Ultrasonic tissue characterization of the heart

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ULTRASONIC TISSUE CHARACTERIZATION OF THE HEART

ULTRASONIC TISSUE CHARACTERIZATION OF THE HEART

PROEFSCHRIFT

ter verkrijging van de graad van doctor
aan de Rijksuniversiteit Limburg te Maastricht,
op gezag van de Rector Magnificus, Prof. Dr. F.I.M. Bonke,
volgens het besluit van het College van Dekanen,
in het openbaar te verdedigen
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To claim that we understand something, we must understand the rudiments.

Aristotle

..... Dedicated to my father and to the memory of my mother.

T.T.

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Chapter 1

INTRODUCTION

Since the earliest work of Edler and Hertz¹ who recorded mitral valve motion with ultrasound, echocardiography has become well established as a useful tool in the evaluation of cardiovascular disease. The advent of two-dimensional echocardiography has further added to its applications.² Ultrasound, by definition, is sound with frequency greater than 20 thousand cycles per second, above the audible range. The suitability of ultrasound for medical diagnosis depends essentially on the characteristics of tissue interfaces which reflect ultrasound.

Ultrasound energy propagates in the form of a mechanical disturbance, or pressure wave, through a medium. The most common way these waveforms have been produced has been through the pulsing of Piezo electric crystal transducers. When the crystal is electronically pulsed, it oscillates mechanically in an expanding and contracting motion that produces a pressure wave. This wave then propagates into the object of interest (a patient or test object) where it interacts with the object to produce echoes. These echoes, or backscattered signal, are the result of the interactions between the waveform and the many acoustical characteristics of the object of interrogation and, therefore, contain the information that is needed to image the object as well as other information that may be related to the state of the tissue.

M-mode Echocardiography

When the ultrasonic energy returns as an echo and strikes the transducer a radiofrequency is created, which will be processed and displayed on an oscilloscope in time-motion or M-mode. This type of recording is best suited for the constantly moving heart structures.³ The principle of M-mode display is shown in Figure 1. The position of the echoes which originate from the different interfaces of the heart structures is continuously plotted against time yielding its motion pattern.

The M-mode echocardiogram is documented with a stripchart recorder

Figure 1: Schematic cross-section of the heart, parallel to the long axis of the left ventricle (LV), showing the structure which the sound beam passes when the transducer (T) is placed in a left parasternal acoustic position (third or fourth intercostal space) and is directed (4) at the left ventricular cavity (long axis plane). Ao: aorta; CW: chest wall; aML: anterior mitral leaflet; IVS: interventricular septum; pML: posterior mitral leaflet; LA: left atrium; PW: posterior wall; RV: right ventricle.

which provides a hard copy of the M-mode data along with the electrocardiogram, phonocardiogram, etc. (Figure 2).

2-Dimensional Echocardiography

Real time, two-dimensional echocardiographic visualization of cardiac anatomical features has now been available for diagnostic cardiology

Figure 2: M-mode scan from a normal person. The direction of the sound beam labelled 1 to 4 in Figure 1 corresponds to the area labelled 1 to 4 in this M-mode scan. ENa: amplitude of posterior wall endocardium; LSa: amplitude of left septal endocardium; LVIDd: diastolic left ventricular internal diameter. For abbreviations see Figure 1.

since the early seventies. The images obtained with a commercially available two-dimensional echocardiographic system are displayed on an oscilloscope and are stored on video tape for subsequent analysis. Utilizing a Polaroid camera, hard copies of stop frame images can be obtained (Figures 3-5).

Two-dimensional echocardiographic examinations are performed with the patient in the supine or left lateral decubitus position. The latter is preferable, because the heart shifts laterally from beneath the sternum. Examination of the left ventricle begins by placing the transducer in the left parasternal region (Figure 6), usually in the third or fourth left intercostal space. An identical acoustical window to that used for M-mode scanning of the heart is thus utilized. From this position, an image of the heart along its long- and short-axis can be obtained. A parasternal long-axis view of the left ventricle usually reveals portions of the aortic root, left atrium, mitral valve leaflets and the base of the left ventricle (Figure 3A). From this position, it is not possible to visualize the cardiac apex.

Short-axis views of the left ventricle are obtained by rotating the transducer clockwise, so that the plane of the ultrasonic beam is approximately perpendicular to the plane of the long-axis of the left

Figure 3: Transducer position, schematic diagram and stop frame of a parasternal (A) long- and (B) short-axis echocardiogram of the heart in a normal person. For details see text. A: anterior; Ca: caudal; Cr: cranial; L: left; MV: mitral valve; R: right; RVOT: right ventricular outflow tract; P: posterior. For abbreviations see Figure 1.

Figure 4: Diastolic and systolic parasternal long axis echocardiograms of a normal cardiac apex. The diagram shows the diastolic and systolic cardiac line.

Figure 5: Apical transducer position together with corresponding diagram and stop frame of two (2C), three (3C) and four (4C) chamber view. RA: right atrium; TV: transpid valve; RV: right ventricle; LV: left ventricle; LA: left atrium; MV: mitral valve; AO: Aorta.

Figure 6: Schematic diagram of the standard nomenclature of the various transducer positions on the chest wall.

ventricle. With the transducer perpendicular to the chest wall a short-axis image of the left ventricle is obtained at the level of the mitral valve (Figure 3B). From this position, the transducer is tilted upward to the base of the heart and downwards to the region of the papillary muscles. Cross sections of the cardiac apex can only be obtained by moving the transducer down the chest wall and placing it over, or in the immediate vicinity of the point of maximal apical impulse. Figure 4 shows parasternal long-axis echocardiograms of the cardiac apex in a normal person.

From the apical location, a cross-section is obtained which displays all four cardiac chambers (Figure 5). This four-chamber view is of particular value in patients with coronary artery disease, since it displays the interventricular septum to a large extent. A two and three-chamber view is obtained by rotating this transducer approximately 60 degrees

clockwise and counter-clockwise, respectively (Figure 5). This apical approach is of particular value in patients with acute and chronic myocardial infarction, as the apex of the left ventricle is often involved.^{4,5}

In summary, the tomographic sections obtained by two-dimensional echocardiography, display the cardiac anatomy,⁶ and provide excellent information about size, shape and wall dynamics of the entire ventricle.^{7,8}

ULTRASONIC TISSUE CHARACTERIZATION IN CARDIOLOGY

The standard clinical applications of echocardiography involve display of the smooth surfaces of the heart, such as the endocardium, epicardium and valve leaflets. A relatively new area in cardiac ultrasound is the attempt to evaluate myocardial tissue composition and/or structure based on the acoustic properties of the heart. The rationale for this area of study which is termed *ultrasonic tissue characterization* is that ultrasound interacts (is absorbed or reflected) differently with abnormal myocardium than with normal myocardium. Several approaches to the evaluation of tissue composition with ultrasound include the display of acoustically abnormal tissue using gray scale or color encoding, the measurement of classical acoustic variables (such as backscatter and attenuation) and computer-based analysis of ultrasound signals and images. Promising results have been obtained through the use of ultrasound tissue characterization methods to identify ischemia, acute and chronic myocardial infarction, cardiomyopathies, myocardial contusion and intracardiac masses. Certain structural abnormalities of the myocardium may be associated with alterations in the character of reflected ultrasound. For example, calcified valvular or annular tissue exhibits a bright, homogeneous appearance⁹; the ventricular septum in patients with hypertrophic cardiomyopathy displays an unusual pattern of echo amplitudes¹⁰; amyloid infiltrated myocardium has been described as "sparkling"¹¹, and scarred myocardium occasionally is recognized as an area of intense echoes.¹² These and other similar observations give impetus to the attempt to identify abnormal myocardial structure based upon the analysis of myocardial acoustic properties i.e. ultrasonic tissue characterization.¹³⁻¹⁵

In summary, ultrasonic tissue characterization can be defined as:

1. The assessment of certain physical attributes of a volume of tissue by evaluation of the characteristics of ultrasound that is propagated through or is reflected by the tissue.
2. The study of the relation of these attributes to potential disease processes in the tissue.

The field of ultrasonic tissue characterization has been applied to

abnormalities of eye,¹⁶ liver¹⁷ and breast.¹⁸ Evaluation of myocardial acoustic properties has been more difficult but several recent observations suggest that tissue characterization by ultrasound may soon be of routine clinical use. Before further discussing the specific approaches to ultrasound tissue characterization, a few definitions are in order:

Acoustic velocity (c) is related to the density (ρ) and compressibility (β) of the tissue through which ultrasound is propagating. The relationship can be expressed as:

$$c = (\rho\beta)^{-1/2}$$

Characteristic acoustic impedance (Z) is defined as the product of density and acoustic velocity:

$$Z = \rho c$$

Absorption coefficient (α_A) is an index of the amount of ultrasonic energy lost by conversion to other forms of energy, chiefly heat.

Scattering cross section per unit volume (α_S) is an index of the ultrasonic energy lost by being redirected (reflected or scattered) away from the direction of the propagating ultrasound beam, due to inhomogeneities in the tissue.

Attenuation coefficient (α_T) is an index of the total energy loss per unit distance as the ultrasound beam propagates through tissue. The attenuation coefficient is the sum of the absorption coefficient and scattering cross section per unit volume:

$$\alpha_T = \alpha_A + \alpha_S$$

Thus, the attenuation of ultrasound by myocardium is due both to absorption and scattering and can be represented as:

$$A(z) = A_0 e^{-\alpha_T z}$$

where $A(z)$ is the amplitude of a plane wave propagating in the z direction and α_T is the attenuation coefficient (including the effects of both absorption and scattering). Different workers have calculated the attenuation coefficient in various fashions.^{3,19} Similarly, ultrasound backscatter measurements have been derived in various ways.^{20,21} Suffice it to say that the various approaches to ultrasound tissue characterization have largely been developed based on the display or measurement of ultrasound attenuation or scattering in soft tissue such as the myocar-

dium. It is important to note that acoustic velocity, attenuation and scattering (and indices derived from them) are importantly affected by other variables such as the temperature of the specimen and the angle with which the ultrasound beam meets the muscle fiber. Regarding this last point, since materials such as heart muscle have well-defined and varying fiber directions, the velocity, scattering and attenuation may all be anisotropic (i.e. depend upon the direction of the fibers in relation to that of the ultrasonic beam).

A detailed description of the qualitative and quantitative methods used in cardiac tissue characterization is discussed in Chapter 6. Although a large number of methods have been developed to extract information on tissue structure from transmitted or reflected ultrasound, three broad categories exist:

I. Visual Methods

Peculiar regional patterns (texture) of ultrasound reflection have been commonly noted in hypertrophic cardiomyopathy²² and amyloidosis.¹¹

A variety of observations have been made utilizing qualitative or quantitative analysis of image displays. The strength of these methods lies in their relative simplicity and their dependence, in general, upon the use of standard or nearly standard echocardiographic imaging systems. The main disadvantage of display-based methods consists of their dependence on imaging system adjustments such as overall gain, time-gain-compensation, compression curves and post-processing gray scale transfer functions. Thus, since these imaging system adjustments can greatly alter image appearance, it is important to note the settings at the initial examination and use the same setting for serial or follow-up studies in the same individual. It should be noted that the use of color encoding of echocardiographic signals is an approach to visual tissue characterization which seeks to increase human perception of regional differences in echo reflectivity, through the use of different colors for differing echo amplitudes.

A color encoded echocardiogram is one in which variations in echo amplitude are displayed as variations in color intensity and hue, instead of variations in gray. The rationale for using color is based on the ability of the human visual system to distinguish a larger number of colors than of shades of gray.

II. Measurement of Acoustic Variables

A vast literature has been compiled on ultrasonic velocity, attenuation and scattering from tissues.^{23,24} Many of these measurements have been

performed *in vitro*. However, one must be aware of the influence of temperature and other variables on these acoustic parameters.²⁵ Furthermore, attempts to quantify ultrasound interactions with biological tissue are affected by several physical phenomena inherent in the ultrasound imaging process. A good example of these phenomena is the occurrence of phase cancellation or "speckle" in ultrasound imaging.^{26,27} Despite these caveats, it should be acknowledged that a growing number of observations suggest that *in vivo* measurements of attenuation and especially of backscatter, may be used to characterize various myocardial abnormalities. One example is that of the now well accepted alteration in tissue acoustic properties caused by acute myocardial infarction. The acoustic impedance,²⁸ attenuation²⁹ and backscatter³⁰ of ultrasound by myocardial tissue all change within hours of experimental coronary occlusion in the dog. Thus, measurements of acoustic variables appears to be a very promising method of tissue characterization.

III. *Digital Image/Signal Analysis*

A third fundamentally different approach to the ultrasound characterization of tissue composition consists neither of evaluation of image displays nor of measurement of the "classic" acoustic variables such as attenuation or backscatter. Instead, the rationale of digital signal/image analysis is that sufficient information is available in the "pattern" of ultrasound signals or images to differentiate different tissue types and different pathological condition of a given tissue type. Thus, this empirical approach has been referred to as "tissue signature", since the goal of signal and image analysis is the categorization of tissue abnormalities based on characteristic patterns of ultrasound reflection. A large literature exists using digital signal analysis in the evaluation of diseases of other organs such as eye¹⁶ and breast.¹⁸ A more modest experience has been garnered in cardiac tissue characterization using digital image or signal analysis, but initial results have been promising. Specifically, quantitative analysis of the 2D distribution of echo amplitudes (referred to as tissue texture) has been shown capable of identifying a variety of abnormalities including acute ischemic and non-ischemic injury in dogs,³⁰⁻³² chronic cardiomyopathies,³³ mitral valve prolapse,^{34,35} and infective endocarditis.³⁶ Analysis of the overall distribution of echo amplitudes has also proved useful in identifying the nature of intracardiac masses (thrombi) in humans.²²

SUMMARY AND FUTURE EXPECTATIONS

Promising data on ultrasonic tissue characterization have been obtained so far. Although much of the previous work on tissue characterization was empirical, recently some interesting and reproducible clinical results have been obtained. It is likely that tissue characterization may soon make a routine contribution to clinical diagnosis in cardiovascular diseases.

This thesis is a compilation of studies concerning the application of ultrasonic tissue characterization both in the experimental and clinical setting. Much data are available today concerning the application of ultrasonic tissue characterization methods in the detection of cardiovascular disease. Limited studies, however, have focussed on color encoded two-dimensional echocardiography and quantification of changes in differentiating between various disease states of the cardiac tissue.^{32,34-36} Color encoding has the advantage over conventional gray-scale imaging because the eye can distinguish considerably more colors than shades of gray. Differentiation of subtle differences of gray-level is not an easy task, even for the experienced echocardiographer. Quantification of mean pixel intensity of color encoded two-dimensional echocardiographic images adds more strength to the applicability of ultrasonic tissue characterization methods in the detection of cardiovascular disease.

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Chapter 2

IDENTIFICATION OF TEXTURAL DIFFERENCES OF THE MITRAL LEAFLETS IN SUBSETS OF PATIENTS WITH MITRAL VALVE PROLAPSE

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SUMMARY

We previously described a subset of patients with mitral valve prolapse and thickened mitral leaflets (MVP-TL) who had an increased incidence of severe mitral regurgitation. To assess whether textural differences exist between patients with MVP-TL and mitral regurgitation and those with nonthickened leaflets (MVP-NTL), 2-dimensional echocardiography was done on 10 normal subjects, 10 patients with MVP-NTL and 7 patients with MVP-TL and severe mitral regurgitation. The 2-dimensional echocardiographic image was digitized, and the mean pixel intensity of the mitral leaflet (PI-ML) and the posterior wall of the aorta (PI-Ao) was determined. To negate the effects of changes in gain settings, PI-ML was expressed as a ratio of PI-Ao.

The PI-ML in the normal group was 29.0 ± 4.0 and the ratio of the mean pixel intensity of mitral leaflet to that of the aortic root was 0.92 ± 0.03 . In the group MVP-NTL, the PI-ML was 29.5 ± 2.1 and the ratio of mean pixel intensity of mitral leaflet to that of the aortic root was 0.86 ± 0.02 . In the group MVP-TL, the PI-ML was 22.1 ± 4.8 and the ratio of mean pixel intensity of mitral leaflet to that of the aortic root

0.53 ± 0.09 . The PI-ML and the ratio of the mean pixel intensity of mitral leaflet to that of the aortic root were both significantly lower in the group MVP-TL when compared to MVP-NTL and the control group.

We conclude that the lower PI-ML in patients with MVP-TL is probably caused by more extensive myxomatous change of the mitral leaflets. Digital imaging enables differentiation of subsets of patients with MVP based on the differences in the mean pixel intensity.

Key Words: Mitral Valve Prolapse, Echocardiography, Digital Image Processing, Mitral Regurgitation.

Introduction

Mitral valve prolapse (MVP) is a common condition seen frequently in young adults.¹ Its prevalence is reported to range from 5 to 20% in the general population.¹⁻³ Although most patients with MVP have a benign clinical course, in some patients significant cardiovascular complications develop, such as cerebral embolic events, progressive mitral regurgitation and infective endocarditis.⁴ Some patients with MVP have abnormally thickened mitral leaflets on echocardiography and are reported to have a higher incidence of cardiovascular complications, including severe mitral regurgitation.⁵ The present study was undertaken to ascertain whether textural differences of the mitral leaflets in subsets of patients with MVP can be differentiated by digital image processing of 2-dimensional (2-D) echocardiograms.

Materials and methods

Between 1983 and 1986, MVP was diagnosed by 2-D echocardiography in 7 patients with clinical evidence of severe mitral regurgitation as defined by a loud holosystolic murmur and a third heart sound. This group consisted of 3 men and 4 women, mean age 65 years (range 40 to 82 years). Two-dimensional echocardiographic examination revealed MVP and thickened mitral leaflets in this subset of patients.

Ten patients (3 men, 7 women) mean age 30 years (range 20 to 48 years), had a midsystolic click and late systolic murmur on physical examination. On 2-D echocardiographic examination, MVP and nonthickened mitral leaflets were seen in this group.

A third group consisting of 10 healthy volunteers (5 men, 5 women), mean age 57 years (range 49 to 69 years), had normal findings on physical examination and 2-D echocardiography.

Prolapse of the mitral valve was diagnosed by 2-D echocardiograms only during sinus beats and was defined as protrusion of one or both

mitral leaflets beyond the plane of the mitral annulus into the left atrium during systole.⁶ One patient with leaflets thickened by MVP underwent angiography and subsequent mitral valve replacement for severe mitral regurgitation.

The leaflets were considered to be thickened when they were more than 5.1 mm in absolute thickness (by using a pair of calipers) or when the ratio of mitral thickness to posterior aortic wall root thickness was more than 1.4 in the parasternal long-axis view. The latter criterion was used to negate the effects of different gain settings used for different patients. The thickness of the mitral leaflets was measured at its thickest point in diastole (onset of QRS complex), whereas the thickness of the posterior wall of the aortic root was measured near its origin.

Data were analyzed using analysis of variance. P values <0.05 were considered significant.

Image acquisition system:

Selected series of videotaped images were digitized using a Diasonics Cardio Revue Center. The Cardio Revue Center has a JVC model VHS videocassette recorder and a Sony model video motion analyzer. The Cardio Revue Center consists of, among other elements, a Datacube model VG-120 video frame digitizer, a keyboard and a cathode ray tube display. The Datacube model VG-120 digitizes a full field of analog video data with a resolution of 320 horizontal by 240 vertical picture elements (pixels) by 64 gray levels.

Two-dimensional echocardiograms were standardized because ultrasound machines and videotape recorders vary in regard to video signal level. To negate the effects of these variations, a standard video signal was presented to the image digitizer using a video processing amplifier and an oscilloscope. Each digitized video frame was transmitted through a highspeed serial data link to a second computer for further processing.

Pixel intensity at any given location could be ascertained using a high-resolution light pen. The computer had the capability to average pixels at any given area of interest. The number of pixels averaged and mean pixel intensity of the areas studied could be displayed. When pixel averaging, we tried to avoid specular reflections from the leading and trailing edges of the valve. To negate the effects of gain settings, the ratio of the mean pixel intensity of the mitral valve to that of the posterior wall of the aortic root was determined. Mean pixel intensity of the mitral leaflet was measured from a diastolic frame obtained at the onset of the QRS on the electrocardiogram.

Table I: Summary of Findings

Group	No. of Pts	Mean Age (yrs)	Thickness (mm)			Mean Pixel Value	
			ML	Ao	ML/Ao	PI-ML	PI-ML/ PI-Ao
Normal	10	57±8	3.70 ±0.90	3.90 ±0.30	0.95 ±0.20	29.00 ± 4.10	0.92 ±0.30
MVP-NTL	10	32±6	3.90 ±1.30	4.00 ±1.10	0.98 ±0.10	29.50 ± 2.10	0.86 ±0.20
MVP-TL	7	65±9	7.00* ±1.70	4.00 ±0.90	1.80* ±0.40	22.12* ± 4.80	0.53* ±0.10

* $P < 0.05$ vs MVP-NTL and normal group (by analysis of variance).

Values are mean $\pm$ standard deviation.

Ao = aortic root (posterior wall); ML = mitral leaflet; ML/Ao = ratio of ML to Ao; MVP-NTL = mitral valve prolapse and nonthickened MLs; MVP-TL = MVP and thickened MLs; PI-ML = pixel intensity of ML; PI-ML/Pi-Ao = ratio of pixel intensity of ML to Ao.

Figure 1. Mean pixel intensity (PI) ($\pm$ standard deviation) of mitral leaflets in normal persons (N) and 2 subsets of patients with mitral valve prolapse. MVP-TL = MVP and thickened mitral leaflets; MVP-NTL = MVP and nonthickened leaflets; NS = not significant.

Figure 2. Ratio of pixel intensity (PI) of mitral leaflet and posterior aortic root wall ($\pm$ standard deviation) in normal subjects (N) and subsets of patients with mitral valve prolapse (MVP). NS = not significant.

Results

The data are summarized in Table I. Mean pixel intensity of the mitral leaflet in the normal group was 29.0 and the ratio of pixel intensity of the mitral leaflet to that of the aortic root was 0.92 (Fig. 1 and 2). In the group with MVP and nonthickened leaflets, mean pixel intensity of the mitral leaflet was 30.0 and the ratio of pixel intensity of mitral leaflet to that of the aortic root 0.86. In patients with MVP and thickened leaflets, mean pixel intensity of the mitral leaflet was 22.1 and the mean ratio of pixel intensity of mitral leaflet to that of the aortic root 0.53.

Statistical analysis revealed a significantly lower pixel intensity of mitral leaflet and the ratio of pixel intensity of mitral leaflet to that of the aortic root in the group with MVP and thickened leaflets as compared to those with MVP and nonthickened leaflet and normal control subjects ($p < 0.05$). However, no significant differences existed between those with MVP and nonthickened leaflets and normal control subjects (Fig. 1).

Discussion

Since MVP is a common condition with a benign prognosis in most patients, identification of a subset of patients with an increased incidence

of cardiovascular abnormalities is important. In a previous study, we showed that patients with MVP and thickened leaflets had a higher incidence of severe MR, tricuspid valve prolapse and aortic root abnormalities.⁵ Subsequent studies by Nishimura⁴ and Barletta⁷ and their coworkers show that patients with MVP and thickened mitral leaflets have a higher incidence of infective endocarditis, sudden death and cerebral thromboembolic events. In this study, we further attempted to characterize the thickened mitral leaflets in patients with MVP in a select group with MVP and severe mitral regurgitation by applying digital image processing techniques.

Our study shows that the mitral valves of patients with MVP and thickened leaflets have lower tissue density and significantly lower mean pixel values than those in normal subjects and those with MVP and nonthickened leaflets. To negate the effects of gain settings, the ratio of the mean pixel intensity of the mitral valve to that of the posterior wall of the aorta was determined. The ratio of pixel intensity of the mitral leaflet to that of the aortic root was also significantly lower in the group of patients with MVP and thickened leaflets.

Thickening of the mitral valve leaflets may occur in a variety of conditions including rheumatic mitral valve disease and MVP. We previously showed that the pixel intensity of rheumatic mitral valves is higher than that of normal mitral leaflets, probably because of increased fibrous tissue and calcification in rheumatic valves.⁸ In contrast to rheumatic valves, the thickened leaflets in patients with MVP and severe mitral regurgitation had a pixel intensity less than that of normal valves. This is probably because of extensive myxomatous degeneration with increased water content resulting in reduced tissue density of these valves. King et al⁹ observed that the density of valves excised at the time of mitral valve replacement for mitral regurgitation and severe MVP was lower than the density of normal valves. These data also indicate that severe myxomatous degeneration of the mitral valve is associated with decreased tissue density.

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Chapter 3

VALUE OF DIGITAL IMAGE PROCESSING OF TWO-DIMENSIONAL ECHOCARDIOGRAMS IN DIFFERENTIATING ACTIVE FROM CHRONIC VEGETATIONS OF INFECTIVE ENDOCARDITIS

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SUMMARY

The ability of a computer-based image digitizing system to differentiate "active" from "chronic" valvular vegetation was evaluated. Twenty-two patients with newly diagnosed infective endocarditis were studied prospectively by serial two-dimensional echocardiograms. Two comparable images (active and chronic) from the same patient taken several weeks apart (range 4-17 weeks) after initiation of therapy were obtained at end diastole with identical gain settings. The images were digitized, magnified, and displayed on a high-resolution color monitor. The mean pixel intensity of the valvular vegetation in the active stage was 20.6 ± 3.6 (mean $\pm$ SD), which increased to 34.4 ± 4.3 in the chronic stage ($p < 0.01$). Because there was some overlap of values, an additional standardization procedure was used in the last eight patients. In these eight patients, the mean pixel intensity of the vegetations in the active stage was 17.5 ± 1.1 , and it increased at 4 weeks to 25.0 ± 1.2 ($p < 0.05$) and further increased in the chronic stage to 33.6 ± 3.1 ($p < 0.05$). There was no overlap of values between the active and chronic stages. Three clinical states are of

particular interest: 1) The mean pixel intensity of the vegetation remained unchanged (23 vs. 24) in one patient who did not respond to antibiotic therapy 2) The mean pixel intensity of the vegetation fell from 35 to 24 when one patient developed reinfection of the same valve, and with treatment, mean pixel intensity once again increased to 34. 3) The mean pixel intensity of the vegetations in five patients with culture negative infective endocarditis was low (21.6 ± 1.9) and increased (33.6 ± 6.0 ; $p < 0.05$) during the chronic stage. The mean vegetation size was 0.73 ± 0.30 cm² in the active stage and decreased to 0.56 ± 0.24 cm² ($p < 0.05$) in the chronic stage; however, in six of 22 patients (27%), vegetation size did not decrease with clinical cure. We conclude that digital image processing of two-dimensional echocardiograms can differentiate active from chronic vegetations of infective endocarditis and is a promising new technique that has the potential of being a useful clinical tool in selected patients with infective endocarditis, for example, in those in whom there is a need to distinguish active from cured infective endocarditis.

Keywords: Infective Endocarditis, Two-dimensional echocardiography, Valvular disease, Ultrasonic tissue characterization.

Introduction

Infective endocarditis (IE) most often involves the cardiac valves and can be detected by M-mode and two-dimensional echocardiography; the sensitivity of M-mode and two-dimensional echocardiograph for the diagnosis of IE has ranged from 34% to 80%.¹⁻⁷ In the majority of patients, valvular vegetations (VEG) that are visualized on the initial echocardiography persist after successful antibiotic treatment of IE.⁷ A non-invasive method that could distinguish "active" from "chronic" VEG of IE is sometimes highly desirable, for example, when a clinical response to antibiotic therapy is inconclusive and the diagnosis of recurrent endocarditis is uncertain or when blood cultures remain negative.

The purpose of this study was to evaluate the ability of a computer-assisted image digitizing system to differentiate active from chronic valvular VEG of IE.

Patients and methods

Patient Selection

In this prospective study, 22 patients with a clinical diagnosis of IE (for

criteria and clinical details see below) with complete and good quality two-dimensional echocardiograms and with well-defined VEG on native valves were included. None of these patients had a previous history of IE. However, one patient from this group subsequently had recurrent endocarditis on the same valve (tricuspid valve) and had a second series of echocardiograms performed during the second admission. For purposes of the study, we have referred to VEG at the time of admission as "active" and those after bacteriological cure as "chronic." Bacteria were cultured from blood in 17 of the 22 patients (Table 1). *Staphylococcus aureus* was isolated in eight patients, *Staphylococcus epidermidis* in one patient, *Streptococcus viridans* in six patients, *Streptococcus pneumoniae* in one patient, and *Hemophilus influenza* in one patient. Five patients had negative blood cultures. The prodromal symptoms of lethargy and fever before admission ranged from 1 to 3 weeks.

There were 13 men and nine women with a mean age of 35.6 years (range, 21-69 years). The VEG were located on the mitral valve in eight patients, the tricuspid valve in ten patients, and the aortic valve in four patients.

In 17 patients with positive blood cultures, the diagnosis of IE was based on: general malaise, fever, new regurgitant cardiac murmur, three or more abnormal blood chemistries (elevated erythrocyte sedimentation rate, leucocytosis, circulating immune complexes, and anemia), and

Table 1: Clinical Features of Patients With Infective Endocarditis

Clinical feature	Active	Chronic
Chills	18	0
General malaise	22	0
Fever	22	0
Splenomegaly	6	1
Cardiac murmur	22	22
Anemia	21	1
Positive blood culture	17	0
Elevated ESR	22	0
Leucocytosis	22	0
CIC	8	0

Active, valvular vegetations observed at time of clinical admission; Chronic, valvular vegetations observed after bacteriological cure. ESR, Erythrocyte sedimentation rate. CIC, Circulating immune complexes.

positive blood cultures. In the five patients with negative blood cultures, the diagnosis of IE was based on general malaise, fever, a new regurgitant murmur, and three or more abnormal blood chemistries (elevated erythrocyte sedimentation rate, leucocytosis, circulating immune complexes, and anemia).

Echocardiographic Examination

Serial two-dimensional echocardiographic images were performed in each patient. A complete two-dimensional echocardiographic examination consisting of a parasternal long-axis view of the left ventricle, parasternal short-axis and parasternal long-axis view of the right ventricle, apical four-chamber view, and apical two-chamber view, with a 2.25-MHz transducer and an IREX Meridian echocardiographic machine (New Jersey) was performed and recorded on a standard VHS videocassette tape.

Two comparable images (which maximally visualized the valve leaflets) from the same patient, taken several weeks apart with a mean period of 9 weeks (range, 4-17 weeks) after initiation of therapy and from the same echocardiographic window and in the same phase of the cardiac cycle (end diastole), were digitized, magnified, and displayed on a high-resolution color monitor. The mean pixel intensity (MPI) of the VEG was obtained by painting the VEG with a light pen. Specular reflections from the leading and trailing edges of the VEG were excluded. The first study, which was considered for analysis (active phase), was performed on the day of admission to the hospital in 12 patients, 1 day after admission in three patients, 2 days after admission in six patients, and 3 days after admission in one patient. The study considered for analysis (chronic phase) was performed after a mean period of 8 weeks (range, 4-17 weeks) after hospitalization and initiation of antibiotic treatment. The cross-sectional areas of the VEG were measured digitally on the echocardiographic machine (IREX Meridian) at end diastole (onset of QRS on electrocardiogram) by two independent observers.

Standardization of Two-Dimensional Echocardiograms

To enable comparison of serial studies, standardization of two-dimensional echocardiograms was performed. This was done by recording the time-gain compensation settings and other controls on the echocardiographic machine at the time of the first study, by keeping these settings identical in subsequent studies in a particular patient, and by presenting a standard video signal to the image digitizer with a video processing amplifier and an oscilloscope.

Table 2. Summary of Clinical Data

Patient Number	Age (Yrs)	Bloodculture positive	Valve affected	Two-dimensional echocardiogram		Vegetation size (cm ²)		Mean pixel intensity	
				Active phase (days)	Chronic phase (weeks)	Active	Chronic	Active	Chronic
1	40	Yes	TV	ADM	4	1.02	0.53	24±3.2	31±5.2
2	24	No	AV	ADM	4	0.67	0.32	21±2.9	34±7.2
3	36	No	AV	ADM	7	0.48	0.42	24±3.4	40±3.7
4	69	Yes	MV	2 Days	4	0.40	0.28	23±4.6	32±3.7
5	21	Yes	MV	ADM	14	0.98	0.45	30±5.4	41±9.6
6	35	Yes	TV	ADM	13	0.80	0.61	25±6.2	36±2.5
7	34	Yes	MV	ADM	17	0.74	0.32	23±6.2	40±13.0
8	47	Yes	MV	ADM	6	0.30	0.33	25±7.7	34±7.8
9	25	Yes	MV	ADM	13	0.89	0.65	18±4.5	29±10.4
10	35	Yes	TV	2 Days	14	1.60	1.28	19±4.5	40±8.2
11	52	No	TV	ADM	8	0.77	0.79	21±3.8	33±6.1
12	32	No	MV	1 Day	4	0.63	0.51	23±3.2	24±4.1
13	38	Yes	TV	ADM	7	1.11	1.32	18±3.2	35±4.2
14	27	Yes	AV	ADM	7	0.54	0.55	20±4.1	38±3.9
15	22	Yes	TV	2 Days	5	0.52	0.58	18±2.7	31±3.8
16	26	Yes	TV	ADM	9	0.58	0.32	17±3.3	30±2.8
17	40	Yes	TV	1 Day	10	0.82	0.74	16±2.9	40±3.4
18	42	Yes	TV	2 Days	6	0.82	0.62	18±2.9	34±7.6
19	24	Yes	AV	3 Days	5	0.61	0.56	19±2.8	32±3.5
20	38	Yes	MV	2 Days	4	0.54	0.47	17±3.1	32±2.3
21	41	No	MV	1 Day	8	0.43	0.39	19±2.9	37±4.1
22	36	Yes	TV	2 Days	5	0.54	0.56	16±3.2	33±3.8
Mean (±SD)	35.6				8.04	0.73±0.30	0.55±0.24	20.6±3.5	34.4±4.3

TV, tricuspid valve; AV, aortic valve; MV, mitral valve; ADM, two-dimensional echocardiography performed on day of admission; 1, 2, 3, two-dimensional echocardiography performed 1, 2, 3 days, respectively, after admission.

Since MPI values in the first 14 patients did not reliably distinguish between active and chronic vegetations, we devised an additional procedure for standardizing gain settings for the initial echocardiographic study. The procedure consisted of turning the gain settings on the echocardiographic machine down to zero and then gradually increasing the gain until the valve leaflet first became visible. This gain setting was noted, and the echocardiogram was performed. The same gain settings were then used for subsequent studies in the same patient.

Data Analysis System

Selected series of videotaped images were digitized with a Diasonics Cardio Revue Center (Diasonics Inc., Milpitas California). The Cardio Revue Center has a JVC model BR-6400U VHS videocassette recorder and a Sony model SVM-1010 Video Motion Analyzer. The Cardio Revue Center consists of a magnetic videodisk, a Polaroid camera, and an 8080-based microcomputer incorporating a five-megabyte hard disk, a Datacube model VG-120 video frame digitizer and a graphic display controller, dual 5-inch floppy disk drives, a dot-matrix printer, a keyboard, and a cathode ray tube display. The Datacube model VG-120 digitizes a full field of analog video data (RS-170 E1A standard analog signal) with a resolution of 320 horizontal by 240 vertical picture elements (pixels) by 64 gray levels, and it downloads the binary result of on-board random access memory in real time while simultaneously converting the binary data in the random access memory back into analog video data viewable on a monitor. In addition, the microprocessor has read and write access to any single pixel contained in the on-board random access memory. Standardization was accomplished with a video processing amplifier and an oscilloscope.

Each digitized video frame was transmitted through a high-speed serial data link to a second computer for further processing. This computer, a Compupro model (Oakland Airport, California) 816/B, is an 8085/8088-based microcomputer incorporating 64 kbytes of random access memory, dual 8-inch 1.2-megabyte floppy disk drives, a Scion Microangelo color graphics subsystem, a red, green, and blue high-resolution video display monitor, and a high-resolution light pen. The digitized video frames were stored on floppy disks after transfer from the Cardio Revue Center and could be retrieved at any time thereafter for redisplay or processing. The Microangelo system stored digitized video images with a resolution of 480 horizontal by 512 vertical pixels.

The pixel intensity of any given location could be ascertained with a high-resolution light pen. The computer had the capability to average

pixels at any given area of interest such as valvular leaflets or the myocardium. The number of pixels averaged and the MPI (value) of the areas studied could be displayed. When averaging pixels, we tried to avoid specular reflections. Sixty-four colors indicated pixel intensity or pixel value; blue and green color represented low pixel values, while yellow and red color represented high pixel values.

The MPI of the VEG, pixel histograms showing the relative frequency of occurrence of pixels, and pixel maps showing a three-dimensional representation of the pixels in the VEG were obtained in all patients.

The VEG size was measured by digital analysis with an IREX Meridian echocardiographic machine at end diastole (onset of QRS complex) in the same view in which the image was digitized. To determine intraobserver and interobserver repeatability of measurement of VEG area, two independent observers measured VEG size, and one observer measured it on two occasions. To determine intraobserver and interobserver repeatability of measurement of MPI, two independent observers measured MPI, and one observer measured it on two occasions.

A paired Student's *t* test was used to evaluate significance of results. For repeated measures, analysis of variance was used (Table 3).

Results

The salient clinical features of the patients during the active and chronic phases are summarized in Table 1. One hundred and five two-dimensional echocardiograms examinations were performed (mean, 4.7/patient) at intervals ranging from time of admission to hospitalization and initiation of antibiotic therapy and to 17 weeks later. Digital imaging processing was performed on all echocardiograms. An unprocessed echocardiogram (black and white) of a patient with tricuspid valve VEG (patient 10, Table 2) is shown in Figure 1. The color picture, pixel histograms, and pixel maps of the VEG from the same patient are shown in Figures 2, 3, and 4, respectively.

The details of the specific valve that was affected, timing of MPI during the active and chronic phases, and the MPI are listed in Table 2. The MPIs of the VEG in the active and chronic stages were 20.6 ± 3.6 vs. 34.4 ± 4.3 (mean $\pm$ SD), $p < 0.01$ (Figure 5). The MPI of the last eight patients who were studied with a special standardization procedure (See "Materials and Methods") showed an increase from time of hospital admission to 4 weeks later (17.5 ± 1.1 vs. 25.0 ± 1.2 , $p < 0.05$) and a further increase to the chronic stage (25.0 ± 1.2 vs. 33.6 ± 3.1 , $p < 0.05$) (Figure 6, Table 3). There was no overlap of values between the active and chronic stages for those patients with standardized initial gain settings.

14W

6W

ADM

Figure 1

14W

6W

ADM

MPI = 40

MPI = 26

MPI = 19

Figure 2

One patient did not respond to antibiotic therapy for 4 weeks (patient 12) and required surgery. In this patient, the MPI remained consistently low (MPI, 23 vs. 24) throughout the 4 weeks after the initiation of therapy. Another patient (patient 13) had recurrent IE on the tricuspid valve. In this patient, the MPI of the VEG increased from 18 to 35 after treatment of the initial infection. After recurrence of infection, the MPI decreased to 24, and with cure of the reinfection, the MPI increased once again to 34 (Figure 7). The pixel map after recurrence of infection was characterized by green and yellow (representing low pixel intensity) superimposed on the preexisting red area (Figure 8). In the five patients (patients 2, 3, 11, 12, and 21; Table 2) with culture negative endocarditis, the MPI of the VEG on admission was low (21.6 ± 1.9). After therapy and clinical cure, the MPI increased to 33.6 ± 6.0 ($p < 0.05$); the pixel maps changed from predominantly blue and green (active stage) to red and yellow (chronic stage).

The mean VEG size was $0.73 \pm 0.30 \text{ cm}^2$ in active vs. $0.55 \pm 0.24 \text{ cm}^2$ in the chronic stage ($p < 0.05$). In six patients who were clinically cured of IE, the VEG size showed a slight increase in the chronic versus active stage ($0.66 \pm 0.3 \text{ cm}^2$ vs. $0.69 \pm 0.4 \text{ cm}^2$, $p = \text{NS}$); however, the MPI showed a significant increase in this period, which was consistent with clinical cure of the IE.

The intraobserver and interobserver repeatability of the measured VEG size revealed correlation coefficients of $r = 0.95$ and $r = 0.96$, respectively. The standard error of the estimate (SEE) for interobserver and intraobserver repeatability of measurement of VEG sizes was 0.07 cm^2 and 0.05 cm^2 , respectively.

The correlation coefficient for intraobserver repeatability of MPI in the active stage was $r = 0.95$ ($\text{SEE} = 1.2$, $p < 0.05$); the correlation coefficient for interobserver repeatability was $r = 0.89$ ($\text{SEE} = 1.8$, $p < 0.05$). The correlation coefficient for intraobserver repeatability of MPI in the chronic stage was $r = 0.92$ ($\text{SEE} = 1.9$, $p < 0.05$); the correlation coefficient for interobserver repeatability was $r = 0.83$ ($\text{SEE} = 2.7$, $p < 0.05$).

← **Figure 1:** Unprocessed two-dimensional echocardiographic image (black and white) of a valvular vegetation of patient referred to in Figure 2. Note that differences of gray level are not as apparent as in the color encoded image.

← **Figure 2:** Example of valvular vegetation (VEG) (in color) on the tricuspid valve (TV) showing the mean pixel intensity (MPI) at admission, at 6 weeks and at 14 weeks. With bacteriological cure, the mean pixel intensity of the VEG gradually increased from 19 to 40. A black and white image of the vegetation in the same patient is shown in Figure 1.

Discussion

The clinical usefulness of two-dimensional echocardiogram in the diagnosis of IE has already been established.^{3,5,8,9} Vegetations on the aortic and mitral valves were first described echocardiographically by Dillon et al in 1973.¹⁰ Although previous investigators had noted an increase in intensity of VEG after bacteriological cure, no attempt was made to keep the echocardiographic gain settings constant.^{11,12} Since changes in gain settings may produce striking changes in the intensity of VEG and because the above observation were subjective, no firm conclusions could be drawn from these studies.^{7,14-17} The 22 patients presented in this study constitute a consecutive series of patients with a clinical diagnosis of IE on native valves; they were selected by strict criteria and were followed on prospective basis. All recording variables on the echocardiographic machine were noted at the time of the initial study and were kept identical in follow-up studies.

In the first 14 patients, there was a partial overlap of data points between the active and chronic VEG probably due to the use of excessive gain setting (without standardization) at the time of the first echocardiogram. We therefore instituted a standardization procedure for setting the gain for the first echocardiogram machine down to zero, and then the gain was gradually increased until the valve leaflet first became visible in each patient. This gain setting was noted, and the echocardiogram was performed. The same gain settings were used for subsequent studies in the same patient. Eight patients were studied with this standardization technique. There was no overlap between the MPI in the active and chronic VEG in this group. Because we studied these eight patients weekly, we first observed a statistically significant increase in the MPI of the VEG 4 weeks after beginning therapy (Table 3).

A fresh VEG of IE consists of neutrophils, clumps of bacteria or fungi coated with erythrocytes, fibrin, and platelet thrombi.^{13,18,19} This mass is attached to the degenerated native valve tissue. After therapy and during the process of bacteriological cure, the composition of the VEG changes; that is, polymorphonuclear leukocytes invade the area, and eventually, the infective organism disappears. Thereafter, fibroblasts infiltrate the area, and the VEG undergo fibrosis, collagen deposition, hyalinization, and calcification. The remnant of the VEG is finally covered with a layer of endothelium. These pathological changes probably account for the increase in the pixel intensity that we observed in chronic VEG.

Figure 3: Representative example of pixel histograms obtained in the patient referred to in Figure 1 at the time of admission (ADM), at 6 weeks (6W) and at 14 weeks (14W). Note the relative preponderance of blue/green bars (representing low pixel values) on admission and the relative increase in red bars (representing high pixel values) at the end of 14 weeks.

ADM

6W

14W

Figure 4: Representative example of pixel maps obtained in the patient referred to in Figure 1 at the time of admission (ADM), at 6 weeks (6W) and at 14 weeks (14W). There is a preponderance of blue/green pixels (representing low pixel values) on admission, and the increase in red pixels (representing high pixel values) at the end of 14 weeks. Note, images at 6W and 14W were originally magnified x3.

Figure 5: Mean pixel intensity in the “active” and “chronic” stage of VEG in all patients ($N = 22$) with infective endocarditis. Mean values $\pm$ standard deviation are displayed. The increase was significant at the 0.01 level. $\circ$ --- $\bullet$, the mean pixel intensity in one patient who did not respond to antibiotic therapy and required surgery.

Figure 6: Plot of mean pixel intensity in the “active” and “chronic” stage and at 4 weeks following initiation of antibiotic therapy in 8 patients, who were studied using a new standardization technique. Mean values $\pm$ standard deviation are displayed. The increase was significant at the 0.05 level.

Table 3: Data From Standardized Echocardiographic Images

Patient	Mean Pixel Intensity				
	ADM	2 Weeks	3 Weeks	4 Weeks	Chronic
15	18	16	16	25	31
16	17	16	18	26	30
17	16	15	16	24	40
18	18	19	19	24	34
19	19	18	...	26	32
20	17	16	17	27	32
21	19	18	17	23	37
22	16	17	17	25	33
Mean±SD	17.5±1.1	16.9±1.2	17.1±0.9	25.0±1.2*	33.6±3.1†

Mean pixel intensity in eight patients studied with the standardization technique.

ADM, on admission; 2, 3, and 4 weeks after initiation of therapy; Chronic, after 4 weeks of initiation of therapy.

* $P<0.05$; 4 Weeks vs. Admission, 2, and 3 Weeks; and † $P<0.05$: Chronic vs. admission, 2, 3, and 4 Weeks by analysis of variance.

Figure 7: Plot of mean pixel intensity (MPI) in 1 patient with recurrent infective endocarditis (Rec. IE). Note that there is an increase in MPI in the chronic phase of the first episode of endocarditis. In the active phase of Rec. IE there is a striking decrease in MPI, which increases during the chronic phase of Rec. IE.

A **B** **C**

Figure 8: Pixel maps of vegetation in patients with recurrent infection. Panel A: "Active" stage (MPI, 18) Panel B: "Chronic" stage (MPI, 35); Panel C: After recurrence of infection (MPI, 24). Note, images A and B were originally magnified x2.

This study indicates that VEG of IE show a gradual increase in the MPI with clinical cure. The sequential acquisition of baseline pixel intensities, pixel maps, and pixel histograms of the VEG may likely be of clinical value. Several patients in this series illustrate the potential clinical value of this line of investigation. 1) One patient did not respond to antibiotic therapy and needed surgical treatment; the MPI remained persistently low. The pixel map showed a preponderance of green (low pixel intensity) during the entire period of antibiotic therapy. This suggests that failure of MPI to increase is a sign of persistent infection. 2) One patient in our series with recurrent endocarditis showed a reduced MPI in the active stage of recurrent infection compared with the value obtained during the chronic phase of the first infection. This patient showed a pixel map in the chronic stage (i.e., before recurrence) characterized by red and yellow (high pixel intensity); the pixel map of the VEG after recurrence of infection showed a green area (low pixel intensity) superimposed on a preexisting red area, which was suggestive of recurrent endocarditis. These findings suggest that recurrence of active IE changes the MPI and pixel map. 3) In patients with culture-negative IE, the MPI increased with therapy, indicating that the VEG was in active phase initially. Although three cultures were negative, these patients clinically had active endocarditis. These three clinical examples indicate that measurement of MPI, pixel histograms, and pixel maps have the potential of assessing antibiotic response in patients with IE, of diagnosing active VEG that are superimposed on healed VEG, and of diagnosing active VEG in culture-negative IE.

In this study, we also observed a reduction of the mean VEG size (Table 2) from the active to the chronic stage. However, in six of 22 (27%) patients, the VEG showed a slight increase compared with the active stage. Other investigators have noted either an increase in VEG size,^{10,11} a decrease in VEG size,²⁰ or no change in VEG size³ after therapy and bacteriological cure. Other reports have shown no apparent correlation between the clinical response and change in VEG size during treatment of IE.^{14,15,21} assessing the efficacy of antibiotic treatment.

In summary, we observed an increase in MPI of valvular VEG after clinical cure of IE. This is probably attributable to an increase in density of the VEG during the chronic phase. A significant increase in MPI first occurred 4 weeks after initiation of therapy. In the three clinical situations of uncontrolled infection, reinfection, and culture-negative IE, pixel averaging and pixel maps provided clinically useful information. The mean VEG size decreased in size with clinical cure in only 73% of patients. We believe digital image processing of two-dimensional echocardiograms of VEG is a promising new technique that could

provide additional diagnostic information of clinical value in selected patients with IE.

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Chapter 4

ULTRASONIC TISSUE CHARACTERIZATION OF ACUTE CANINE MYOCARDIAL INFARCTION.

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SUMMARY

This study assessed changes in left ventricular (LV) texture on 2-dimensional echocardiography (2DE) after experimental myocardial infarction (MI). In 13 dogs, the left anterior descending coronary artery (LAD) was occluded (OC) for 3 hours, followed by 1 hour of reperfusion (REP) and sacrifice. 2DE were performed pre-LAD OC, 3 hours post-OC and 1 hour after REP by placing a 5 MHz transducer on the chest wall. After sacrifice, triphenyl tetrazolium chloride (TTC) staining was performed on 1 cm. thick LV cross-sectional slices. Five dogs served as controls (shams). 2DE were digitized and in the region of LV asynergy (area of MI), and adjacent normal area, the mean pixel intensities ($\pm$ SD) were calculated.

There was no significant change in the mean pixel intensity in the lateral (22.8 ± 1.3 and 23.4 ± 1.8) and anteroseptal (23.2 ± 1.9) regions from 0 through 4 hours in sham-operated dogs. In dogs undergoing LAD occlusion, the mean pixel intensity from the pre- to post-occlusion

period: 1) Showed no significant change in the lateral (normal) area, 24.4 ± 2.7 vs 24.7 ± 2.9 ; 2) In the area of wall motion abnormality (area of myocardial infarction) the mean pixel intensity increased from 24.5 ± 2.7 to 33.7 ± 4.5 , $p < 0.01$. There was no significant change in the mean pixel intensity between the 3-hour post-occlusion and post-REP period in either the lateral (normal) or antero-septal areas of LV; and 3) The area of LV asynergy corresponded to the area of myocardial infarction on TTC stain.

Our study demonstrates that: 1) 2DE showed increased mean pixel intensity in the area of MI; reperfusion did not alter these changes; and 2) The area of myocardial infarction on TTC corresponded to the area of LV asynergy seen by 2DE.

Key Words: Echocardiography, Myocardial Infarction, Ultrasonic Tissue Characterization, Triphenyl Tetrazolium Chloride.

Introduction

Echocardiography has been used extensively for the evaluation of left ventricular function in patients with myocardial infarction. It is now well established that decreased regional wall motion occurs in the presence of ischemia and infarction¹⁻⁶. Other investigators have noted decreased wall thickening and wall thinning in ischemic and infarcted tissue^{5,7,8}.

Thus, these echocardiographic findings are not specific for infarction and cannot distinguish ischemic from infarcted tissue which limits to some extent their clinical utility. Digital image processing of 2D echocardiograms has been shown to differentiate between normal and healed myocardial infarction in patients⁹. The objective of the present study were to assess whether digital image processing of 2D echocardiograms would detect changes in mean pixel intensity in the setting of an acute myocardial infarction induced in dogs.

Methods

Animal Preparation:

Eighteen adult mongrel dogs (10 males, 8 females) weighing between 30-50 kilograms were used in this study. The study protocol was approved by the Animal Ethics Review Board of the University of Southern California School of Medicine, Los Angeles. The dogs were studied in agreement with the regulations stipulated by the Animal Ethics Review Board. They were given morphine sulphate 1 mg/kg i.m. and

sodium pentobarbital 30 mg/kg i.m. one-half hour prior to surgery. An intravenous line was started, ECG leads were placed and the trachea was intubated. Ventilation was controlled with a respirator receiving 100% inspired oxygen concentration. Supplemental morphine sulphate was given in small, incremental doses by intravenous injection up to a total dose of 5 mg/kg as needed. Two-dimensional echocardiograms were performed with the dogs lying in the right lateral position and by placing a 5 MHz transducer (focussed in narrow band) directly on the chest wall.

The chest was entered through an incision in the left 5th intercostal space, the pericardium incised and the beating heart exposed. Five dogs served as controls (sham operated dogs). In 13 dogs undergoing intervention, a bolus injection of lidocaine 1 mg/kg was given to all dogs prior to clamping the left anterior descending coronary (LAD) artery. The LAD was isolated approximately 1 cm from the ostium of the left main coronary artery, proximal to the first large diagonal branch. The LAD was occluded with a vascular clamp. Following this, the dogs were maintained on lidocaine 2-4 mg/kg with a constant drip through a venous catheter. Additional bolus injections of lidocaine (1 mg/kg) were given in the event an episode of ventricular tachycardia occurred in the anesthetized dog. At the end of 3 hours of LAD Occlusion, the vascular clamp was released and reperfusion permitted for a maximum period of 1 hour.

All dogs were sacrificed at the end of the study by injecting a 5-10 mg/kg bolus dose of sodium pentobarbital, while ventilation was concomitantly discontinued. The chest was then opened, and the hearts were removed for additional investigations as described below.

Echocardiographic Data:

Prior to any manipulation of the LAD, baseline parasternal short axis views at different levels of the left ventricle were obtained in all dogs by placing a 5 MHz transducer directly on the chest wall. The time-gain-compensation settings (TGC) and all other controls on the echocardiographic machine were kept identical in all studies in all dogs. Images were recorded on standard VHS video tapes.

In addition, two-dimensional echocardiograms (2DE) in the short axis view at various levels of the left ventricle were performed prior to chest incision, pre-LAD occlusion, 3 hours post-LAD occlusion and 1 hour after reperfusion. In sham operated dogs, serial 2DE was performed at 0 hours and at the end of 4 hours.

The 2DE videotaped images gated in the end-diastolic phase (onset of

QRS complex) of the cardiac cycle were digitized using a Diasonics Cardio Revue Center (CRC). The CRS has a JVC Model BR-6400U VHS video cassette recorder and a Sony Model SVM-1010 video motion analyzer. The CRC consists of a magnetic video disk, a Polaroid camera and a 8080-based microcomputer incorporating a 5-megabyte hard disk, a Datacube Model VG-120 digitizer and graphic display controller, dual 5-inch floppy disk drives, a dot-matrix printer, a keyboard and a cathode ray tube display. The Datacube Model VG-120 digitizes a full field of analog video data (RS-170 EIA Standard Analog Signal) with a resolution of 320 horizontal by 240 vertical picture elements (pixels) by 64 gray levels and downloads the binary result to on-board random access memory (RAM) in real time, which simultaneously converts the binary data in the RAM back into analog video data viewable on a CRT monitor. In addition, the microprocessor has read/write access to any single pixel contained in the on-board RAM.

We performed standardization of 2DE because ultrasound machines and video tape recorders vary in regard to video signal level⁹. To negate the effects of these variations on color encoding, a standard video signal is presented to the image digitizer. This is accomplished by using a video processing amplifier and an oscilloscope. Each digitized video frame was then transmitted through a high speed serial data link to a second computer for further processing. The second computer, a Compupro Model 816/B, is an 8085/8088-based microcomputer incorporating 64 kbytes of RAM, dual 8-inch, 1.2-megabyte floppy disk drives, a Scion Microangelo® color graphic subsystem, an RGB high resolution video display monitor and a high resolution light pen. The digitized video frames are stored on floppy disks after transfer from the CRC and can be retrieved at any time thereafter for display or processing. The Microangelo® color system stored digitized color video images with a resolution of 480 Horizontal by 512 Vertical pixels by 64 user-definable colors and simultaneously converts the video data contained in the RAM into analog video signals capable of driving a high resolution color monitor.

Sixty-four selected colors indicate pixel intensity (pixel value) with purple representing a pixel value of 0, and white representing a pixel value of 63. The pixel intensity at any given location could be ascertained by using a high resolution light pen. Two observers who were blinded to the results of histopathology, assessed and agreed on the area of wall motion abnormality which was subsequently digitized. The entire area of interest, i.e. the area of wall motion abnormality representing the area of infarction and a comparable area of adjacent normally moving

myocardium (control region), was demarcated and the mean pixel intensity (value) obtained. When recording pixel values, we tried to avoid specular reflections from the leading and trailing edges of the myocardium.

Triphenyl Tetrazolium Chloride Staining

The slices of dog heart were incubated in a freshly prepared solution of triphenyl tetrazolium chloride (TTC) for 20 minutes at 37°C to delineate infarcted tissue. After staining, the slices were fixed in formaldehyde solution and photographs of all specimens were obtained for documentation purposes. Normal tissue was stained brick-red, whereas necrotic zones remained unstained.

Results

Echocardiographic Findings:

The echocardiographic data are summarized in tables I and II. The number of pixels which were averaged ranged from 800-1500. In sham operated dogs, the mean pixel intensity in the lateral (22.8 ± 4.0 vs 23.1 ± 3.8) and antero-septal (23.2 ± 3.5 vs 22.6 ± 3.9) regions showed no significant changes from 0 to 4 hours (Table I).

In dogs undergoing LAD occlusion (Table II, Figures 1, 2A and 2B) there was a significant ($p < 0.01$) increase at 3 hours in the mean pixel intensity (33.7 ± 4.5) in the area of wall motion abnormality (area of infarction) compared to the same area during the pre-occlusion phase (25.4 ± 2.7). There was a significant difference in the mean pixel intensity at 3 hours post-LAD occlusion, between the antero-septal and the lateral (control) region 33.7 ± 4.5 vs 24.7 ± 2.9 , $p < 0.02$). The mean pixel intensity in the lateral region (control region) did not change significantly from baseline to 3-hours post-LAD-occlusion. No significant change was observed in the mean pixel intensity between 3-hours post-LAD occlusion and 1-hour post-reperfusion in either the infarcted (33.7 ± 4.5 vs 33.3 ± 4.4) or lateral (control) areas (24.7 ± 2.9 vs 24.3 ± 2.4).

Table 1: Echocardiographic data in sham dogs

Dog. #	LATERAL REGION		ANTEROSEPTAL REGION	
	Mean Pixel Intensity 0 Hrs	Mean Pixel Intensity 4 Hrs	Mean Pixel Intensity 0 Hrs	Mean Pixel Intensity 4 Hrs
1.	23	24	24	22
2.	21	21	22	22
3.	22	23	21	20
4.	24	23	23	24
5.	24	26	26	25
Mean±SD	22.8±1.3	23.4±1.8	23.2±1.9	22.6±1.9

Abbreviations: 0 Hrs = baseline values
 4 Hrs = At the end of 4 hours
 p=NS between 0 hours and 4 hours in lateral and anteroseptal regions

Figure 1: Plot of mean pixel intensity measured prior to LAD-occlusion, 3 hours post-LAD occlusion, and at 1 hour after reperfusion in the anteroseptal and lateral regions * p<0.02 compared to pre-occlusion and vs lateral region.

Table II: Echocardiographic data pre- and post-LAD occlusion

Dog #	LATERAL REGION			ANTEROSEPTAL REGION		
	Mean Pixel Intensity			Mean Pixel Intensity		
	(Pre-Occl)	(Post-Occl)	(Post-Rep)	(Pre-Occl)	(Post-Occl)	(Post-Rep)
1.	22	24	23	25	31	28
2.	24	24	24	23	28	33
3.	24	22	21	25	32	31
4.	26	29	26	29	38	39
5.	27	26	28	25	33	34
6.	31	31	23	30	45	44
7.	22	21	21	24	29	31
8.	20	21	27	28	34	34
9.	23.	24*	†	23	29*	†
10.	25	24	26	26	37	29
11.	25	26	27	24	33	32
12.	22	24	22	25	34	31
13.	24	25	24	23	35	34
Mean±SD	24±2.7	24.7±2.9	24.3±2.4	25.4±2.7	33.7±4.5	33.3±4.4

Abbreviations: Pre-Occl = pre-occlusion of the left anterior descending artery (LAD); Post-Occl = 3 hour post-LAD occlusion; Post-Rep = 1 hour post-reperfusion, † = this dog died approximately 2 hrs after LAD occlusion; * = post-Occl. values obtained after ± 2 hrs of LAD occlusion.

Post-Occl and post-Rep values in the *lateral* region were compared to pre-Occl values ($p=NS$). There was no significant difference between post-Occl and post-Rep values.

Post-Occl and post-Rep values in the *anteroseptal* region were compared to pre-Occl values ($p<0.01$). There was no significant difference between post-Occl and post-Rep values.

2A

2B

Figure 2: A representative example of 2D echocardiograms obtained in a dog prior to LAD occlusion and post-LAD occlusion. Note that the mean pixel intensity prior to LAD occlusion (2A) in the anteroseptal area is 27, whereas after LAD occlusion (2B) this has increased to 34.

Figure 3: Cross-sectional slice of dog heart after immersion in TTC stain. Note that the necrotic area (devoid of stain) corresponds to the area of LV asynergy. N=Necrotic Area

Triphenyl Tetrazolium Chloride Staining:

This revealed lack of staining in the anteroapical region of the left ventricle (area of infarction). The non-infarcted regions showed a brick-red color (Fig. 3) whereas the infarcted zone was devoid of stain.

Statistical Analysis:

The mean pixel intensity of the region of left ventricular asynergy (area of myocardial infarction) and adjacent normal area were compared and a Student's paired t-test (corrected for multiple comparisons by the Bonferroni correction) applied to evaluate significance of results. Probability-values of <0.05 were considered significant.

Discussion

Experimental studies have used different indices of ultrasonic tissue characterization for diagnosing myocardial infarction. These studies have focussed on attenuation^{10,11} backscatter¹²⁻¹⁴, amplitude variation of radiofrequency signal and frequency shift¹⁵, and color encoding of analog 2DE images¹⁶. Recent studies in patients have shown increased myocardial echo intensities or brightness in areas of chronic infarcts⁹ and in several other myocardial diseases¹⁷.

Although interesting results have been obtained, both attenuation and backscatter have certain limitations. For measuring or estimating attenuation, one has to make use of transmission methods, i.e. interposing the tissue specimens between separate transmitting and receiving crystals (transducers), which is not practical in the clinical setting. Recently, techniques have been proposed for measuring attenuation from the reflected signal¹⁸, but these techniques have not as yet been applied to the study of acute myocardial infarction. To measure backscatter, the raw radiofrequency signal has to be acquired and digitized using a special data acquisition system. Skorton et al¹⁴ and Haendschen et al¹⁹ studied acute myocardial infarction in closed chest dog experiments. The former group noticed changes in gray-level distribution in areas of necrosis in dogs with 2-day old infarcts; the latter group did not notice changes in gray-level distribution in the 3 hours post-LAD occlusion period but only after reperfusion of the ischemic areas.

The detection of acute myocardial infarction within a few hours after its onset by placing the transducer directly on the chest wall and utilizing video data for analysis of results has been previously described²⁰⁻²². Fraker et al used a visual analog and noted an increase in apparent brightness of myocardium quite early after acute canine myocardial infarction²¹. However, the measurement of mean pixel intensity and quantification of altered signal intensity to our knowledge, has not been previously described. In the present study, an approach using color encoding of 2DE to differentiate acutely infarcted myocardium from adjacent normal areas was evaluated. Since the human eye can distinguish only a limited number of gray levels, subtle variations in shades of gray may be missed. In contrast, the human eye can distinguish many more variations of the color spectrum. The use of color encoding, thus, offers a partial solution to this problem. Furthermore, equipment used in our laboratory has the ability to perform objective analysis of regional abnormalities of cardiac tissue by determining the mean pixel

intensities (values) in any given area of a digitized 2DE image.

In our experiments, ultrasound studies were performed prior to coronary artery occlusion, at 3 hours post-LAD occlusion and 1 hour after reperfusion. The area of wall motion abnormality on the 2DE image was assumed to be the area of infarction and the calculated mean pixel intensity of this area showed a significant increase with respect to pre-LAD occlusion values and values obtained from adjacent normally moving myocardium. These results are in contrast to results obtained by Haendschen et al¹⁹ who did a similar study on dogs and observed no change in mean pixel intensity at 3 hours of post-LAD occlusion, but a significant increase in mean pixel intensity was noted after reperfusion. A possible explanation for the disparity in results could be the difference in the equipment used for data acquisition.

We also utilized triphenyltetrazolium chloride (TTC) staining to confirm the location of the area of myocardial infarction which showed increased mean pixel intensity on the digitized 2DE images. TTC staining has been used extensively *in vitro* in delineating normal myocardium from areas of myocardial infarction. Several studies have shown that this technique is an accurate and economical method for detecting myocardial infarction (*in vitro*) as early as 3-6 hours after coronary artery occlusion, a time prior to the development of well defined histologic necrosis^{23,25}. Tetrazolium dyes form colored precipitate (TTC-red) in the presence of intact dehydrogenase enzyme systems. Areas of necrosis lack dehydrogenase activity and, therefore, fail to stain. Our results showed that areas of left ventricular myocardium which were devoid of stain (abnormal areas) corresponded to the area of increased pixel intensity on the digitized 2DE image.

In summary, our data demonstrate that analysis of digitized and color encoded 2DE performed by placement of the transducer on the chest wall, provide a useful and noninvasive method for detecting acute changes in mean pixel intensity caused by myocardial infarction. Additional studies are needed to see how soon after coronary artery occlusion the textural changes are first seen and to quantitate the volume of the infarcted tissue. Should this technique become applicable to man, the ability to diagnose an acute MI early could be important.

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Chapter 5

EVIDENCE OF DECREASED TISSUE DENSITY IN MITRAL VALVES WITH SEVERE MYXOMATOUS DEGENERATION AND INCREASED TISSUE DENSITY IN RHEUMATIC MITRAL VALVES: IN VITRO OBSERVATIONS

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SUMMARY

We have previously shown that patients with mitral valve prolapse and thickened mitral leaflets (MVP-TL) have an increased incidence of severe mitral regurgitation (MR). To assess whether digital image processing of 2D echocardiograms (2DE) can detect differences in tissue density between normal valves, MVP-TL and rheumatic mitral valves (RH-MV), we studied 10 normal mitral valves excised at autopsy, 10 myxomatous mitral valves and 5 surgically excised rheumatic mitral valves (RH-MV). The valves were examined by 2DE in a water tank with a 2.25 mHz transducer and all 2DE recording parameters were kept identical. The 2DE images were digitized. The thickness of the mitral valves and the mean pixel intensity (MPI) were determined in the three groups of specimens. The maximum thickness of the normal valves was significantly less than MVP-TL [0.37 ± 0.05 vs 0.63 ± 0.06 cm (mean $\pm$ SD), $p < 0.05$] and

RH-MV [$p.37 \pm 0.05$ vs 0.70 ± 0.04 cm, $p < 0.05$]. The MPI was significantly lower in MVP-TL group compared to normals [21.0 ± 1.9 vs 27.9 ± 1.5 , $p < 0.05$]. However, the MPI of RH-MV was significantly higher than the normal mitral valves [42.0 ± 3.4 vs 27.9 ± 1.52 , $p < 0.05$] and MVP-TL [42.0 ± 3.4 vs 21.0 ± 1.9 , $p < 0.05$].

The MPI, which reflects tissue density, was significantly lower in MVP-TL group probably because of severe myxomatous changes. In the RH-MV group, the MPI was significantly higher than controls and MVP-TL. Thus, digital image processing of echocardiograms enables further characterization of the thickened leaflets in MVP and RH-MV and provides a method for distinguishing thickened mitral leaflets due to myxomatous changes from that due to rheumatic mitral valve disease.

Key Words: Echocardiography, Mitral Valve Prolapse, Digital Image Processing.

INTRODUCTION

Mitral valve prolapse, which frequently affects young adults, in particular young women and girls, is a common abnormality, and its prevalence ranges between 5-20%.¹⁻⁴ Significant cardiac arrhythmias, progressive mitral regurgitation, infective endocarditis and neurologic impairment may occur in association with mitral valve prolapse.⁵ However, the incidence of these complications is small, thus, it is important to identify subgroups of patients that are at high-risk for developing complications.⁶

We and others have shown that patients with mitral valve prolapse and thickened mitral leaflets have an increased incidence of cardiovascular abnormalities.^{5,7} However, thickening of mitral valves also occurs in rheumatic mitral valves. Thickening of the mitral leaflets in mitral valve prolapse is due to severe myxomatous degeneration, whereas, in rheumatic mitral valve disease increased mitral valve thickness is the result of fibrosis and calcification of the valve. The present in-vitro study was undertaken to determine whether the thickened leaflets of patients with mitral valve prolapse and rheumatic mitral valve disease have textural characteristics which are different from each other and from those of normal valves.

MATERIALS AND METHODS

Control Population

Ten patients with a mean age of 47.0 years (range 22-78 years) who had no history of cardiovascular disease and had a noncardiovascular death

were selected as controls. The mitral valve was excised at autopsy and histology revealed a normal mitral valve.

Patient Population

Ten patients included in this study, had a mean age of 61.7 years, (range 47-78 years) and had clinical and echocardiographic evidence of mitral valve prolapse and severe mitral regurgitation on physical examination and noninvasive testing. The diagnosis was confirmed by cardiac catheterization with angiography and by histological examination of the excised valves at time of valve replacement for severe mitral regurgitation.

The valves were stained with hematoxylin and eosin, the Verhoff's Van Gieson stain for elastin and collagen and the colloidal iron stain for acid mucopolysaccharides. Patients who had all 3 of the following criteria were considered to have myxomatous degeneration of the mitral valve: (1) Disruption of the zona fibrosa layer; (2) Expansion of the zona spongiosa layer with acid mucopolysaccharides; and (3) > 50% of the valve involved with acid mucopolysaccharide deposition as shown by the colloidal iron stain.

Five patients with a mean age of 40 years (range 36-55 years) with rheumatic heart disease (mitral stenosis and mitral regurgitation) who had mitral valve replacement were studied. The diagnosis was confirmed by noninvasive tests, cardiac catheterization with angiography and histologic examination which showed thickening, fibrosis and calcification.

Echocardiographic Data

The thickness and mean pixel intensity of the mitral valves were studied in the *in-vitro* situation.

The valves or portions of the valvular leaflets were suspended horizontally (with Catgut) in a water bath at 32°C. The transducer was allowed to touch the surface of the water and images obtained a depth of 4 cm and recorded on standard VHS videocassette tapes. An IREX Meridian echo machine and a 2.25 MHz transducer was used to obtain echocardiographic images. The gain settings in the echo machine were kept constant for all the studies. The images were digitized (see data acquisition system) and the mean pixel intensity calculated. The maximum thickness of the mitral valve was measured (with calipers) from the echocardiographic image.

Image Acquisition System:

The 2DE videotaped images were digitized using a Diasonics Cardio

Revue Center (CRC). The CRC has a JVC model BR-6400U VHS videocassette recorder and a Sony model SVM-1010 video motion analyzer. The CRC consists of a magnetic video disk, a Polaroid camera and an 8080-based microcomputer incorporating a 5-megabyte hard disk, a Datacube model VG-120 video frame digitizer and a graphic display controller, dual 5-inch floppy disk drives, a dot-matrix printer, a keyboard and a cathode ray tube display. The Datacube model VG-120 digitizes a full field of analog video data (RS-170 EIA standard analog signal) with a resolution of 320 horizontal by 240 vertical picture elements (pixels) by 64 gray levels and downloads the binary result of on-board random access memory (RAM) in real time, while simultaneously converting the binary data in the RAM back into analog video data viewable on a CRT monitor. In addition, the microprocessor has read/write access to any single pixel contained in the on-board RAM.

We performed standardization of 2D echocardiograms because ultrasound machines and video tape recorders vary in regard to video signal level. To negate the effects of these variations, a standard video signal was presented to the image digitizer. It was accomplished using a video processing amplifier and an oscilloscope.

Each digitized video frame was transmitted through a high-speed serial data link to a second computer for further processing. This computer, a Compupro model 816/B, is an 8085/8088-based microcomputer incorporating 64 kbytes of RAM, dual 8-inch 1.2-megabyte floppy disk drives, a Scion Microangelo® color graphics subsystem, an RGB high-resolution video display monitor and a high-resolution light pen. The digitized video frames were stored on floppy disks after transfer from CRC and could be retrieved at any time thereafter for display or processing. The Microangelo system stored digitized video images with a resolution of 480 H by 512 V pixels.

The pixel intensity at any given location could be ascertained using a high-resolution light pen. The computer had the capability of average pixels at any given area of interest. The number of pixels averaged and the mean pixel value of the areas studied could be displayed. When pixel averaging, we avoided specular reflections from the leading and trailing edges of the valve. The MPI of the mitral valve was obtained by painting a 2D image (blow-up) of the mitral valve with the light pen. The entire mitral leaflet was taken into account for measurement of MPI.

Analysis of variance was used to evaluate significance of results. P-values of < 0.05 were considered significant.

Table I: Summary of findings

	No. of Pts	Mean Age (Yrs)	Maximum Thickness of MV (cm) $\pm$ SD*	Mean Pixel Intensity of MV $\pm$ SD
Normals	10	47.2	0.37 $\pm$ 0.05	27.9 $\pm$ 1.5
MVP-TL	10	61.7	0.63 $\pm$ 0.06	21.0 $\pm$ 1.9
RH-MV	5	40.0	0.70 $\pm$ 0.04	42.0 $\pm$ 3.4

MVP-TL=mitral valve prolapse with thickened mitral leaflets; RH-MV=rheumatic mitral valves; MV=Mitral Valve; *=mean values; pts=patients; Yrs=Years

RESULTS

The data are summarized in Table 1. The histological appearance of a prolapsing mitral valve is shown in figure 1. The maximum thickness (mean $\pm$ SD) of the excised mitral leaflets of control subjects was

Figure 1: Histological appearance of a prolapsing mitral valve in a 47 year old man. Note the area of myxomatous degeneration between arrows (magnification $\times 25$).

Figure 2: Maximum mitral valve (MV) thickness in cm in normal, myxomatous and rheumatic mitral valves. The difference was significant ($p < 0.05$).

0.37 ± 0.05 cm (range 0.30-0.45 cm). The maximum thickness (mean $\pm$ SD) of mitral leaflets in the myxomatous mitral valves was 0.63 ± 0.08 cm (range 0.53-0.70 cm) and was significantly higher than normals ($p < 0.05$). The maximum thickness of the rheumatic mitral valves was 0.70 ± 0.04 cm (range: 0.65-0.78 cm) and was significantly thicker than normals ($p < 0.05$) (Figure 2). The mean pixel intensity $\pm$ SD of the normal valves was 27.9 ± 1.5 (range 26.0-31.0 cm). The mean pixel intensity in the myxomatous mitral valve group was 21.0 ± 1.9 (range 18.0-23.0 cm) and was significantly lower than normals ($p < 0.05$); in the rheumatic mitral valve group, it was 42.0 ± 3.4 (range 38.0-47.0 cm) and was significantly higher than normals ($p < 0.05$). There was no overlap of values for mean pixel intensity in normal valves, myxomatous valves, and rheumatic mitral valves (Figure 3). There was a statistically significant difference ($p < 0.05$) between rheumatic, myxomatous and normals in mean pixel intensity and mitral valve thickness (Figure 3).

Figure 3: Mean pixel intensity in normal, myxomatous and rheumatic mitral valves (MV). The difference was significant ($p < 0.05$).

DISCUSSION

Previous investigators have shown that myxomatous degeneration of the mitral valve is a common cause of mitral valve prolapse.⁸ Although the majority of patients with mitral valve prolapse have a benign prognosis, serious complications may occur in some patients. We have shown that patients with mitral valve prolapse and thickened mitral leaflets have a high incidence of cardiovascular complications when compared to patients who have mitral valve prolapse and normal leaflet thickness.^{7,9} Nishimura and his colleagues noted that patients with mitral valve prolapse and thickened mitral leaflets have a much higher incidence of progressive mitral regurgitation, sudden death, cerebral embolic events and infective endocarditis as compared to patients with mitral valve prolapse and normal mitral leaflet thickness.⁵ Barletta and his coworkers observed that patients with multiple valve prolapse or diffuse valvular thickening have an increased incidence of neurological complications.¹⁰ Thus, patients with mitral valve prolapse and thickened mitral leaflets,

are more likely to have serious complications.

We have previously reported that the mitral valves (studied *in-vivo*) of patients with mitral valve prolapse and severe mitral regurgitation had textural abnormalities and lower mean pixel intensity in addition to thickening of the valve leaflets.⁹ Since values for mean pixel intensity *in-vivo* are influenced by a variety of factors, such as gain settings, the present *in-vitro* study was undertaken. This *in-vitro* study confirms that myxomatous mitral valves have a significantly lower mean pixel intensity than normal mitral valves. These data are consonant with findings of King and associates who noted that the density of myxomatous valves was less than that of normal valves.⁸ Extensive myxomatous degeneration of the mitral valve is the probable cause of the decreased tissue density that we noted. The mean pixel intensity of the rheumatic mitral valves was significantly higher ($p < 0.05$) than normal mitral valves and myxomatous mitral valves. This is probably attributable to the extensive fibrosis and calcification present in these valves.

In summary, patients with mitral valve prolapse and severe mitral regurgitation had mitral leaflet thickness which was greater than that of normal valves. The thickened mitral valves with MVP showed a mean pixel intensity which was less than that of normal valves. The reduction in mean pixel intensity of the valves in patients with mitral valve prolapse is probably attributable to reduced tissue density resulting from extensive myxomatous degeneration of the valve. The thickened rheumatic mitral valves in contrast to normals and prolapsing mitral valves, showed increased mean pixel intensity, which is probably due to extensive fibrosis and calcification of these valves.

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Chapter 6

ULTRASONIC TISSUE CHARACTERIZATION METHODS IN THE DIAGNOSIS OF CORONARY ARTERY DISEASE

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During the last decade, echocardiography has become increasingly popular and is currently one of the most widely used tools in cardiovascular diagnosis.¹⁻³ Ultrasound examination of the heart is a fairly simple process and, in comparison to other imaging techniques, the instrumentation is relatively inexpensive. With the development of new and far more expensive imaging techniques, such as computerized tomography and nuclear magnetic resonance, the comparison of cost-benefit ratios has become even more obvious.

The first in vivo ultrasound measurement for the purposes of tissue differentiation and characterization was made by Wild and Reid in 1952.⁴ They observed distinct quantifiable differences between A mode waveforms of normal breast tissue and malignant and benign breast lesions. Subsequent widespread use of these and other techniques now associated with ultrasonic tissue characterization was delayed for over 25 years. Even today, our knowledge of the propagation of ultrasound in tissue and the development of ultrasound technology, which serves as the basis for ultrasonic tissue characterization, is still a major research topic.

This chapter reviews the role of cardiac tissue characterization in the evaluation of coronary artery disease. To accomplish this task, the feasibility of ultrasonic tissue characterization is discussed, present techniques undergoing evaluation are reviewed, and future developments are assessed.

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Acoustic properties of tissue

Major factors that describe the propagation of ultrasound in tissue include the density, the elasticity, and the velocity of sound in tissue, the specific acoustical impedance (ratio of pressure to particle velocity at the point of interest), the absorption of sound by tissue, and the scattering of sound by tissue structure inhomogeneities.^{2,5,6} Absorption refers to energy loss as a result of a transformation to other energy forms. Various relaxation, viscous, and thermal mechanisms are responsible for absorption in tissue.

Absorption in tissues is primarily related to the protein content, although there is a component of absorption that is apparently related to other constituents.⁷ In general, it appears that acoustic absorption in tissue can be described by a relaxation process, which is defined by the molecular properties of the material.⁷⁻¹⁰

Since live human tissue is generally at a constant normal body temperature of 37°C, there is little need in present diagnostic regimens to consider effects of temperature on the acoustic properties of tissue. This is not to imply that such temperature effects should be ignored, since interesting possibilities utilizing temperature changes have been suggested for experimental studies with the aim of possible future medical applications.^{11,12}

Attenuation

When an ultrasonic pulse propagates in tissue, some of the energy is lost via absorption or the transformation to other energy forms, and some of the energy is scattered and/or redirected along alternative paths. Attenuation refers to energy loss in the propagation of sound as a result of both scattering and absorption. From in vitro and in vivo studies spanning over 60 years, it is clear that attenuation is an important parameter for the description of the propagation of ultrasound in tissue.¹³⁻¹⁶ Significant and medically useful correlations have already been demonstrated between attenuation and pathology, and it seems likely that the in vivo measurement of attenuation will play a major role in any ultrasonic tissue characterization scheme.^{11,14} The attenuation of sound in tissue is a frequency-dependent process. Thus, as the ultrasound pulse propagates, less energy is available to penetrate deep into the body. The penetration of ultrasound decreases as the frequency increases,¹⁷ and attenuation increases almost linearly with increasing frequency. A term used to express the amount of absorption and attenuation of ultrasound in tissue is the *half-value layer* or *half-power distance*.¹⁸ These terms refer to the distance that ultrasound will travel in a particular tissue before its

Table 1: Acoustic properties of several common materials: these represent averaged values from the literature which were measured in vitro by transmission methods

Material	Propagation velocity (m s^{-1})	Characteristic impedance ($10^6 \text{ kg m}^{-2} \text{ s}^{-1}$)	Attenuation at 1 MHz (dB cm^{-1})	Frequency dependence of attenuation
Water (20°C)	1480	1.48	0.0022	f^2
Aluminum (bulk)	6300	17.0	0.018	f
Air (at standard temp. and pression)	343	0.000415	12	f^2
Blood	1570	1.61	0.18	f^2
Brain	1541	1.58	0.85	$\sim f$
Fat	1450	1.38	0.63	$\sim f$
Human soft tissue (mean value)	1540	1.63	0.81	$\sim f$
Muscle	1585	1.70	1.3-3.3	$\sim f$
Skull bone	4080	7.80	13	f^2
Lung	650	0.26	41	$1/f$

Reproduced by permission from Jones and Leeman [1].

Figure 1: Regional variation of the slope of the attenuation (between 2 and 10 MHz) in normal dog myocardium. N, number of samples. Reproduced by permission from O'Donnell et al. [35]

energy is attenuated to half its original value. Table 1 shows the characteristic acoustic properties of several common materials. Attenuation at 1 MHz is lowest in water, relatively higher in blood and brain tissue, and highest in the lung.

Different investigators have used various methods of measuring or estimating attenuation.^{13,14,19-22} Such measurements are important to cardiac tissue characterization because changes in frequency dependence of attenuation in dog myocardium are correlated with regional creatine kinase (CK) depletion associated with myocardial infarction.^{23,24} The measurement of CK depletion is a standard biochemical marker of the infarction process.

Figures 1 and 2 show the slope of the ultrasonic attenuation for normal and for normal versus ischemic dog myocardial tissues determined at specified intervals following coronary occlusion. Although significant differences are noted between normal and ischemic tissues, the behavior of the attenuation slope suggests a complex biological process and a complex relationship between the tissue state and tissue acoustic properties.

Figure 2: Slope of the ultrasonic attenuation (between 2 and 10 MHz) for normal and ischemic dog myocardial tissue determined at specified intervals following coronary occlusion. N, number of samples. Reproduced from O'Donnel et al. [35]

Backscatter

When discussing ultrasonic echoes, it is important to distinguish between specular reflections and scattering. Specular reflections are produced by objects that are large with respect to the wavelength of sound and present a relatively smooth surface to the ultrasonic beam.^{2,3} Such objects reflect the ultrasonic energy in accordance with geometric acoustics (which is equivalent to Snell's Law in optics). With specular reflection, there exists a wide range of angles for which virtually none of the ultrasound energy returns to the transducer. The angles of reflection are predicted by the spatial orientation and shape of the reflecting object or interface. To date, echocardiography has been principally concerned with specular echoes originating from cardiac valves and walls.³ Specular reflectors are angle dependent, e.g., when imaging a cardiac structure, such as the posterior wall of the left ventricle, the endocardium will appear unusually bright during a certain phase of the cardiac cycle and less bright in another phase when surface angles change with respect to the incident beam.

When ultrasound waves impinge on surfaces or structures that are much smaller than their wavelength, the incident energy is scattered in all directions. Although scattered echoes are more difficult to record because of their lower amplitude, they are ever-present and, in contrast to specular reflections, are not angle dependent. In addition, scattering from tissue or parenchyma is important for visualizing objects essentially parallel to the ultrasonic beam. It is now generally acknowledged that, by studying the pattern of ultrasound echoes that are *backscattered* to the transducer, certain cellular structures or pathologically altered tissue states can be adequately differentiated.

Basic concepts of digital computation in echocardiography image digitization

The term *image processing* refers to the manipulation and analysis of pictorial data. There are three general methodologies that have been employed for image processing:

1. *Optical processing*, as the name implies, uses lenses and other, less familiar optical components to manipulate optical images. This method of processing has the advantage of being extremely fast.
2. *Analog processing* refers to the processing of image data by analog electronic circuitry; therefore, this method can only be applied to images that are in analog electronic form. The electronic processing of video images is the most common example of analog image processing.
3. *Digital image processing*. With the advances in computer technology, digital processing has become the dominant method for manipulating

and analyzing pictorial data. Digital processing employs general or specialpurpose digital computers, or computer peripherals, to process discrete images. Such an image is represented as a discrete matrix of picture elements (pixels), with each element having one of a finite number of values (gray levels or intensities).

The process of digitization can be described as dividing an image into a grid or matrix of discrete positions (pixels), each position located by a pair (x, y) of position coordinates. At each pixel position, an attribute of the image is measured (sampled) and assigned a discrete value (quantized). In ultrasound imaging, the attribute of interest is the local echo amplitude, which is displayed as a particular gray level (or a particular color). Thus, the digitized echocardiogram may be described as a matrix of pixels, each pixel having a discrete gray level or echo amplitude value.²⁵

There are two important questions to be addressed when designing the echocardiographic digitization process. First, how many pixels should the image be partitioned into and, second, how should one measure gray level or echo amplitude?

Digitizing an image into a fine matrix of several (small) pixels will tend to produce a more visually pleasing digital image, whereas fewer (larger) pixels cause a "blocky" or "pixelated" appearance.²⁵

The number of gray levels necessary for optimal visualization will probably be less than that necessary for quantitative analysis, in particular those aimed at ultrasound tissue characterization. Commonly available digitalbased echocardiographs quantize gray level (echo amplitude) into four bits (16 gray levels), six bits (64 gray levels), and eight bits (256 gray levels).

Image storage

The analog two-dimensional images have usually been stored on videotape cassettes. This method provides for relatively inexpensive storage of large numbers of images and for convenient review (slow speed, fast speed, real time, stop frame) of the recorded image data. The quality of stored data when played back on the videotape is adversely affected by two factors. The first is a loss in image quality that occurs when recording images on videotape, especially when viewing images in the stop-frame mode. The second is the relatively poor temporal stability of the playback mechanism in the videotape recorder.

An alternative approach is to store the image in digital form within the scanner itself. Commercially available echocardiographic instruments typically have enough internal digital memory to store one or two images. It is thus possible to transfer into digital memory a systolic and a diastolic

image from the same cardiac cycle. With this arrangement, however, it is not possible to acquire a large number of echocardiographic images in real time.

Image display and enhancement

Digitized echocardiographic images are displayed on a high-resolution video monitor, similar to an ordinary television set. A video monitor is a faster display device in the sense that horizontal lines are displayed (left to right) one after the other from the top to the bottom of the screen. Following scan conversion, each pixel is displayed on the video monitor with an intensity corresponding to the value of the pixel (i.e., echo amplitude).

Enhancement refers to the application of one or more processing steps to an image for purposes of improving the quality by reducing noise or, in some other fashion, making features of the image easier to appreciate.

Image enhancement can be further subdivided into image smoothing and contrast enhancement.

Image smoothing

The most straightforward approach to image smoothing is to replace each pixel in an echocardiographic image with the average of the value of the pixels in its immediate surrounding neighborhood. For instance, the area of interest might consist of the center pixel in question and its eight immediately adjacent and clearly defined neighbors. Thus, each pixel would be replaced by the average of nine pixels.

Contrast enhancement

The rationale behind contrast enhancement is to make some image features, such as endocardial or epicardial borders, more visually apparent. The simplest way to achieve this is to perform background subtraction, i.e., subtracting a fixed gray level from the value of all pixels in the image. This *background* gray level may be selected arbitrarily or may correspond to the average gray level of some region of the image. If this region is selected within the cavity, background subtraction will have the effect of improving contrast between myocardium and cavity.

Edge detection

Edge detection is the process of identifying borders or edges of interest in an image; this enables determination of all pixels encompassed by an edge. The converse is also possible. Several edge-detection algorithms have been devised and applied to both medical and other types of images,

the two most important being parallel and sequential edge-detection algorithms. As the names imply, parallel edge detection attempts to find complete object borders simultaneously, while sequential techniques search for one point on the border at a time. An image edge can be defined as a transition in gray level. Two important factors that complicate edge detection are the presence of image noise and gray level transitions that do not correspond to desired borders.

COMMONLY USED GEOMETRIC TRANSFORMATIONS IN ANALYZING TWO-DIMENSIONAL IMAGES

Image zoom

The simplest geometric transformation is *image zoom*, in which the image (or portion of the image) is magnified, usually by a factor that is a multiple of 2. The most straightforward procedure for performing image zoom by a factor N is referred to as *pixel replication* and consists of replacing each pixel with an "X" block of pixels of the same gray level. A disadvantage of this system is that it tends to produce "blocky" images.

An alternative procedure employs interpolation to produce a more pleasing image. Image interpolation is the process of calculating a gray level value for a point whose gray level is not defined from the gray level values of the neighboring pixels.

Pixel histogram

This gives the relative frequency of occurrence of gray levels (pixels) in a particular area being studied. The area can be delineated with a high-resolution light pen by painting the desired area into the image on the monitor screen.

Pixel map

This gives a three-dimensional reproduction of the number of pixels in a desired area on the picture screen. The area of interest is painted into the picture or demarcated by a line by using a high-resolution light pen.

Pixel averaging

The entire area of interest (several pixels) on the picture screen can be included for pixel averaging by demarcating it with a high-resolution light pen.

Image acquisition/processing system

We now describe the methods that we use at the Los Angeles County-University of Southern California Medical Center for color encoding and signal processing of two-dimensional echocardiograms.

Videotaped images are digitized using a Diasonics Cardio Revue Center (CRC). The CRC has a JVC model BR-6400U VHS videocassette recorder and a Sony model SVM-1010 video motion analyzer. The CRC consists of a magnetic video disk, a Polaroid camera, and an 8080-based microcomputer incorporating a 5-megabyte hard disk, a Datacube model VG-120 digitizer and graphic display controller, dual 5-inch floppy disk drives, a dot matrix printer, a keyboard, and a cathode ray tube (CRT) display. The Datacube model VG-120 digitizes a full field of analog video data (RS-170 EIA standard analog signal) with a resolution of 320 horizontal (H) by 240 vertical (V) picture elements (pixels) by 64 gray levels, and downloads the binary result to on-board random access memory (RAM) in real time, which simultaneously converts the binary data in the RAM back into analog video data viewable on a CRT monitor. In addition, the microprocessor has read/write access to any single pixel contained in the on-board RAM.

The two-dimensional echoes have to be standardized because ultrasound machines and videotape recorders vary in regard to video signal level. To negate the effects of these variations on color encoding, a standard video signal is presented to the image digitizer. This is accomplished by using a video-processing amplifier and an oscilloscope. Each digitized video frame is then transmitted through a high-speed serial data link to a second computer for further processing. The second computer, a Compupro model 816/B, is an 8085/8088-based microcomputer incorporating 64 kilobytes of RAM, dual 8-inch 1.2-megabyte floppy disk drives, a Scion Microangelo color graphics subsystem, an RGB high-resolution video display monitor, and a high-resolution light pen. The digitized video frames are stored on floppy disks after transfer from the CRC and can be retrieved at any time thereafter for display or processing. The Microangelo color system stores digitized color video images with a resolution of 480 H by 512 V pixels by 64 user-definable colors and simultaneously converts the video data contained in the RAM into analog video signals capable of driving a high-resolution color monitor.

Three to five gated video frames from each patient can be averaged to produce a smooth image. Pixel intensity (pixel value) is indicated by 64 selected colors. The pixel intensity at any given location can be ascertained by using a high-resolution light pen. The entire area of

interest (several pixels) is demarcated and the mean pixel value obtained. When recording pixel values, we try to avoid specular reflections from the leading and trailing edges of the structure being imaged.

Ultrasonic tissue characterization

As indicated earlier, ultrasonic tissue characterization encompasses a wide variety of techniques and methods that seek to extract and separate from an interrogating ultrasonic wave the medically significant features of ultrasound interactions and to display ultrasonic tissue signatures appropriate for a differential diagnosis or some other medical purpose.

To be useful and significant, such signatures or measures must be related to tissue pathology or physiology. Tissue characterization is broad in scope and, in certain cases, can include aspects of qualitative and quantitative imaging. At its simplest, tissue characterization involves merely a redisplay or recombination of ultrasound information in a way that is more pathology specific.

CARDIAC TISSUE CHARACTERIZATION

Importance of standardization

The influence of changes in gain setting on echo intensity must be borne in mind when analyzing echocardiograms. An unusually high gain setting may result in an artifactual increase in echo intensity, especially in anteriorly situated structures such as the interventricular septum. This phenomenon is unlikely to be important when comparing structures at the same depth such as different areas of the posterior wall of the left ventricle in the parasternal long-axis view. It should be emphasized that, in order to compare serial studies in the same patient, the same gain settings have to be used for all of the studies. This can be achieved by noting the gain settings used for the first study and using the same settings for subsequent studies.

Problems with cardiac tissue characterization

Cardiac tissue characterization can be achieved with the use of specially modified equipment in order to extract and analyze echo signals that have not been subjected to the gross distortions common in commercial M- and B-scan equipment. Even given that such technical problems are resolved, there are some difficulties associated with the heart itself that demand a more careful design of a tissue characterization investigation than is required for most other organs in the body. These are briefly reviewed here.

Motion

The heart, by its very nature, is constantly in rapid motion. This makes it difficult (and with standard methods, impossible) to track a particular tissue segment to ensure that echoes from that region only are being analyzed. The difficulty may be overcome to some extent, in most patients, by simultaneously monitoring the electrocardiogram, for example, and ensuring that echoes acquired only during a particular phase of the cardiac cycle are analyzed. Such a procedure clearly simplifies the comparison of results between different patients, observers, and even from the same patient, if the time course of some condition is being investigated.

Cycling

Even if a specified segment of cardiac tissue could be tracked throughout the cardiac cycle, it is highly unlikely (as experiments with canine hearts confirm) that its measurable acoustic properties and tissue signatures would remain constant throughout the contraction and relaxation phases of the muscle.^{17,26} Here again, utilizing the electrocardiogram as a "clock" in order to analyze data from only known phases of the cardiac cycle would be advantageous. A direct consequence of this approach is to compare results throughout the cardiac cycle in order to follow the changes in measured properties. Significance is then attached also to the time variation of the tissue characterization indices rather than only to their absolute value at a particular cardiac phase. Necrotic or ischemic regions would be expected to show considerably less time variation than normal regions — due to lack of thickening! It is also very difficult, in such a dynamic tissue characterization approach, to distinguish between the significant cycling and the less-important motion effects. In practice, it may be more convenient to compare results in two phases only (e.g., end diastole and end systole) rather than mapping the full-time course throughout the cardiac cycle. Olshansky et al.²⁶ have studied the variation of left ventricular myocardial gray level on two-dimensional echocardiograms as a result of cardiac contraction. These investigators found a significant decrease in gray level from parasternal shortaxis images digitized at end diastole (175 ± 58.0) to end systole (59 ± 2.5 , $p < 0.005$). These cyclic changes were observed in the left ventricular posterior wall; findings in the ventricular septum were less consistent.

Acoustic window

The human heart may be ultrasonically viewed *in vivo* only through a limited number of relatively small acoustic windows. This restricts the

full range of information that can be acquired for tissue characterization and may well exclude some cardiac regions from such investigations.

Cardiac size

Some tissue characterization methods, which are of potential value in cardiac studies such as attenuation estimation from backscattered echoes, require fairly long tissue segments for implementation. Such techniques, developed for relatively large organs such as the liver, need considerable refinement before they can be implemented on the short data sets obtainable from the myocardium.

QUALITATIVE METHODS USED IN CARDIAC TISSUE CHARACTERIZATION

Visual methods

Textural abnormalities on visual inspection of echocardiograms have been described in a variety of disease states in humans. A characteristic granular appearance to the interventricular septum has been described in hypertrophic obstructive cardiomyopathy (HOCM).²⁸ Highly refractile echoes (HREs) have been noted in other infiltrative and noninfiltrative cardiac disease.²⁸⁻³⁰ Myocardial scar tissue produces a dense echo pattern.³¹ However, the process of directly imaging acoustically abnormal tissue is somewhat unpredictable, due to the several image manipulations routinely performed during the echocardiographic image acquisition process. Such instrument controls as gain and time-gain compensation substantially alter the image appearance masking or mimicking "diagnostic" changes in the reflective properties of the tissue.³² Further, the variability of chest wall and other tissue intervening between the transducer and the region of interest will often alter the echocardiographic appearance of even normal myocardium. Thus, the appearance of the myocardium on standard echocardiograms may not always be a reliable indicator of abnormal cardiac tissue structure.

M-mode echocardiograms

Some investigators have used M-mode echocardiograms to study myocardial scar tissue characteristics. Rasmussen et al.³¹ and others²⁹ noted increased signal intensity in the interventricular septum several weeks after anteroseptal myocardial infarction. They also demonstrated a high degree of correlation between the presence of high-intensity echoes and the presence or absence of scar at surgery and at autopsy.

Two-dimensional echocardiography

Several studies indicate that cardiac tissue changes can be detected by two-dimensional echocardiography in certain disease states and in patients with acute myocardial infarction.^{27,33-38} Two-dimensional echocardiographic examination has revealed highly refractile HREs in infiltrative and noninfiltrative disorders of the heart.^{30,31} Rogers et al.⁴⁰ showed that useful information about the ultrasonic reflectivity of atherosclerotic coronary artery lesions can be extracted from two-dimensional echo-imaging systems by digital processing of the two-dimensional echocardiographic images.

Color encoding of the analog images

Color processing can increase the dynamic range of echo information because the eye can distinguish colors more easily than shades of gray. While color encoding has been accomplished with M mode echocardiography, two-dimensional echocardiograms allow a spatial integration difficult to achieve with M-mode echocardiography.

Parisi et al.⁴¹ produced acute myocardial infarction in 26 dogs by ligation of the left anterior descending artery. Two-dimensional echocardiograms were performed through the chest wall before and serially after coronary ligation. The dogs were then killed in four groups at intervals from 24 h to 8 weeks. Each two dimensional echo was processed through a video quantizer, which encoded echo amplitudes progressively into eight regions of color. The myocardium was graded with respect to color composition in regions that showed any abnormally contracting segment (ACS). The ACS exhibited a progressive increase in echo intensity that became maximal 6-8 weeks following coronary ligation. However, in ten of 13 dogs, myocardial echo intensity increased to some degree within 48 h after coronary ligation.

Color encoding digitized echocardiograms

Recent advancement in computer sciences have made digital image processing of two-dimensional echocardiograms possible. In our laboratory, the analog two-dimensional image is digitized by one computer and then the digitized data are transmitted to a second computer for color encoding (see *Image acquisition/processing system*). Each pixel is assigned a value from 0 to 63, corresponding to 64 colors. The area of interest can be demarcated by a light pen and can subsequently be analyzed by determining the mean pixel intensity, kurtosis, and skewness. Data generated in our own laboratory indicate that scar tissue resulting from old myocardial infarction can be detected

Figure 3: Parasternal long-axis view of patient with inferior myocardial infarction (*chronic stage*) and adjacent normal myocardium. Note the increase in mean pixel intensity at the site of infarction. Reproduced by permission from Chandraratna et al.⁴²

by color encoding and signal averaging of two-dimensional echocardiograms.⁴² The processed image showed clear demarcation of the area of infarction as shown by a color difference in the area of wall motion abnormality (area of infarction) as compared with adjacent normally moving muscle (figure 3). This subjective impression of a color change in the area of infarction was confirmed by objective determination of pixel intensity in the infarcted area and comparing it with adjacent muscle. Areas of infarction in the “chronic” phase exhibited higher pixel intensities than did adjacent normal muscle (figure 4).

QUANTITATIVE METHODS USED IN CARDIAC TISSUE CHARACTERIZATION

Analysis of video image

Several studies⁴²⁻⁴⁴ have used digital image processing of echocardiograms in differentiating cardiac structure and for quantitative analysis of cardiac abnormalities in man. The video image was digitized and each gray level was assigned a finite pixel value.

Figure 4: Pixel intensity in patients with new and old myocardial infarction; comparison of normal with infarcted myocardium. Reproduced by permission from Chandraratna et al [42]

In other studies related to (experimental) acute myocardial infarction in dogs,⁴⁵ the mean pixel intensity of the area with wall motion abnormality (area of infarction) was compared with an adjacent normal area. Statistically significant differences in the mean pixel intensity were noted between the region with wall motion abnormality and adjacent normally moving myocardium. Skorton and co-workers⁴⁶ analyzed two-dimensional echo images of dogs (closed-chest experiment) prior to and 2 days after occluding the circumflex artery. These investigators observed quantitative changes as depicted by a significant decrease in kurtosis (i.e., peakedness of gray-level distribution) in the infarcted region in comparison to adjacent normal areas. The distribution of gray level present in each region of interest was displayed as a gray-level histogram, showing the frequency of occurrence of each gray level within that region. Before and 2 days after coronary artery occlusion, 256 gray-level histograms were generated. Average gray level increased significantly from preocclusion to postocclusion images in the infarcted regions, but not in the control regions. Average gray level was calculated for each region of interest, and the shape of the gray-level distribution was analyzed by calculation of skewness and kurtosis, and by qualitative features of shape of gray-level histograms. Skewness measured the symmetry and kurtosis measured the peakedness of gray-level distribution. A decrease in kurtosis indicates flattening and broadening of the gray-level distribution, whereas reduced skewness values are seen in less-skewed histograms.

Another group has investigated the pattern of echoes from within the myocardium in order to arrive at a tissue characterization index. Joynt et al.⁴⁷ have analyzed the distribution of echo amplitudes obtained from normal human subjects and patients with myocardial infarction. Although the number of subjects was very small, these authors felt that the analysis provided a differentiation between normal and affected myocardium.

Analysis of radiofrequency signal

The radiofrequency (RF) signal, produced by the transducer when it transforms the ultrasonic echoes into an electrical waveform, must be subjected to simple processing such as envelope detection in order to produce an image on the video screen. More extensive processing and filtering are used to produce a visually pleasing image. This process of manipulation of the RF signal results in a loss of phase information and, therefore, a loss of much information regarding tissue signature. Thus, for purposes of tissue characterization, analysis of the RF signal prior to

processing is important for many applications. To utilize the RF signals effectively, the echocardiographic machine must be specially modified and interfaced to an appropriate data acquisition system that digitizes and stores the RF signals.⁴⁸ Digitization rates of 20-30 MHz are typical. Very fast memory, 1-2 megabytes in size, is required for recording the digitized data; larger but slower memory is required for data storage and analysis.

An example of the application of statistical signal analysis to RF data can be found in the work of Joynt and co-workers,^{36,49} who digitized portions of single A lines (corresponding to single lines from two-dimensional echocardiograms) and analyzed the amplitude distributions of the RF signal from a selected region of myocardium. This relatively simple evaluation of the distribution of echo amplitudes in a local region allowed the investigators to differentiate cardiac thrombus from artifact. Using a somewhat similar approach, Dines and co-workers⁵⁰ were able to identify acutely infarcted myocardium based on a set of statistical descriptors of the RF waveforms; a further statistical analysis of these RF signals also allowed some estimation of the relative scatterer spacing within the myocardium. It is also of note that several groups have shown that analysis of RF signal patterns is an efficacious approach to tissue characterization in other organs^{13,51-53} (e.g., liver and eye), adding credence to the use of these techniques in the myocardium.

Although the use of RF data is attractive and has many advantages, it also has significant drawbacks. Radiofrequency data must be sampled at a very rapid rate (usually 20 MHz). The amount of data stored and processed when using RF data is thus much larger than that manipulated when using image or video data. Moreover, standard echocardiographic instruments do not allow direct access to the ultrasound signal in its RF form.

MEASUREMENT OF ACOUSTIC PROPERTIES OF THE MYOCARDIUM

The measurement of the acoustic properties of the myocardium has proved to be a sensitive method for detecting pathologic abnormalities. The acoustic parameters that have most often been measured include attenuation,^{34-36,56} attenuation coefficient, and integrated ultrasonic backscatter.⁵⁹⁻⁶³ Such may be derived from the RF signal.

In one of the early studies of myocardial tissue characterization, Namery and Lele⁵⁶ noted a change in acoustic impedance in excised hearts 30 min after acute experimental myocardial infarction. Thus, changes in elastic properties may prove to be a useful measure for characterizing the myocardium.^{64,65}

Measurement of attenuation

Mimbs and co-workers found a significant decrease in attenuation of ultrasound passing through acutely infarcted myocardium when compared with normal tissue.³⁴ Interestingly, the acoustic alterations were noted as soon as 15 min after acute experimental coronary occlusion in open-chest dog studies. The measurements in this study were performed *in vitro* using excised tissue specimens. The attenuation remained less than normal in the infarcted region for the first 24 h after infarction. Measurements performed more than 3 days after coronary occlusion, however, showed an increase in attenuation through the infarcted tissue relative to the normal myocardium.¹⁵ Thus, not only did the acoustic analysis allow very early identification of ischemic tissue, but the age of the ischemic injury affected the direction of the change in attenuation. Although of great theoretical and physiologic interest, these initial excised tissue studies of attenuation were performed in transmission mode, i.e., by interposing tissue specimens between separate transmitting and receiving crystals. Such studies would clearly not be done *in vivo*. Although techniques have recently been developed for measuring attenuation in reflection mode, i.e., using the same crystal as transmitter and receiver,⁶⁷ these latter techniques have only recently been applied to myocardial studies.

Measurement of backscatter

Many promising studies in ultrasound tissue characterization have been based upon the measurement of the backscatter coefficient or the integrated backscatter, parameters that may be derived from the echo signal.

O'Donnel et al,⁶¹ using in part a concept discussed by Siegelmann and Reid,⁶⁸ introduced the measurement of integrated backscatter, which in an estimation of the energy within an ultrasound signal returning from a portion of tissue, compared with the energy in a signal returning from a "perfect" reflector. Measurement of integrated backscatter as early as 1 h after acute occlusion of a coronary artery in an open-chest dog allowed identification of an increase of backscatter from infarcted compared with normal myocardium.⁶² Therefore, this presumably clinically applicable measurement was able to identify acute infarction. In a subsequent study, the authors were able to use integrated backscatter to identify myocardium that was rendered abnormal by administration of the cytostatic agent doxorubicin.⁶⁰

One of the problems that has limited the development of clinical methods of ultrasound myocardial tissue characterization is a method of accounting for alterations in the ultrasound beam as it passes through

tissues that are interposed between the transducer and the region of interest. This intervening tissue absorbs and scatters ultrasound, acts as a low-pass filter (since attenuation of ultrasound is directly related to frequency), and causes diffraction effects. At least three methods have recently been reported that have attempted to correct for the effects of intervening tissue and thus set the stage for clinical measurements of myocardial acoustic properties. Cohen and co-workers measured the acoustic properties of excised chest-wall specimens⁶⁹ and utilized these measurements to derive a *correction factor* for use in adjusting backscatter measurements for the effect of the chest wall. Using this correction factor, these investigators were able to identify acute ischemia through the chest wall in an animal model and showed a high correlation between measurements of backscatter through the chest wall in an animal model and showed a high correlation between measurements of backscatter through the chest wall and those obtained directly from the epicardium over the site of injury. O'Donnel has attempted to correct for the effects of intervening tissue by developing quantitative volume backscatter images.⁶³ Melton and Skorton have reported a hardware-based method of altering the gain-compensation profile individually along each ultrasound line of site within a two-dimensional echocardiographic frame, based on differential amplification of signals of varying amplitude.⁵⁸ These and other methods of accounting for intervening tissue will probably in the near future allow reasonable estimates of integrated backscatter to be made through the chest wall in a clinically relevant fashion.

Mimbs and co-workers⁶² studied a series of open-chest dogs and observed that integrated backscatter was increased by 200% compared with values in control regions within 1 h after coronary occlusion, and by 400% within 6 h. Factors responsible for alteration of ultrasonic properties in the acute phase of myocardial infarction include the development of interstitial and intracellular edema, changes in concentration of cytoplasmic proteins, differences in tissue volume between ischemic and non-ischemic regions, and changes in concentration of potential ultrasonic contrast agents such as blood and calcium content. Although characteristic histologic changes are not seen in the very early phases of myocardial infarction by light microscopy, swollen organelles, mitochondrial densities, and myofibrillar relaxation are evident within 1 h of coronary occlusion by electron microscopy.⁶² In addition, the authors noted an increase in cardiac fluid content as reflected by an increase in wet to dry weight ratio in the acute phase of infarction and they felt that this was an important determinant of increased ultrasonic backscatter that was observed. Mimbs and co-workers³⁶ did a series of experiments in which

hearts from 21 rabbits were studied for 5-7 weeks after coronary occlusion. Ultrasonic attenuation and ultrasonic backscatter were increased significantly when compared with nonischemic regions from the same rabbits. An increase in the collagen content within the infarcted tissue was considered to be an important determinant of the increase in attenuation and backscatter.

SUMMARY AND FUTURE EXPECTATIONS

There is now sufficient laboratory evidence that various indices of ultrasonic tissue characterization can provide adequate information in the diagnosis of myocardial infarction. With some modification of existing equipment, it may be possible to acquire data for tissue characterization during routine echocardiographic examinations without causing any inconvenience to the patient.⁴⁸

It is conceivable that the widespread use of real-time gray-scale scanners and color-encoded images, and the availability of powerful low-cost computers, will aid the further development of ultrasonic tissue characterization.

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Chapter 7

DETECTION OF EARLY MYOCARDIAL TISSUE CHANGES IN ACUTE CANINE MYOCARDIAL INFARCTION BY ULTRASONIC TISSUE CHARACTERIZATION METHODS

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ABSTRACT

To determine the time after left anterior descending artery (LAD) occlusion that changes in myocardial texture first occur, we performed 2-D echocardiograms (2DE) on 5 sham operated dogs and 8 dogs before and after proximal LAD occlusion. Serial 2DE were performed every 15 minutes by placing a 5 MHz transducer directly on the chest wall. The dogs were sacrificed at the end of the study, and the area of myocardial infarction was confirmed by triphenyl tetrazolium chloride staining. The 2DE images were digitized. The mean pixel intensity (MPI) $\pm$ SD in the area of myocardial infarction (region of asynergy) and a normally (control region) moving area equidistant from the transducer were calculated; thus each dog served as its own control.

In sham operated dogs, no significant change in MPI, skewness, or kurtosis were observed. There was a significant increase in MPI in the infarcted regions (anteroseptal) compared to values obtained in the normally moving (lateral) regions at 30 minutes (MPI = 25.2 ± 23.1 vs 23.0 ± 0.7 , $p < 0.05$), and this difference persisted at 45 minutes (MPI =

25.8±0.9 vs 22.6±0.5, $p < 0.01$), 60 minutes (MPI = 27.2±1.4 vs 22.8±0.8, $p < 0.01$), 90 minutes (MPI = 28.6±1.6 vs 22.5±0.9, $p < 0.01$) and 180 minutes (MPI = 28.7±1.8 vs 21.3±1.4, $p < 0.01$). In addition, kurtosis showed a statistically significant decrease between infarcted (anteroseptal) and normal (lateral) regions starting at 45 minutes (-0.256±0.3 vs 0.280±0.6, $p < 0.05$) and persisting to 180 minutes (0.090±0.4 vs 0.030±0.2, $p < 0.05$).

We conclude that digital image processing of 2DE obtained through the chest wall enables the early detection of myocardial infarction, that is, at 30 minutes.

Key Words: Echocardiography, Myocardial Infarction, Ultrasonic Tissue Characterization.

INTRODUCTION

Chronic myocardial infarction has been occasionally detected by noting areas of increased brightness on M-mode¹ and 2D echocardiography.^{2,3} Fraker et al⁴ noticed an increase in apparent brightness of infarcted areas of dog hearts after acute myocardial infarction; however, their findings were subjective in nature and no objective quantification of acoustic reflectance was attempted in their study. Other investigators have analyzed radiofrequency signal⁵ and gray level run-lengths⁶ from acutely infarcted canine myocardium to identify acute myocardial ischemia. Color encoding of ultrasound amplitudes (i.e. replacing gray-level variations with variations in color intensity and hue) has proved useful in studying the evolution of myocardial infarction.^{3,7} Skorton et al⁸ have evaluated the quantitative statistical characteristics of echocardiographic intensities (gray-levels) from standard echocardiograms of canine myocardium before and two days after experimental coronary occlusion. The distribution of gray-levels (shape of the gray-level histograms) changed significantly with acute infarction. In another study, a significant increase in mean pixel intensity was noted at the end of 3 hours of coronary artery occlusion in the anteroseptal region (infarcted) compared to noninfarcted regions.⁹ Since the naked eye cannot detect subtle changes in gray-levels, a digital image processing method developed in our laboratory was utilized to determine how *early* after coronary artery occlusion a myocardial infarction could be diagnosed in dogs.

Methods

Animal Preparation

Thirteen adult mongrel dogs weighing between 25-50 kg were used in this study. The study protocol was approved by the Institutional Review Board of the University of Southern California School of Medicine. They were given morphine sulphate 1 mg/kg i.m. and sodium pentobarbital 30 mg/kg i.m. one-half hour prior to surgery. An intravenous line was started, ECG leads were placed and the trachea intubated. Ventilation was controlled with a respirator and the dogs received 100% inspired oxygen. Supplemental morphine sulphate was given in small, incremental doses by intravenous injection up to a total dose of 5 mg/kg. Two-dimensional echocardiograms were performed with the dogs lying in the right lateral decubitus position by placing a 5 MHz transducer directly on the chest wall.

The chest was entered through an incision in the left fifth intercostal space, the pericardium incised and the beating heart exposed. Five dogs served as controls (sham operated dogs). In 8 dogs undergoing intervention, a bolus injection of lidocaine 1 mg/kg was given prior to clamping the left anterior descending coronary artery (LAD). The LAD was isolated approximately 1 cm from its origin proximal to the first large diagonal branch. The LAD was occluded with a vascular clamp. Following this, the dogs were maintained on lidocaine 2-4 mg/kg with a constant drip through a venous catheter. Additional bolus injections of lidocaine (1 mg/kg) were given in the event an episode of ventricular tachycardia occurred in the anesthetized dog.

All dogs were sacrificed at the end of the experiment by injecting a 5-10 mg/kg bolus dose of sodium pentobarbital, while ventilation was concomitantly discontinued. The chest was then fully opened, and the hearts were removed for additional investigations as described below.

Echocardiographic Data

Prior to any manipulation of the LAD, baseline parasternal short axis views at different levels of the left ventricle were obtained in all dogs by placing a 5 MHz transducer (narrow band, focussed) directly on the chest wall. The time-gain-compensation setting (TGC) and all other controls on the echocardiographic machine were kept identical in all studies. Images were recorded on standard VHS video tapes. Image analyses were performed on videotaped studies.

Standardization of 2D Echocardiograms

In order to enable comparison of serial studies, standardization of 2D echocardiograms was performed. This was done 1) By noting the time-gain compensation (TGC) settings and other controls on the echo machine at the time of the first study; 2) By keeping these settings identical in subsequent studies; and 3) By presenting a standard video signal to the image digitizer using a video processing amplifier and an oscilloscope.

Data Analysis System

Selected series of videotaped images were digitized using a Diasonics Cardio Revue Center (CRC). The CRC has a JVC Model BR-6400U VHS video cassette recorder and a Sony Model SVM-1010 Video Motion Analyzer. The CRC consists of a magnetic video disk, a Polaroid camera and an 8080-based microcomputer incorporating a 5-megabyte hard disk, a Datacube Model VG-120 video frame digitizer and a graphic display controller, dual 5-inch floppy disk drives, a dot-matrix printer, a keyboard and a cathode ray tube (CRT) display. The Datacube Model VG-120 digitizes a full field of analog video data (RS-170 E1A standard analog signal) with a resolution of 320 horizontal (H) by 240 vertical (V) picture elements (pixels) by 64 gray levels and downloads the binary result of on-board random access memory (RAM) in real time, while simultaneously converting the binary data in the RAM back into analog video data viewable on a CRT monitor. In addition, the microprocessor has read/write access to any single pixel contained in the on-board RAM. Standardization was accomplished using a video processing amplifier and an oscilloscope.

Each digitized video frame obtained at end-diastole (beginning of R wave on ECG) was transmitted through a high-speed serial data link to a second computer for further processing. This computer, a Compupro Model 816/B, is an 8085/8088-based microcomputer incorporating 64 kbytes of RAM, dual 8-inch 1.2-megabyte floppy disk drives, a Scion Microangelo® color graphics subsystem, an RGB high-resolution video display monitor and a high-resolution light pen. The digitized video frames were stored on floppy disks after transfer from the CRC and could be retrieved at any time thereafter for redisplay or processing. The Microangelo® system stored digitized video images with a resolution of 480 H by 512 V pixels.

The pixel intensity of any given location could be ascertained using a high-resolution light pen. The computer had the capability to average pixels at any given area of interest such as valvular leaflets or the

myocardium. The number of pixels averaged and the mean pixel value of the areas studied could be displayed. The entire area of interest i.e. the area of wall motion abnormality representing the area of infarction and a comparable area of adjacent normally moving myocardium (control region) was demarcated and the mean pixel intensity obtained. When pixel averaging, we tried to avoid specular reflections from the leading and trailing edges of the myocardium.

"Skewness" measured the asymmetry of the shape of the distribution and "kurtosis" measured the peakedness of the distribution relative to the length and size of its tails.

The calculations of skewness and kurtosis were done as follows:

Values for skewness and kurtosis of the pixel distributions were calculated using the standard statistical definitions and incorporating them in our computer software package. First a standardization variable, Z , is computed

$$Z = (x - \bar{x})/S$$

where $\bar{x}$ = mean of the distribution and S = standard deviation of the distribution.

The following calculations are then performed:

$$\text{Skewness} = \frac{N}{(N-1)(N-2)} \sum Z^3$$

$$\text{Kurtosis} = \frac{N(N+1)}{(N-1)(N-2)(N-3)} \sum Z^4 - \frac{3(N-1)(N-1)}{(N-2)(N-3)}$$

Triphenyl Tetrazolium Chloride Staining

After sacrificing, the heart was rapidly excised and sliced into 1 cm. thick transverse sections from apex to base parallel to the atrioventricular groove. The slices of dog heart were immersed in a freshly prepared solution of triphenyl tetrazolium chloride (TTC) for 10-15 minutes at 32°C.^{10,11} After staining, the slices were fixed in formaldehyde solution and photographs of specimens were obtained for documentation purposes and for later comparison with ultrasound images.

Statistical Analysis

The mean pixel intensity of the region of left ventricular asynergy (area of myocardial infarction) and a normally moving area were compared and a Student's paired *t*-test applied to evaluate significance of results. Probability values of <0.05 were considered significant.

RESULTS

Echocardiographic Data

The mean pixel intensity, kurtosis, skewness were calculated in all dogs. The results are shown in figure I and tables I to IV.

In *sham* operated dogs, no significant change in mean pixel intensity was

MEAN PIXEL INTENSITY IN SHAM OPERATED DOGS AND DOGS UNDERGOING LAD OCCLUSION MEAN VALUES $\pm$ SD ARE DISPLAYED

Figure 1: Mean pixel intensity in 5 sham operated dogs and 8 dogs undergoing left anterior descending artery occlusion. Mean $\pm$ SD are displayed. AS= anteroseptal area; Lat.= lateral area.

Asterisks indicate values that differ from the corresponding time intervals at $p < 0.05$ between the anteroseptal (infarcted) and the lateral (normal) regions.

noted (at each point in time) from baseline values to 30, 45, 60, 90 and 180 minutes between the *anteroseptal* region and *lateral* regions. In addition, skewness and kurtosis were measured and showed no statistically significant difference in sham operated dogs.

However, in dogs undergoing LAD occlusion, a statistically significant difference was noted in mean pixel intensity between the *infarcted* (*anteroseptal*) and *normal* (*lateral*) regions of the myocardium at 30 minutes ($MPI=25.2\pm2.1$ vs 23.0 ± 0.7 , $p < 0.05$) and this difference

Table I: Kurtosis in sham operated dogs

Mean Values $\pm$ SD

Time	Kurtosis (AS)	Kurtosis (LAT)	P Value
Baseline	0.81 ± 0.01	0.75 ± 0.08	NS
15 min	0.59 ± 0.38	0.41 ± 0.43	NS
30 min	0.47 ± 0.68	0.34 ± 0.92	NS
45 min	0.74 ± 0.09	0.55 ± 0.84	NS
60 min	0.70 ± 0.23	0.87 ± 0.05	NS
90 min	0.73 ± 0.16	0.51 ± 0.59	NS
180 min	0.38 ± 0.26	0.23 ± 0.37	NS

NS=not significant; AS=anteroseptal region, LAT=lateral region

Table II: Skewness in sham operated dogs

Mean Values $\pm$ SD

Time	Skewness (AS)	Skewness (LAT)	P Value
Baseline	-0.17 ± 0.28	-0.41 ± 0.71	NS
15 min	-0.40 ± 0.82	-0.21 ± 0.43	NS
30 min	-0.39 ± 0.54	0.11 ± 0.31	NS
45 min	-0.47 ± 0.56	0.24 ± 0.34	NS
60 min	-0.29 ± 0.23	0.07 ± 0.31	NS
90 min	-0.20 ± 0.40	0.15 ± 0.51	NS
180 min	0.11 ± 0.30	0.25 ± 0.31	NS

NS=not significant; AS=anteroseptal region, LAT=lateral region

persisted at 45 minutes ($\text{MPI}=25.8\pm0.9$ vs 22.6 ± 0.5 , $p<0.01$), 60 minutes ($\text{MPI}=27.2\pm1.4$ vs 22.8 ± 0.8 , $p<0.01$), 90 minutes ($\text{MPI}=28.6\pm1.6$ vs 22.5 ± 0.9 , $p<0.01$) and 180 minutes ($\text{MPI}=28.7\pm1.8$ vs 21.3 ± 1.4 , $p<0.01$) (Figure I).

In addition, in dogs undergoing LAD occlusion, kurtosis showed a statistically significant decrease between *infarcted* (anteroseptal) and *normal* (lateral) regions; starting from 45 minutes (-0.256 ± 0.3 vs 0.289 ± 0.6 , $p<0.05$) to 180 minutes (0.090 ± 0.4 vs 0.300 ± 0.2 , $p<0.05$).

Table III: Kurtosis in dogs undergoing LAD occlusion

Mean Values $\pm$ SD

Time	Skewness (AS)	Skewness (LAT)	P Value
Baseline	1.26 ± 1.16	0.59 ± 0.74	NS
15 min	0.32 ± 0.38	0.40 ± 0.40	NS
30 min	0.14 ± 0.75	-0.01 ± 0.42	NS
45 min	-0.25 ± 0.32	0.28 ± 0.64	0.03
60 min	-0.59 ± 0.33	0.58 ± 1.08	0.01
90 min	-0.37 ± 0.34	0.34 ± 0.61	0.04
180 min	0.09 ± 0.43	0.30 ± 0.23	0.04

NS=not significant; AS=anteroseptal region, LAT=lateral region

Table IV: Skewness in dogs undergoing LAD occlusion

Mean Values $\pm$ SD

Time	Skewness (AS)	Skewness (LAT)	P Value
Baseline	-0.82 ± 0.43	-0.62 ± 0.24	NS
15 min	0.29 ± 0.43	0.21 ± 0.36	NS
30 min	-0.24 ± 0.35	0.23 ± 0.38	NS
45 min	-0.12 ± 0.35	0.00 ± 0.64	NS
60 min	-0.03 ± 0.28	0.01 ± 0.45	NS
90 min	-0.11 ± 0.37	0.40 ± 0.40	NS
180 min	0.25 ± 0.35	0.16 ± 0.75	NS

NS=not significant; AS=anteroseptal region, LAT=lateral region

Changes in skewness showed no statistically significant difference in dogs undergoing LAD occlusion.

Triphenyl Tetrazolium Chloride Staining

This revealed lack of staining in the anteroseptal region of the left ventricle (area of infarction). The noninfarcted regions showed a brick-red color.

DISCUSSION

Ischemic myocardium changes the nature of the reflected ultrasound signal, and this change is detectable.^{9,11-17} In canine preparations, Mimbs et al^{16,17} showed increased integrated backscatter in ischemic myocardium *in-vivo* as early as one hour after coronary occlusion. Several studies have shown that integrated backscatter undergoes variation throughout the cardiac cycle with a decrease in backscatter from diastole to systole.¹⁸⁻²⁰

Madaras et al²¹ have recently reported that integrated ultrasonic backscatter measured in open-chest dogs varies with the phase of cardiac contraction in normal myocardium. Olshansky et al²² have shown that left ventricular myocardial gray-levels obtained by 2-dimensional echocardiograms vary from end-diastole to end-systole. Fraker et al⁴ noted an increase in apparent brightness of infarcted areas of dog hearts after acute myocardial infarction; however, their findings were subjective in nature and no quantification of acoustic reflectance was attempted.

Skorton et al⁸ have evaluated the (quantitative) statistical characteristics of echocardiographic intensities (gray-levels) from standard echocardiograms of canine myocardium before and two days after experimental coronary occlusion. The distribution of gray-levels (shape of the gray-level histograms) changed significantly with acute infarction. Utilizing quantitative measures of echocardiographic image texture which were previously used in the analysis of nonmedical images, McPherson et al²³ have identified acutely ischemic myocardium 1-2 hours after coronary occlusion in dogs.

Results of the present study indicate that measurement of mean pixel intensity of digitized 2DE images can detect myocardial ischemia as early as 30 minutes after coronary artery occlusion in dogs. Our findings corroborate those of some other investigators^{5,16,24} who, while using more cumbersome approaches which involved acquiring radiofrequency data, found significant changes at an early stage following occlusion of the coronary circulation in experimental studies. The current data

were obtained by placing a 5.0 mHz transducer directly on the chest wall. In this study, the first significant increase in mean pixel intensity in the infarcted region was observed at 30 minutes following LAD occlusion. The mean pixel intensity in the infarcted region continued to increase over time after occlusion and further significant differences were noted between 30 to 180 minutes post-occlusion between the infarcted and normal regions. No significant difference in mean pixel intensity between antero-septal and lateral regions was seen in the sham operated dogs in the three hour study period. Kurtosis (peakedness of mean pixel intensity) showed a significant decrease at 45 minutes between the antero-septal and lateral regions following LAD occlusion, and this difference remained significant until 180 minutes. In sham operated dogs, no difference in kurtosis was observed between the lateral and antero-septal regions. Our observations that the MPI in the region of the wall motion abnormality at 15 minutes was not significantly different when compared to a control region, indicate that the changes in MPI observed at subsequent times was probably not caused by the wall motion abnormality per se, because wall motion abnormalities occur immediately after coronary occlusion.

Although the current data are expressed as mean $\pm$ SD, and our method required a fair amount of averaging of data, the measurement 30 minutes after coronary artery occlusion was statistically significantly different from normally moving areas for mean pixel intensity and at 45 minutes for kurtosis. The explanation for the altered mean pixel intensity and kurtosis in infarcted regions of the myocardium is not entirely clear. Mimbs et al¹⁷ noted an increase in myocardial fluid content reflected by the wet-to-dry weight ratio in their experiments involving ultrasonic backscatter. Schnittger et al⁵ observed changes by electron microscopic slides at the subcellular level, and this may alter scattering properties. Myocardial cells or bundle of cells can be looked upon as individual scatterers, and as found by others using erythrocytes,²⁵ when the density of scatterers changes, the backscatter may change.

In conclusion, the results of the present study demonstrate that digital image processing of 2-dimensional echocardiograms is an additional useful tool in diagnosing myocardial infarction in its very *early* stages in dogs. Its applicability to man remains to be further explored.

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Chapter 8

APPRAISAL OF THE RESEARCH PERFORMED

The present research was conducted from September 1985 to June 1988, at the Echocardiographic Laboratory of the Section of Cardiology, Department of Medicine, Los Angeles County — University of Southern California School of Medicine, Los Angeles, under the supervision of Dr. P.A.N. Chandraratna (Professor of Medicine, Director of Echocardiography and Graphics).

Two dimensional echocardiography provides a great deal of information about the size and volume of various cardiac chambers and about regional and global left ventricular function. In addition to the above, information regarding the structure of the cardiac tissue (e.g. myocardium and valves) can sometimes be obtained by analysis of 2 dimensional echocardiograms, for instance, when gross calcification and fibrosis can be seen by the naked eye. However, in other instances when mild and subtle abnormalities are present, a more sophisticated diagnostic approach is needed.

A relatively new area of endeavor in cardiac ultrasound is the attempt to evaluate myocardial tissue composition and/or structure based on the acoustic properties of the heart. The rationale for this area of study — termed “Ultrasonic Tissue Characterization”, is that ultrasound interacts (is absorbed or reflected) differently with abnormal myocardium than with normal myocardium. This rationale stems from the common clinical observation that certain abnormalities of the heart (calcification, myocardial ischemia, amyloidosis and others) affect the appearance of echocardiographic images. Several approaches to the evaluation of tissue composition with ultrasound include the display of acoustically abnormal tissue using gray scale or color encoding, the measurement of classic acoustic variables, and computer based analysis of ultrasound signals and images. Promising results have been obtained through the use of ultrasound tissue characterization methods to identify acute ischemia, acute and chronic myocardial infarction, cardiomyopathies, myocardial contusion and intracardiac masses.

The purpose of this research was to investigate the ability of a

computer based image digitizing system developed in our laboratory to: 1) quantitate textural abnormalities of valvular leaflets in patients with mitral valve prolapse and rheumatic mitral valves; 2) differentiate "active" from "chronic" vegetations in patients with infective endocarditis; and 3) differentiate normal from infarcted myocardium in dogs. To realize this goal, our computer system was designed to measure mean pixel intensity $\pm$ SD, kurtosis and skewness in a given area of interest in the normal and abnormal tissue. In addition, it has the capability of drawing pixel histograms and pixel maps of desired areas on the picture screen and perform certain other functions such as image zoom, signal averaging, edge detection, etc. Sixty-four colors indicated pixel intensity or pixel value; e.g. blue and green color represented low pixel values, while yellow and red color represented high pixel values. Using this methodology, we were able to detect (early) infarcted from normal myocardium in dogs in two separate studies, differentiate "active" from "chronic" vegetations of infective endocarditis and establish that a subset of patients with severe myxomatous degeneration have a lower mean pixel intensity than normal mitral valves, whereas, patients with rheumatic mitral valves have a higher mean pixel intensity than normal and myxomatous mitral valves.

There is now ample laboratory evidence, based on in vitro and in vivo animal studies, together with some confirmatory studies in humans, to suggest that cardiac tissue characterization is feasible. With some modification to existing equipment, it is possible to acquire data for tissue characterization during routine echocardiographic examinations without causing any inconvenience to the patient. Unfortunately, such modified equipment is unavailable commercially at the present time and research instruments provided by some companies have proven inadequate to the task. Thus, all tissues characterization studies carried out to date have been performed with equipment either constructed or modified by individual investigators.

The future development of cardiac tissue characterization is clearly tied to the availability of appropriate instrumentation. The widespread use of real time, gray-scale instruments and the availability of powerful, low-cost microcomputers will almost certainly kindle enthusiasm amongst cardiologists and scientists alike and will lead to a new generation of ultrasound systems that are appropriate for tissue characterization studies. Such studies have the potential for making a substantial contribution to diagnostic medicine, in general, and to echocardiography, in particular. It is conceivable that, in the not too distant future, ultrasonic tissue characterization will become one of the more powerful and routine applications of echocardiography.

SUMMARY

The first chapter gives an introduction to the basic principles and applications of ultrasonic tissue characterization in the detection of cardiovascular diseases. Three modalities of this technique are discussed:

1. Visual methods.
2. Measurement of acoustic variables.
3. Digital image/signal analysis.

A more detailed description of the qualitative and quantitative methods used in cardiac tissue characterization is discussed in chapter 6. The methods of two-dimensional echocardiographic recordings are also described in this chapter. The aim of this thesis was to investigate the ability of a computer based image digitizing system developed in our laboratory to differentiate normal from abnormal cardiac tissue by digital image processing of two-dimensional echocardiographic images.

In Chapter 2, two groups of patients with mitral valve prolapse, one with thickened mitral leaflets (MVP-TL), and the other with normal leaflet thickness (MVP-NTL) were compared to each other and with a normal control group. The two-dimensional echocardiographic images were digitized and the mean pixel intensity of the mitral leaflets (PI-ML) and the posterior wall of the aorta (PI-Ao) was determined. The mean pixel intensity in the MVP-TL and ratio PI-ML/PI-Ao was compared to the MVP-NTL group; the mean pixel intensity in the MVP-TL group was significantly lower than that of MVP-NTL group. In addition, the mitral leaflets of the MVP-TL group were significantly thicker than those of the MVP-NTL group and normal controls. The lower mean pixel intensity in the MVP-TL group is probably attributable to more extensive myxomatous change in this group of patients.

In Chapter 3, the ability of a computer based image digitizing system to differentiate active from chronic vegetations of infective endocarditis by digital image processing of two-dimensional echocardiograms was evaluated. This study was performed on a prospective basis on 22 patients who were studied serially for a mean period of 8 weeks (range 4-22 weeks) after initiation of antibiotic therapy. This method enabled us to reliably differentiate "active" from "chronic" vegetations; the first significant

increase in mean pixel intensity occurred at 4 weeks following therapy. Vegetation size decreased in only 73% of the patients, whereas in the remaining 27% of the patients the vegetation size did not change significantly from the active to the chronic stage.

In Chapter 4, experimental myocardial infarction was induced in 13 dogs by ligation of the left anterior descending coronary artery (LAD). Five sham operated dogs served as controls. The mean pixel intensity measured in the anteroseptal region (infarcted region) was significantly higher in dogs undergoing LAD occlusion for 3 hours, as compared to a normally moving (lateral) region. Triphenyl tetrazolium chloride staining confirmed the region of myocardial infarction suspected on two dimensional echocardiograms and digitized two dimensional images. In sham operated dogs no change in mean pixel intensity was noted at 4 hours.

In Chapter 5, the results of an in-vitro study which was performed on excised mitral valves with myxomatous degeneration and severe mitral regurgitation, rheumatic mitral valves, and normal mitral valves of persons with a negative cardiovascular history are presented. Mitral valve thickness and mean pixel intensity were determined in all three groups. The mitral valve thickness in the excised rheumatic and myxomatous valves were both significantly higher than that of normal valves. The mean pixel intensity in the myxomatous valves was significantly less than that of rheumatic mitral valves and normal mitral valves. The decrease in the mean pixel intensity in myxomatous valves with severe mitral regurgitation is probably due to decreased tissue density in these valves. The increased mean pixel intensity found in rheumatic mitral valves when compared to myxomatous mitral valves and normal mitral valves reflects increased tissue density probably as a result of fibrosis, and calcification.

Chapter 6, reviews the present knowledge of the application of ultrasonic tissue characterization methods in the diagnosis of coronary artery disease. This technique has been used both in the experimental and clinical setting and some very promising results have been obtained. The importance of image standardization in ultrasonic tissue characterization is also discussed in detail. It appears from the literature that this technique of ultrasonic tissue characterization is sensitive in detecting ischemic changes in the myocardium.

In the study described in chapter 7 the feasibility of digital image processing in the very early stages of acute myocardial infarction in an animal model is discussed. The mean pixel intensity, kurtosis, and skewness were measured in 8 dogs who were subjected to left anterior descending artery occlusion. Five sham operated dogs served as controls. Serial 2-dimensional echocardiographic studies were performed every 15 minutes for the first hour and every 30 minutes thereafter for the remaining 2 hours of the study. There was a significant increase in mean pixel intensity between infarcted and non-infarcted regions beginning at 30 minutes which persisted until the end of 3 hours of LAD occlusion. Kurtosis showed a significant decrease in the infarcted versus non-infarcted regions beginning at 45 minutes and continued until the end of the study in dogs undergoing LAD occlusion. Kurtosis did not change significantly in sham operated dogs. There was no change in skewness between the anteroseptal and lateral regions in dogs undergoing LAD occlusion or in sham operated dogs.

In Chapter 8, the institution where the research was performed, purpose of research, methodology used, as well as a general background of ultrasonic tissue characterization are described. In addition, an appraisal of the work performed and the future of this noninvasive diagnostic method is presented.

SAMENVATTING

Hoofdstuk 1: Inleiding.

In dit hoofdstuk wordt de theoretische achtergrond alsmede de waarde van ultrasonore weefseltyping beschreven bij de opsporing van cardiovasculaire ziekten. Een literatuuroverzicht van deze techniek en de algemene toepassing van M-Mode en tweedimensionale echocardiografie wordt aangegeven. Drie modaliteiten van ultrasonore weefseltyping worden besproken.

A. Visuele methoden

B. Meting van acoustische variabelen

C. Digitaal beeld/Signaal analyse.

Een uitgebreide beschrijving van de kwalitatieve en kwantitatieve methoden die gebruikt worden in ultrasonore weefseltyping is gegeven in hoofdstuk 6. Het doel van dit promotieonderzoek is om een nieuw computersysteem dat ontwikkeld is in ons laboratorium te gebruiken en te proberen hiermee cardiovasculaire aandoeningen te diagnostiseren.

Hoofdstuk 2:

Twee groepen patiënten met mitralisklepprolaps: 1. met verdikte klepslippen (MVP-TL) en 2. met niet-verdikte klepslippen (MVP-NTL) werden vergeleken met een controle groep (normalen). De tweedimensionale beelden werden gedigitaliseerd en de gemiddelde pixelintensiteit (MPI) van de mitralisklepslip (PI-ML) en de achterwand van de aortawortel (PI-Ao) werden gemeten. De MPI en de ratio PI-ML/PI-Ao in de drie onderzoeksgroepen werden vergeleken. De MPI en de ratio PI-ML/PI-Ao in de MVP-TL groep waren significant lager dan in beide andere groepen.

De verlaagde MPI in de MVP-TL groep is waarschijnlijk toe te schrijven aan de myxomateuze veranderingen van de mitralisklep.

Hoofdstuk 3:

In dit hoofdstuk wordt de waarde van ons computersysteem t.a.v. het onderscheiden van actieve en chronische vegetaties bij infectieuze endocarditis geëvalueerd. Deze studie werd verricht op een prospectieve wijze bij 22 patiënten over een gemiddelde periode van 8 weken (range 4 tot 18 weken) na het instellen van antibiotische therapie. De gemiddelde pixel intensiteit (MPI) van de actieve en chronische vegetaties op verschillende

kleppen werd seriaalsgewijs gemeten. Deze techniek stelde ons in staat om bij infectieuze endocarditis actieve van chronische vegetaties te onderscheiden. De MPI toonde een significante toename met klinische verbetering. Deze toename in MPI werd voor het eerst gezien vier weken na het instellen van antibiotische therapie. De grootte van de vegetaties vermindert significant bij 73% van de patiënten; bij de overige 27% van de patiënten werd geen significant verschil gemeten in grootte tussen het actieve en het chronische stadium.

Hoofdstuk 4.

Hierin worden de resultaten beschreven van een experimentele studie bij 18 proefdieren (honden). Bij 13 honden vond kunstmatige afsluiting van de ramus descendens anterior plaats (LAD); de overige 5 honden werden als controls gebruikt. De gemiddelde pixel intensiteit (MPI) werd gemeten in het abnormaal bewegend gebied (anteroseptaal) en werd vergeleken met de MPI in het normaal bewegend gebied (lateraal). Er was een significante toename van MPI gemeten drie uur na afsluiting van de LAD in het geïnfarceerde t.o.v. het normaal bewegend gebied ($p < 0.05$). In de controle groep werd geen significant verschil in MPI gezien tussen het anteroseptaal en lateraal gebied. Triphenyl tetrazolium chloride kleuring bevestigde het geïnfarceerde gebied en hielp in het onderscheid maken tussen normaal en abnormaal weefsel.

Hoofdstuk 5.

Dit hoofdstuk geeft de bevindingen weer van een in vitro verrichte studie van mitraliskleppen met: 1. myxomateuze degeneratie gepaard gaande met ernstige mitralisklepinsufficiëntie; 2. reumatische mitraliskleppen en 3. gezonde mitraliskleppen (normaalgroep). Histopathologisch onderzoek bevestigde deze bevindingen. De gemiddelde pixel intensiteit (MPI) en de dikte van de klepslippen werden gemeten in de drie groepen. De reumatische en myxomateuze klepslippen waren beide dikker dan de normale klepslippen ($p < 0.05$). De MPI van de reumatische klepslippen was significant hoger dan die van de normale klepslippen. Daarentegen hadden de myxomateuze klepslippen een significante lagere MPI ($p < 0.05$) dan de normale klepslippen.

Hoofdstuk 6.

Hierin wordt een literatuuroverzicht gegeven van de waarde van ultrasonore weefseltyping bij de diagnose van coronairlijden. Deze techniek heeft toepassing zowel op het experimentele gebied als in de klinische praktijk. De resultaten van de diverse studies zijn in het algemeen gunstig. Verder wordt in dit hoofdstuk de noodzaak van standaardisatie

van de echocardiografische beelden en de verwachtingen m.b.t. de bruikbaarheid van deze techniek besproken.

Hoofdstuk 7.

Digitalisatie van tweedimensionale echocardiografische beelden bij proefdieren (honden) hielp ons met het opsporen van vroege ischemie. De gemiddelde pixelintensiteit (MPI), kurtosis en skewness werden gemeten in 8 honden bij wie de ramus descendens anterior (LAD) was afgesloten. Vijf honden werden gebruikt als controle groep. Bij alle honden werd tweedimensionale echocardiografie seriaalsgewijs verricht iedere 15 minuten gedurende het eerste uur, daarna eens per 30 minuten gedurende de overige twee uur van het onderzoek. Er werd een significante toename van MPI gevonden in de geïnfarceerde gebieden t.o.v. de normaal bewegende gebieden (lateraal) na 30 minuten LAD afsluiting en dit bleef aanwezig gedurende de rest van de tijd van het onderzoek. De kurtosis vertoonde een significant verschil na 45 minuten in de geïnfarceerde gebieden t.o.v. de normaal bewegende gebieden (lateraal). Daarentegen vertoonde de skewness geen significante veranderingen. Er werden geen significante verschillen gevonden m.b.t. deze drie parameters bij de controles wanneer het anteroseptaal gebied vergeleken werd met het laterale gebied.

Hoofdstuk 8.

Hierin wordt het instituut waar het onderzoek plaats vond vermeld en worden doel van research, de gebruikte methodologie, en een algemene achtergrond van ultrasonore weefseltypering beschreven. Daarnaast worden waarde en toekomstperspectief van deze non-invasieve onderzoeksmethode besproken.

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