtosis. The cyclic variation index of the gray level amplitude is calculated according to the formula: $(MGL_{ED} - MGL_{ES})/MGL_{ED})*100$ (Fig. 4). The intraclass correlation coefficient (Bland and Altman procedure[12]) for the septum MGL was 0.92 for the diastolic and 0.90 for the systolic sample. For the posterior wall MGL it was 0.89 for the diastolic and 0.91 for the systolic sample.

Real-time backscatter imaging. Actually there is the possibility of obtaining real time two-dimensional backscatter images. A software package "Acoustic Densitometry" is supplied with Agilent Technologies echocardiographs and it provides an on-line integrated capability to measure, display and analyze the average acoustic image intensity within the user-specified ROI at user-specified trigger intervals. This method allows one to obtain the quantitative analysis of the IBS[5]. IBS is the relative measure of the total ultrasonic energy backscattered by a small volume of the tissue under evaluation. By using special hardware, the IBS signal processor operates on the digitally beam-formed radiofrequency signal in incremental time windows along each acoustic line to generate the real-time IBS image at a frame rate of 25 Hz. The useful dynamic range of the IBS image data is 60 dB[1]. Acoustic Densitometry allows the user to specify a ROI within the IBS image to obtain the average acoustic intensity of the ROI. The Acoustic Densitometry measurement is independent of the non-linear compression and post-processing functions of the ultrasound imaging chain (Fig. 3).

To ensure standardization of the IBS acquisition, the transthoracic echo imaging must be performed in a left ventricular long-axis view; the initial instrument gain settings of transmitter power and receiver time-gain

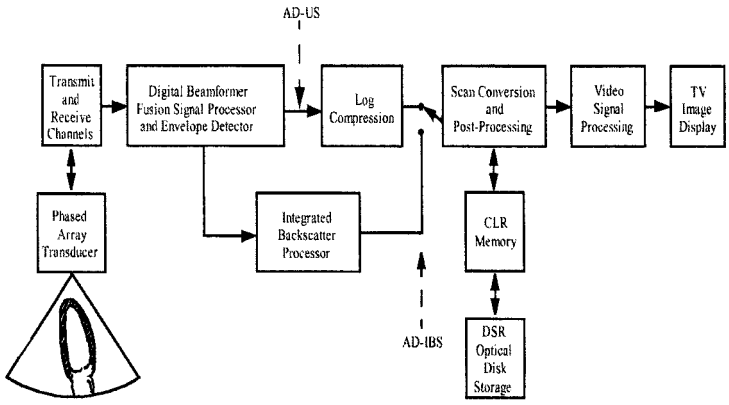

Figure 3. *Block diagram showing conventional and integrated backscatter data flow paths in the Sonos 5500 and Acoustic Densitometry (AD) data measurement nodes. The AD tool can be used with conventional ultrasound imaging data (AD-US) and with integrated backscatter image data (AD-IBS). From D'Sa[5], with permission.*

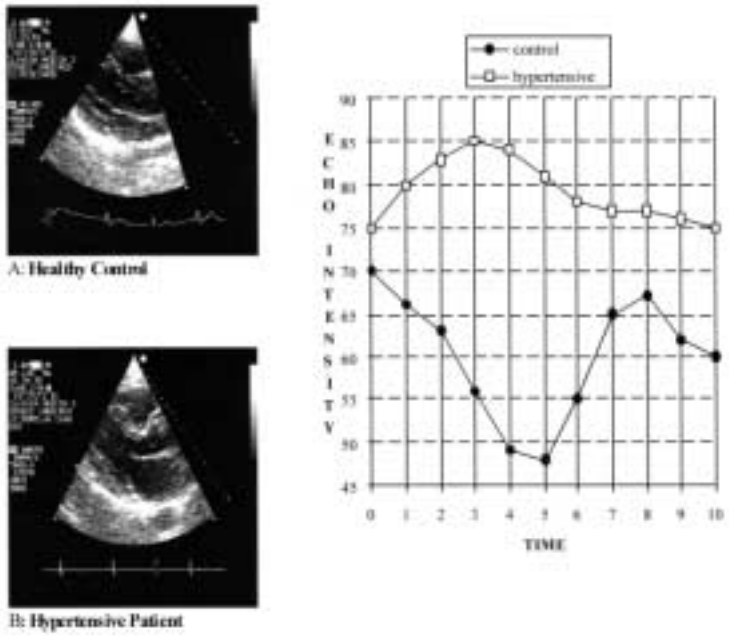

Figure 4. *For the two groups each panel shows* on the left side: *digitized two-dimensional echocardiography images of the left ventricle (parasternal long-axis view);* on the right side: *variations of echo intensity in a region of interest placed at the level of the septum* (on the ordinate) *during one cardiac cycle, arbitrarily divided into 12 frames independently of the heart rate.*

compensation profiles are adjusted to achieve uniform and optimal visualization of the two-dimensional image. The examined structures (septum and posterior wall) must be nearly perpendicular to the ultrasonic beam, thereby obtaining a good signal-to-noise ratio and minimizing problems associated with tissue anisotropy (the cyclic variation of IBS is dependent on the angle between the muscle fibers' orientation and the incident ultrasonic beam). The image disk file is retrieved into a cineloop for quantitative analysis of the backscatter intensity, and an elliptical-shaped ROI of the same size is placed within the myocardium of the mid-septum and of the mid-posterior wall with the major axis of the ROI almost orthogonal to the ultrasonic beam. Care must be taken to exclude specular echoes arising from the myocardial wall boundaries. So as to track and sample the same region of the myocardium throughout the cardiac cycle an experienced echocardiographer manually adjusted the location of the ROI on a frame-by-frame basis[13,14]. The IBS data measurements obtained are then graphically displayed as a time-intensity waveform using an intensity scale range from 0 to 64 dB. The IBS data are analyzed for about 2 or 3 cardiac cycles. The measurements obtained for each cardiac cycle are: the intensity of IBS at end-diastole (IBSed), the intensity of IBS at end-systole (IBSes), the IBS variation (IBSed - IBSes) and the cyclic variation index, which was computed using the formula: (IBSed - IBSes)/IBSed)*100, both for the septum and the posterior wall. The intraclass correlation coefficient was calculated according to Bland and Altman procedure[12], typically using three values of IBS at end-diastole and at end-systole both for the septum and the posterior wall. The intraclass correlation coefficient for septum IBS was 0.95 for the diastolic and 0.93 for the systolic sample, while for posterior wall IBS it was 0.90 for the diastolic and 0.93 for the systolic sample.

Biological basis of ultrasonic tissue characterization

The different structural components of the myocardium can influence its acoustic properties in physiologic and pathologic conditions (Rayleigh scattering)[15]. Collagen is a primary determinant of both scattering[16] and attenuation of myocardial tissue[17,18]; a linear relationship was found between IBS and hydroxyproline content in autopsied human hearts with fibrotic changes associated with remote myocardial infarction[7]; furthermore a significant direct correlation was found between the collagen content as determined at biopsy and the regional echo amplitude[19]. Scattering geometry is another determinant of myocardial reflectivity: in fact, myocardial scattering intensity directly depends on myocyte size; the microstructural arrangement of myocardial cells embedded in a collagen matrix may provide a sufficient local acoustic impedance mismatch to account for the scattering from normal myocardium[20]. Ventricular muscle fiber orientation might influence myocardial acoustic properties. In fact, the insonification angle might greatly influence the magnitude of attenuation and backscatter (the backscatter is maximal when the echo beam is directed perpendicularly to the orientation of the muscle fibers). On the other hand, the middle portion of the left ventricular wall mainly comprises circumferentially oriented fiber bands[21]. The tissue water content and blood flow both influence myocardial at-

tenuation and scattering; the increase in water content (tissue edema) and, to a lesser degree, the reduction in coronary blood flow (myocardial ischemia) might influence the acoustic properties of the myocardium.

Some considerations must be made about the dynamic aspect of scattering: as described by Wickline et al.[8], peak values occurred at end-diastole and minimal values at end-systole. However, these cyclic changes in the echo amplitude are related, although not linearly, to the intrinsic myocardial contractile performance. Masuyama et al.[22] found a reduced cyclic variation of the IBS indices in the septum of a mixed population of patients with hypertensive, valvular hypertrophy and hypertrophic cardiomyopathy, compared with normal subjects.

Myocardial ultrasonic tissue characterization in the hypertensive heart

In essential hypertension, the left ventricular pressure-volume overload *per se* or through the interaction of complex humoral factors, mainly mediated by the renin-angiotensin-aldosterone system, is able to induce some myocardial structural alterations which represent the early stage of organ damage in the evolution towards the hypertensive heart disease[23,24]. It is well known that, in the hypertensive heart, the correct echocardiographic determination of the left ventricular mass and the analysis of its relationship with left ventricular geometry are very important for their diagnostic, prognostic and therapeutic implications. The relevance of the detection of the ultrastructural alterations in the hypertensive heart is confirmed by several morphological features characterized by an increase in the collagen volume fraction in the hypertrophied left ventricles both in human autopsies and in non-human primates: interstitial fibrosis, perivascular fibrosis, replacement fibrosis of necrotic myocytes and plexiform fibrosis are the specific findings of histological analysis. In particular, the myocyte necrosis observed in pathological hypertrophy stimulates fibroblast proliferation and replacement of the muscular component by connective tissue[25,26].

In their pioneering work, Chandrasekaran et al.[27] differentiated amyloid and hypertrophic cardiomyopathy by means of computerized quantitative texture analysis of conventional echocardiographic data, but they were unable to differentiate hypertensive patients from normal subjects (videodensitometry). In a more recent study, Gigli et al.[28] could not differentiate subjects with uncomplicated hypertension and mild to moderate left ventricular hypertrophy from healthy controls using the IBS technique. However, these authors limited the analysis to end-diastolic values. These values were not sufficient for differentiation purposes if the cyclic variation of the backscatter indices was not taken into consideration. Interestingly, Naito et al.[14], using an M-mode format IBS imaging system, observed that hypertrophic cardiomyopathy and ventricular hypertrophy due to hypertension can be differentiated on the basis of the quantitative analysis of the transmural gradient in IBS which was present only in patients with hypertrophic cardiomyopathy.

Our research group has applied ultrasonic tissue characterization in essential hypertension to the pathophysiological model of severe left ventricular hypertrophy[29]. In this study, we have demonstrated, using quantitative texture analysis of the myocardium (videodensitometry), that hypertensive subjects with severe left ventricular hypertrophy and normal left ventricular systolic function showed a significantly lower cyclic variation index of mean gray level, an expression of impaired intrinsic contractility, in comparison with the control group and the athletes' group both for the septum and the posterior wall. On the other hand, no significant difference regarding this parameter was found between athletes and controls. A further observation that in the myocardium of severe hypertensive left ventricular hypertrophy such ultrastructural abnormalities are present, was documented by Lucarini et al.[30] who employed IBS radiofrequency analysis: the authors showed an increased myocardial reflectivity (IBS) in hypertensive severe left ventricular hypertrophy that was not present in subjects with mild degrees of left ventricular hypertrophy. However, the authors limited the analysis to end-diastolic values and did not take the cyclic variation of IBS into account.

Subsequently our group had investigated if ultrasonic modifications in myocardial structure could be detectable in a group of hypertensive patients with and without mild-moderate left ventricular hypertrophy[31] when conventional left ventricular systolic functional indexes are still within the normal range. We demonstrated that abnormalities of the cyclic variation index might be specific to the hypertensive status and substantially independent of the left ventricular mass. In fact, these abnormalities were present in hypertensive patients both when the left ventricular mass was within normal limits as well as in the presence of a mild to moderate degree of left ventricular hypertrophy. In fact, the reduction in cyclic variation index observed in hypertensive subjects with a "normal" fractional shortening, could be considered an "early", independent index of abnormal intrinsic contractility.

Alterations of ultrasonic textural parameters in essential hypertension: possible explanations. In spite of the lack of histopathological (biopsy) data, not ethically acceptable in subjects with this type of disease, on the basis of various experimental and human autopsy studies on the myocardium in hypertension, some hypotheses could be made to explain the alterations in the acoustic properties of myocardium and in particular in the dynamic aspects of scattering:

• the increase of the intermyocytic collagen network which occurs in hypertension[23,25,32] could determine,

in systole, an increase in scattering thus causing a reduction in its normal cyclic variation, despite the reduction in the length of myocardial fibers during contraction, which represents one of the determinants of the systolic decrease in acoustic myocardial reflectivity in normal subjects;

• the pressure-volume overload in hypertension, causing myocardial stimulation mediated by complex mechanical and humoral factors, could moreover determine a change in the orientation, structure or geometry of both the muscle fibers as well as the collagen network (shift from type III to type I collagen molecules), and a myocyte replacement by fibroblasts due to myocyte apoptosis[33], so influencing the acoustic properties of the tissue[24,34,35];

• variations in myocardial blood flow, possibly related to the presence of alterations in the microcirculatory system: a reduction in capillary density in the hypertrophied hypertensive myocardium[36] could at least partly explain the scattering alterations in hypertension.

The decrease in the cyclic variation of the echo amplitude in hypertensive subjects, although in the presence of normal systolic indexes (left ventricular fractional shortening and mid-wall fractional shortening) in a concentric left ventricular hypertrophy model, may suggest that the variation in the echo amplitude could be considered as a distinct, "early" index of an altered myocardial function, and a useful parameter indicating a potential evolution towards hypertensive heart failure. Thus the cyclic variation index is probably a highly sensitive parameter in the identification of the abnormal echodensity in hypertension and in other diseases. If we consider subgroups of hypertensive patients, the cyclic variation index was significantly lower in patients with a left ventricular mass > 175 g/m^2 and in patients with concentric hypertrophy, i.e. the patients with the worst prognosis in terms of morbidity or mortality due to cardiovascular events[37].

The fact that the correlation between the cyclic variation index and the cardiac mass is only partial could be explained by the disproportionate connective tissue content and altered coronary microcirculation present in hypertrophied myocardium. Animal data showed a 2-3-fold increase in connective tissue in advanced hypertensive left ventricular hypertrophy[25]. This evidence was confirmed even in humans[26] on the basis of morpho-functional correlations. Thus, the collagen structure of the heart is likely to be an important determinant of the videodensitometric signal.

Comparison of ultrasonic textural parameters with transmitral Doppler echocardiography. The analysis of the transmitral flow velocity is largely used for the evaluation of the global diastolic function. In the pressure-volume overload of hypertensive subjects, an alteration of the passive end-diastolic phase (increased stiffness) was observed. The E/A ratio is inversely related to left ventricular mass and it is lower in the concentric hypertrophy group, thus selecting the patients with the worst cardiovascular prognosis. Our work has compared the sensitivity of both methods (videodensitometry and transmitral Doppler analysis) for the discrimination, through individual analysis, of hypertensive patients from normal subjects. On the basis of the E/A ratio, only 24% (13/53) of patients are actually discriminated from normal subjects, while individual analysis of the cyclic variation index, both at the level of the septum and of the posterior wall, discriminated 74% (39/53, $p < 0.01$) of patients[38]. These results clearly demonstrate that, compared to the E/A ratio, the cyclic variation index better discriminates normal from hypertensive hearts.

Other applications of myocardial ultrasonic tissue characterization

Athlete's heart. The athlete's heart represents a model of physiological left ventricular hypertrophy and many papers have demonstrated, through conventional echocardiography, that in this model various adjustments, strictly related to the type of exercise (isometric or isotonic), are present. In fact isometric exercise mostly induces concentric hypertrophy while isotonic exercise mostly induces eccentric hypertrophy. With regard to this, ultrasonic tissue characterization added useful information about the physiological features of this form of left ventricular hypertrophy: using radiofrequency studies, our research group demonstrated that the myocardium of the athlete's heart has the same ultrasonic reflectivity as that of healthy sedentary controls. These findings were further confirmed at videodensitometric evaluation[29] and at real-time radiofrequency analysis[39,40].

Cardiomyopathies. *Hypertrophic cardiomyopathy.* Early abnormalities in myocardial texture were found in hypertrophic cardiomyopathy both at qualitative and more recently at quantitative approaches to the evaluation of myocardial structure. Masuyama et al.[22] found in hypertrophic cardiomyopathy a low cyclic variation of the IBS, similar to that found in uncomplicated pressure-volume overload, but significantly lower than in controls. At radiofrequency analysis of IBS, Lattanzi et al.[39] found an increase in the end-diastolic IBS signal in hypertrophic cardiomyopathy. This finding was interpreted as an expression of an increase in collagen content. These findings were confirmed by another study of ours using radiofrequency analysis of the IBS to compare hypertrophic cardiomyopathy patients with athletes and healthy subjects; in this study we found that the athlete's heart with left ventricular hypertrophy preserved a normal myocardial texture. On the other hand, hypertrophic cardiomyopathy showed, in comparison with healthy controls, a higher IBS both in the septum

and the posterior wall. At IBS analysis, Naito et al.[14] demonstrated that in patients with hypertrophied hearts, the cyclic variation of IBS was smaller and the calibrated myocardial IBS was higher than in normal subjects. However, there were no significant differences between hypertensive hypertrophy and hypertrophic cardiomyopathy. Using radiofrequency analysis, Vitale et al.[41] studied the myocardial reflectivity in pediatric and adult patients with hypertrophic cardiomyopathy and demonstrated that in young patients the ultrasonic myocardial reflectivity is normal. This was in contrast to the significant increase observed in adult patients affected by the same disease and highlighted an age-dependent difference in myocardial reflectivity in hypertrophic cardiomyopathy.

Dilated cardiomyopathy. Some experimental observations support the hypothesis that ultrasound tissue characterization may be useful in the early detection of dilated cardiomyopathies. With regard to this, at real-time two-dimensional IBS analysis Vered et al.[42] showed that in dilated cardiomyopathy some abnormalities (impairment) of cyclic dependent changes in ultrasonic myocardial backscatter are present; subsequently Naito et al.[43], using the calibrated IBS, have demonstrated that in dilated cardiomyopathy, myocardial IBS values were higher and the cyclic variation of the IBS was lower in patients with myocardial fibrosis, thus providing information otherwise only available through endomyocardial biopsy. By means of IBS analysis, Fujimoto et al.[44] were able to extend the previous observations in dilated cardiomyopathy, finding a correlation with both the extent of fibrosis in myocardial tissue and the myocyte diameter; the authors concluded that their findings suggested that ultrasonic tissue characterization was a good indicator of the severity of fibrosis and of myocyte atrophy in patients with dilated cardiomyopathy.

Infiltrative cardiomyopathy. It is well known that this disease is able to cause qualitative alterations in myocardial texture. However, the quantitative ultrasonic approach to tissue characterization might add some useful information about the myocardial damage which occurs in these cardiomyopathies. Chandrasekaran et al.[27], using a videodensitometric approach, were able to discriminate hypertrophic cardiomyopathy and amyloid heart disease from normal myocardium by analyzing the gray level texture variables. Pinamonti et al.[45] have demonstrated that in myocardial amyloidosis quantitative texture measurements of second order statistic variable, entropy was significantly and consistently higher in amyloid versus normal patient data. These authors concluded that amyloid-infiltrated myocardial walls showed ultrasound image texture alterations that may be quantified at digital image analysis techniques.

Toxic cardiomyopathy. In experimental models, the use of anthracycline could induce a cardiomyopathy characterized by increased fibrosis substituting myocardial cells and occurring in response to myocardial damage. Some initial observations in humans confirmed these findings. In particular ultrasonic tissue characterization could be useful in the early detection of myocardial damage induced by these drugs. In this respect, Goens et al.[46], using ultrasound myocardial tissue characterization by IBS, conducted a study in children treated with anthracyclines for malignancy; the results indicated that an abnormal cyclic variation of IBS was prevalent both during anthracycline treatment (17%) and also at late follow-up (20%). This report provides preliminary evidence that the cyclic variation index of IBS may be a useful supplement to the non-invasive, echocardiographic evaluation of the heart during anthracycline treatment in children.

Myocarditis. The diagnosis of myocarditis is often difficult and frequently necessitates endomyocardial biopsy. Since conventional echocardiography is non-specific, in particular in the early phases of the illness, ultrasonic myocardial tissue characterization can be helpful. For this purpose, Lieback et al.[47] explored the clinical value of echocardiographic tissue characterization in the diagnosis of myocarditis by comparing the videodensitometric textural findings with endomyocardial biopsy data. The authors have found that three texture parameters were able to differentiate between normal and abnormal myocardium: the mean gray value, i.e. average brightness, was appreciably higher in cases of myocarditis than in control subjects whereas one co-occurrence and one run length feature had markedly decreased. The authors concluded that myocarditis and fibrosis are able to induce changes in echocardiographic image texture, i.e. increase in brightness, heterogeneity, and contrast and that, by performing digital image texture analysis of the echocardiograms, it was possible to distinguish between myocarditis and normal myocardium.

Systemic diseases

Diabetes mellitus. The prevalence and the nature of diabetic cardiomyopathy are controversial and even in this pathological model ultrasonic tissue characterization could clarify the intrinsic mechanisms in a very early phase of the disease (occult cardiomyopathy). With regard to this, Perez et al.[48] have investigated the abnormal myocardial acoustic properties (IBS) in (type II) diabetic patients and their correlation with the severity of disease, in the presence of normal systolic function. The authors found that in diabetic patients, a reduction of the cyclic variation of IBS occurs and that this ab-

normality is greater in diabetic patients who presented with retinopathy or nephropathy. More recently, our group[49] has found a higher myocardial reflectivity (by radiofrequency analysis of IBS) in type I diabetic patients compared to healthy controls; we concluded that an abnormally increased myocardial echodensity, possibly related to collagen deposition, can be detected in asymptomatic diabetic patients with normal rest systolic function. This finding may be considered to be a very early, preclinical alteration, potentially related to the subsequent development of diabetic cardiomyopathy. These findings were confirmed by another study in which videodensitometry was performed in type I diabetic patients; in fact, the authors[50] found that the cyclic variation of the mean gray level of the septal and posterior wall was lower in diabetics than in healthy controls.

Systemic sclerosis. We also investigated the potential myocardial involvement in patients with systemic sclerosis at videodensitometry[51], in an attempt to detect preclinical myocardial alterations in these patients by quantitative evaluation of the two-dimensional echo gray level distribution analysis. Alterations in the cyclic echo amplitude, probably related to myocardial fibrosis, were detected in the large majority of patients with systemic sclerosis (88%). The authors concluded that in patients with systemic sclerosis ultrasonic videodensitometric analysis represents a non-invasive, feasible method able to detect early myocardial alterations which could be related to both fibrosis and microcirculatory abnormalities.

Subclinical hypothyroidism. Another recent field of research by our group[52] was subclinical hypothyroidism. In an attempt to investigate the usefulness of the combined use of conventional echo-Doppler and ultrasonic videodensitometry in the study of myocardial structure and function we found that these patients had a lower cyclic variation of mean gray level both for the septum and the posterior wall; furthermore, cyclic variation index values were found to be significantly and directly related to serum FT_4 and FT_3 concentrations and significantly but inversely related to serum TSH levels. The authors concluded that subclinical hypothyroidism is associated with changes in myocardial structure detectable at ultrasonic videodensitometry; these changes are quantitatively related to the loss of thyroid function and could represent an early sign of myocardial damage in hypothyroidism.

Nephropathy. There are complex relationships between the uremic status and the heart and some authors have hypothesized the existence of an uremic cardiomyopathy. Once again ultrasonic tissue characterization might be useful for the earlier detection of myocardial damage due to uremia. With regard to this, Di Bello et al.[53] investigated the behavior of videodensitometric parameters in dialysis patients, a complex pathophysiological model of pressure-volume overload, and compared them to those observed in patients with essential hypertension with a similar left ventricular mass. Compared with hypertensive subjects and with controls, hemodialysis patients showed a significantly lower cyclic variation of mean gray level both for the septum and the posterior wall. Abnormalities of the two-dimensional echocardiographic gray level distribution were present both in hemodialysis patients and in hypertensive patients, but seem unrelated to the degree of echocardiographic hypertrophy as such and probably represent an early stage of uremic cardiomyopathy.

Coronary artery disease

Many experimental works have been made in this field of research in an attempt to identify a possible application of the ultrasonic tissue characterization which could increase the usefulness of conventional echocardiography in the detection of myocardial infarction, ischemia or viability.

Myocardial infarction, hibernating myocardium and ischemia. At IBS analysis, Milunski et al.[54] found that ultrasonic tissue characterization promptly detects acute myocardial infarction, showing a dramatic reduction of the cyclic variation of IBS in the infarct area and that it might delineate the potential benefit of coronary reperfusion manifested by restoration of the cyclic variation of IBS in the presence of severe wall motion abnormalities. Using the same procedure, Wickline et al.[55] have demonstrated that myocardial infarcts showed an increase in IBS values and a loss of the cardiac cycle dependent variation in backscatter.

The induction of myocardial ischemia causes an increase in the absolute IBS value and a reduction of its cyclic variation; these findings were observed during experimentally induced myocardial ischemia in animal models and during stress tests in humans by both methods, videodensitometric[56] and IBS[57]. Furthermore, it was demonstrated that there was no correlation between the extent of wall motion abnormalities and the degree of blunting of the cyclic variation, although there was a temporal association between the onset of dyssynergy and the onset of alterations in textural indexes.

The detection of viable myocardium is another important field of research[54,58]; in fact, the viable myocardium, despite the reduction in wall motion, preserves the cyclic variation of the IBS signal. Thus, it is possible to differentiate it from myocardial infarction which shows a significant reduction in the cyclic variation of IBS and an increase in the absolute end-diastolic value of IBS. Reperfusion of a viable myocardium at angioplasty prevents the increase in the IBS signal, preserving its cyclic variation.

Other minor applications of ultrasonic tissue characterization are the detection of an intracardiac thrombus[59], myxoma[60], cardiac allograft rejection[61] and the evaluation of the effects of anabolic-androgenic steroids on weight-lifters' myocardium[62].

Comparison with nuclear magnetic resonance. Burden similarities can be found between ultrasonic tissue characterization and myocardial tagging of nuclear magnetic resonance; in fact both techniques are able to explore the intrinsic myocardial motion. Tagged magnetic resonance imaging of the heart is a valuable technique for the quantitative non-invasive assessment of regional myocardial contractile performance[63]. The spatial modulation of magnetization (SPAMM)[64] technique generates two orthogonal sets of parallel planes of magnetic saturation by a sequence of non-selective radiofrequency pulses. The myocardium appears as a spatially encoded pattern that moves with the tissue and can be analyzed to reconstruct myocardial motion. The position of the tags must be measured by means of a tag detection algorithm and myocardial motion computed using the displacement information from each tag. SPAMM-tagged magnetic resonance images correspond to a collection of spectral peaks in the Fourier domain. The inverse Fourier transform of one of these peaks is a complex image, the phase of which is linearly related to a directional component of the tissue displacement. A harmonic phase (HARP) image is the calculated phase of this complex image, which can be used to synthesize conventional tag lines and calculate the two-dimensional myocardial strain. Its use in clinical cardiology is related to the demonstration of its sensitivity to small changes in myocardial strain during pharmacological stress testing and to its ability to accurately index regional wall motion abnormalities. Some observations with myocardial tagging indicated its utility in the detection of early impairment of intrinsic myocardial contractility in hypertension[65], valvular pressure overload, myocardial infarction, myocardial viability and cardiomyopathies.

Conclusions

Further studies are needed to establish the cause of the abnormal echodensity and its real clinical and prognostic value, in the various pathological models. Feigenbaum[66] has recently expressed a favorable opinion about the possibility, with the increasing use of digital recordings, to "anticipate advances in the making of tissue diagnoses using echocardiography". The new, just commercially available, digitized technologies in echocardiography will allow a more standardized approach to ultrasonic tissue characterization (i.e. videodensitometry, IBS, Doppler tissue imaging and myocardial contrast echocardiography) and improved comparison of the results of different laboratories. Furthermore, they will permit a great number of observations and consequently a well-founded debate about the real clinical and prognostic impact of these new textural parameters.

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