QUANTITATIVE CARDIAC ULTRASOUND

The clinical investigations published in this thesis have been realised within the framework of project V and project VIII of the Interuniversity Cardiology Institute of the Netherlands (ICIN).

The studies on ultrasonic myocardial tissue characterisation were supported by the Netherlands Technology Foundation (STW).

Financial support by the Netherlands Heart Foundation for the publication of this thesis is gratefully acknowledged.

Printing by Tripiti, Rotterdam, the Netherlands.

QUANTITATIVE CARDIAC ULTRASOUND

Quantitatieve Echocardiografie

proefschrift

ter verkrijging van de graad van doctor

aan de Erasmus Universiteit Rotterdam

op gezag van de rector magnificus

prof. dr. C.J. Rijnvos

en volgens besluit van het college van dekanen.

De openbare verdediging zal plaatsvinden op

7 november 1990 om 15.45 uur

door

Hans Rijsterborgh

geboren te Amsterdam

PROMOTIECOMMISSIE

Promotoren:	prof. dr. ir. N. Bom
	prof. dr. J.R.T.C. Roelandt
Overige leden:	prof. dr. ir. A.J. Berkhout
	prof. dr. J. Lubsen
	prof. dr. A. Versprille
	prof. dr. C.A. Visser

CONTENTS

PREFACE / ACKNOWLEDGEMENTS

This thesis is about the various aspects of quantitative cardiac ultrasound. The first four chapters are mainly devoted to the reproducibility of echocardiographic measurements. These are focussed on the variation of echocardiographic measurements within patients. An important issue, since this variation determines the distinction between a real change of the measurement in a single patient or a difference caused by chance. The problems of the estimation of the variation of measurements within patients and its clinical implications are elaborated more specifically for Doppler velocity measurements and for cardiac volume determinations using cross-sectional echocardiography. Next, there are two chapters about echocardiographically determined normal ranges of cardiac dimensions. These reference ranges provide for the quantitative distinction between sickness and health. The last two chapters of this thesis deal with the newest aspect of quantitative cardiac ultrasound: non-invasive cardiac tissue characterisation using integrated backscatter measurements. These chapters are focussed on the distinction between normal myocardial tissue and acute ischemic cardiac muscle.

The studies, of which the results are included in this thesis, were performed within the framework of the Interuniversity Cardiology Institute of the Netherlands (ICIN) under the stimulating guidance of Klaas Bom (project V) and Jos Roelandt (project VIII). Without their continuous support this thesis would probably not exist and I am honoured with the fact that they both act now as my 'promotor'.

It will not be unnoticed that the larger part of the conclusions in this thesis is based on statistical analysis. In this regard I am much indebted to Koos Lubsen who not only explained the finer points of medical statistics to me, but also introduced me to the art of 'eye-ball' statistics.

The support of the Dutch Technology Foundation (STW) gave us the unique opportunity to explore the field of myocardial tissue characterisation. This part was also based on another form of cooperation between universities. This study was assisted by prof. dr. ir. A.J. Berkhout of the Delft University of Technology, Department of Acoustics. I hope we will continue this cooperation in the next future.

I wish to express my gratitude to Paul Voogd of the University of Leiden for his cooperation. The amount of work we had to go through at the time of our 'Leiden Study' has been forgotten. But I will remember our fertile scientific evaluations at the spicy Indonesian restaurant, since then closed down for reasons that escaped me.

It is beyond any doubt that this thesis could not have been created without the cooperation of numerous colleagues. It is next to impossible to acknowledge the contributions of all individual persons to the realisation of the subjects dealt with in this thesis. However, remaining grateful for the work of all those unmentioned, I like to express my special gratitude to a few colleagues in particular.

The members of the 'first hour': Jan Honkoop, Frans van Egmont, Wim Vletter, Jackie McGhie, Ton Bom, Gerard van Zwieten and Kees Ligtvoet.

Our distinguished guests from around the globe: Stefano Dominicucci (Italy), Anselmo Mayella (Tansania), Tamas Foster (Hungary), Conny Tirtaman and Donny Romdoni (Indonesia).

And last, but not least, the participants of our effort to characterise myocardial tissue non-invasively: Charles Lancée, Frits Mastik, Nico de Jong, Ton van der Steen, Loes Sassen, Rob van Bremen and Piet Verdouw.

Rotterdam, September 1990 Hans Rijsterborgh

CHAPTER I

AN INTRODUCTION TO THE ASSESSMENT OF THE REPRODUCIBILITY OF QUANTITATIVE CARDIAC ULTRASOUND

INTRODUCTION

Quantitative cardiac ultrasound has become an important diagnostic tool in clinical decision making. The measurements of specific parameters of left ventricular function by cross-sectional echocardiography and by Doppler echocardiography help to differentiate between normal and abnormal ventricular function and to assess the severity of cardiac disease. In the future quantitative backscatter analysis may help to differentiate normal myocardial tissue from diseased tissue. The great advantage of an echocardiographic examination is that it is not invasive, can be performed at the bedside and be repeated as often as needed.

The usefulness of a quantitative method for clinical decision making depends to a large extent upon the accuracy of the measurements and therefore information about the accuracy of quantitative echocardiography is mandatory. In order to assess the accuracy of a given echocardiographic measurement one needs a reference method which is highly accurate. Such a method is commonly referred to as the 'gold standard'. The problem is that in clinical practice, when available, such a method cannot be applied in patients for ethical reasons and is restricted to *in vitro* verification. But this is no proof that the method can be applied to patients with the same results and one has to select other means of *in vivo* verification.

Various methods for *in vivo* verification of an echocardiographic parameter are available. An common approach in the literature is a one-to-one comparison with an accepted method of measurement such as e.g. angiography. The problem is that the basic accuracy of the accepted traditional method is often unknown. The result of such a one-to-one comparison is the difference one could expect when applying quantitative echocardiography instead of the conventional method. In most cases this is useful information. What one really wants to know is which of both methods must be preferred in terms of reproducibility. Reproducibility must be assessed in terms of bias and random error. Different methods are available to estimate the bias and the various components of the random error. Only when the bias and random error of a method of measurement are known quantitative echocardiology can be applied at a patient level. The various methods and their results, the estimators of differences in bias and the estimators of the different components of random error, will be discussed in this chapter.

THE CONCEPTS OF BIAS AND RANDOM ERROR

If a result of a measurement could be compared with the true value, two types of error could be distinguished. A first type of error is a consistent error which is assumed to be constant throughout the ranges measured. This is known as the bias of the method of measurement. Since the bias is constant it will be present in every measurement. Bias can never be reduced by taking more measurements to obtain a mean value. Bias may be introduced into the measurement by the method of measurement itself and by the observer during the interpretation of the cross-sectional echocardiograms or Doppler recordings. In clinical practice, the real bias can never be measured, but differences in bias between two methods of measurement or between two observers can be assessed.

The second type of error is the random error. This is basically the non-consistent component of the difference between the true value and the measurement itself. Random errors can be introduced into the measurement by the method of measurement and by the observer during interpretation. Due to the random nature of this type of error, the influence on an averaged value will be diminished if more measurements are taken. Random errors can be expressed as a variance or as a standard deviation. The magnitude of a random error can be assessed by performing the measurement several times, under the condition that its true value does not change during the experiment, and by subsequently calculating the standard deviation of the measurements. With the assumption (or verification) that the random errors are normally distributed one can calculate the confidence limits of the measurement itself or the confidence limits of a mean value given the number of measurements.

The concepts of bias and random error are graphically illustrated in Fig. I-1. The true value T is measured by two methods of measurement having each their respective bias (B_A and B_B) and random error (e_A and e_B). The measurements can be regarded as the sum of three components:

$$M_A = T + B_A + e_A$$
$$M_B = T + B_B + e_B$$

The value of T is constant, but unknown in medical practice. The bias is assumed to be constant and its magnitude will depend on the method of measurement. The random error is not constant, its magnitude is characterised by a standard deviation and will be related to the method of measurement.

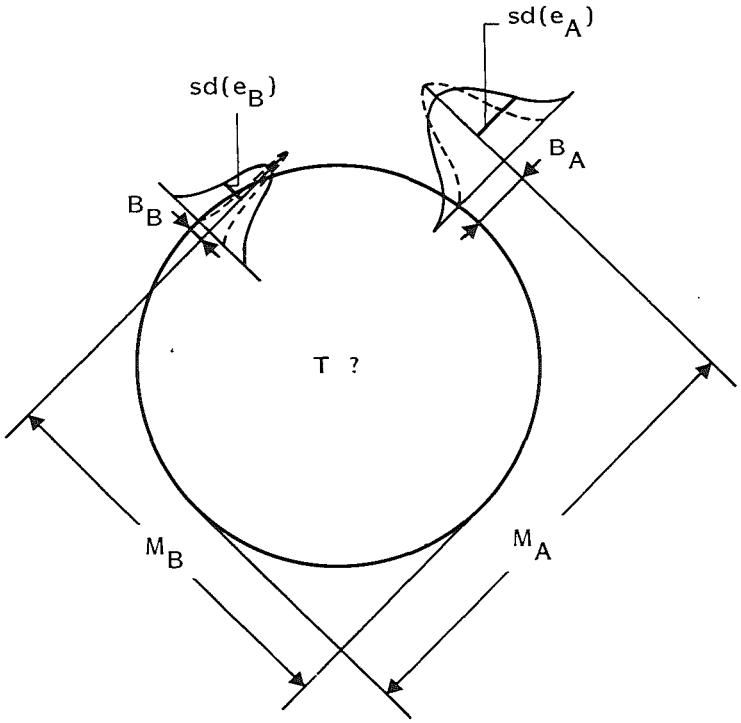

Fig. I-1. Graphical illustration of the measurement of the true value T with two methods of measurement (A and B) having their own specific bias B and random error e. The indices refer to the different methods used. Abbreviation: sd(e) = standard deviation of random error e.

OTHER SOURCES OF VARIATION

If one wants to perform serial measurements in a patient, for instance to reduce the influence of random errors, it cannot be assumed that the true value of the measurement is constant throughout the investigation. Spontaneous fluctuations of the true value may occur due to respiration effect or to changes in heart rate. For instance, it is shown in Chapter II that Doppler echocardiographic measurements depend upon the state of respiration (inspiration or expiration) and upon the duration of the cardiac cycle during atrial fibrillation. These fluctuations are called the beat-to-beat variation and are assumed to be random. The influence of beat-to-beat variation will be reduced if more measurements are taken to obtain an averaged value. The extent of the beat-to-beat variation can be expressed as a variance or as a standard deviation.

THE CLINICAL RELEVANCE OF THE TOTAL RANDOM VARIATION

Every cardiac *in vivo* measurement from cross-sectional echocardiograms or from Doppler recordings will be subjected to beat-to-beat variation and observer random error. Therefore the sum of these random components is of clinical importance. The extent of the total random component determines the confidence limits of a single measurement in a patient, or the confidence limits of a mean value based on a number of measurements.

Otherwise, if the extent of the total random variation is known, one can estimate the number of measurements needed to reduce the confidence limits of a mean value to a desired level.

Another important clinical application of the use of the total random error is in the field of follow-up c.q. interventional studies. In [1] the echocardiographic studies of the cardiac effects of nitrates on left ventricular dimension and volume were reviewed and an attempt was made to answer whether or not echocardiography can allow to monitor the effects of nitrates in patient groups and in individual patients. Here it was shown that changes of 3 mm or less in the left ventricular dimension measured by M-mode echocardiography might fall within an acceptable measurement variability. In one study, half of the patients showed a difference of 3 mm or less, therefore in these patients the one can not assume that the changes were induced by nitrates.

Since the measurement is influenced by a random component differences of measurements in follow-up studies may be caused by the random component itself. If the extent of the random component is known, one can calculate a minimum difference needed for a genuine, or statistically significant, change of the measurement. This is applied in Chapter II for Doppler echocardiographic measurements and in Chapter IV for cross-sectional echocardiographic measurements. This 'threshold' difference is not only determined by the extent of the random error of the measurement. Another contribution may be caused by a difference in bias if different observers are involved. This difference in bias must be taken into account if differences in measurement are to be evaluated.

INTRAOBSERVER AND INTEROBSERVER TESTS

Intraobserver and interobserver tests are performed to assess the differences of measurements due to interpretation of the cross-sectional echocardiograms or Doppler recordings. One observer performs the measurements of exactly the same cardiac cycles again, not knowing his previous results. Or two observers analyse the same cardiac cycles independently of one another. The differences between the paired measurements have to be analysed in terms of a mean value and a standard

deviation. With regard to these tests there is no relevant information in a correlation coefficient of the simple linear regression of the paired measurements. The magnitude of the correlation coefficient is determined, in part, by the variance of the measurements and not by the statistical properties of the differences alone. However, visual inspection of the scatterplots of the paired measurements are often helpful in the evaluation of the comparison.

The mean value of the differences represents the difference in bias between the results of the observers or between the results of the observer at both instances. Consistent observer differences are not an insurmountable problem in quantitative echocardiology. The magnitude of observer differences in bias can easily be estimated. They can be reduced by analysing the possible causes. Standardisation of the measurements may be the magic word here. If the attempts to reduce the differences in bias fail, the differences can be taken into account.

The standard deviation of the paired differences represents the sum of the observer random errors made at both instances. A practical measurement will be influenced by only one observer random error and not by the sum of two. With certain assumptions the standard deviation of the observer random error can be estimated. It can be assumed that the standard deviation of the observer random error, made at both instances, is the same. This is a reasonable assumption. The other assumption is that the covariance of the paired random observer errors is small as compared to the variance of the random observer error. This condition is met if the number of paired measurements is large enough (i.e. larger than 200). If both assumptions are made the standard deviation of the observer random error is equal to the standard deviation of the differences of the paired measurements divided by the square root of two. This method is applied in Chapter II, in which the standard deviation of the observer random error is estimated of several Doppler echocardiographic measurements.

The relevance of the knowledge of the observer random error is that the measurement itself can be evaluated. A measurement with a large random observer error as compared to the range of measurements in clinical practice will not produce reproducible results unless a large number of measurements is taken in one patient. It should be noted that in intraobserver and interobserver tests the method of measurement itself, including the influence of the observer is being evaluated. There is no contribution of beat-to-beat variation since the same cross-sectional images (or the same Doppler curves) are being analysed.

THE ESTIMATION OF THE TOTAL RANDOM VARIATION

The total random variation represents the sum of the beat-to-beat variation and the random observer error. The standard deviation of the total random variation of a measurement in a patient can be estimated by performing serial measurements of consecutive cardiac cycles and calculating the standard deviation of these measurements. If this procedure is repeated in a number of patients one can estimate the total random variation of the method of measurement in an averaged situation. Variance analysis provides the means to accomplish this: the 'variance within patients' can be estimated by using the 'one way analysis of variance' [2].

The standard deviation of the total random variation of the measurement gives relevant information about the confidence ranges of a mean value of a measurement, or otherwise one can estimate the number of measurements needed to reduce the confidence limits to a desired range. With the knowledge of the standard deviation of the total random variation one can calculate a threshold difference needed to detect a genuine, or statistically significant, change in the situation of a patient. These are all important items in the field of clinical decision making at a patient level using quantitative echocardiology.

If the estimation of the total random variation is combined with intraobserver and interobserver tests, the relative contributions of beat-to-beat variation and the observer variation to the total random variation can be evaluated. A large total variation can be caused by either beat-to-beat variation or observer variation. If the large variation is mainly due to observer variation then the method of measurement must be in doubt.

THE COMPARISON OF TWO METHODS OF MEASUREMENT

When a new method of measurement has been developed, the 'classical' study is to perform measurements with this new method and compare the results with the measurements obtained with a conventional method in the same patients. By analysing the paired measurements one can estimate the differences to be expected if the conventional method is replaced by the new one. In terms of bias and random error, these differences will consist of a difference in bias and a random component of the sum of the total random variation of both methods, if the measurements are made non simultaneously.

The drawback of this comparison is that no conclusions can be made which method to prefer in the individual patient for clinical decision making in terms of reproducibility. Since the sum of the total random error is estimated no information is available about the total random errors of each method separately. One must realise that the situation may occur in which the differences found are mainly due

to a large random error of the conventional method and in practice, it appears that some traditional methods have not been tested with regard to reproducibility. It would be unfair with regard to the new method, because the comparative *in vivo* verification will not reveal the separate random errors related to the respective methods of measurement.

A better approach is to perform serial measurements of consecutive cardiac cycles with both methods in the same patients. The paired measurements give the information about the difference in bias related to both methods. Analysis of the serial measurements yields the estimation of the total random error of both methods separately. Intraobserver and interobserver tests performed for both methods will reveal the random errors of the respective methods separately. Based on these studies decisions can be made on which method is to be preferred in terms of reproducibility. If other factors are involved, like non-invasive examinations versus invasive examinations, one can evaluate the trade-offs in a more realistic manner.

ALTERNATIVE COMPARISONS OF DIFFERENT METHODS OF MEASUREMENT

Grubbs' estimators were developed to estimate the variance of the random error of a method of measurement which cannot be repeated a number of times [3,4]. Grubbs initially applied this method in order to evaluate the adequacy of electric clocks measuring burning times of powder train fuzes. Fortunately there are no medical applications for these devices but there are situations in which cardiac measurements cannot be repeated for other reasons of patient discomfort. In these cases the Grubbs' estimators can be applied to solve the problem of random error estimation.

It would be beyond the scope of this introduction to evaluate the Grubbs' estimators in too much detail [5], but a number of relevant properties can be mentioned here.

Applying Grubbs' estimators for the comparison of two methods of measurement one may come across the fact that the variance of one of the random errors has a negative value. This is mathematically correct but it does not make sense statistically. In that case the basic assumption of zero covariance between the true values and the random errors is not met. This may happen if the number of paired measurements is not large enough to diminish this covariance. Therefore, if two methods are compared, the accuracy of the Grubbs' estimators will depend upon the number of measurements, the extent of the random error to be estimated and the range of the true values.

This drawback is not encountered if more than two methods of measurements are compared. In that case the accuracy of the Grubbs estimators depends only on the number of measurements and the magnitude of random errors to be estimated and not on the range of the true values.

The Grubbs' estimators do not give the exact values of the variance of the random errors. It is obvious that the accuracy of the estimation will improve if more measurements are taken. The confidence limits of the estimators can be calculated [6]. But even if the variance of the random errors itself is not precisely estimated, their ratio's can be assessed more accurately under certain conditions [3]. If the relative values of the total random variation of the different methods are known then decisions can be made about which method is to be preferred in terms of reproducibility.

A unique example of this property of the Grubbs' estimators can be given by reanalysing the data published by Erbel et al. [7]. The subject of this study was to evaluate nine echocardiographic and nine angiographic methods to determine the volumes of 22 silicon rubber asymmetric cardiac models having known volumes. Single plane (two different cross-sections) as well as biplane methods were combined with the accepted mathematical cardiac models to determine a volume from cross-sectional images: the disc method (Simpson's rule), area-length and ellipsoid methods. A total number of 18 methods were explored.

Here, in order to simulate the problem in practice, Grubbs' method was applied on this data as if the true values of the volumes were not known. The standard deviations of the random errors as assessed by the Grubbs' estimators are given in the first column of Table I-1. In the second column the actual standard deviations of the random errors, based on the true volumes, are listed. The magnitudes of the standard deviations are not the same. However, it can be appreciated that both conclusions, drawn separately from the respective columns, about the relative random variation of the different methods would be exactly the same.

IN CONCLUSION

In this chapter it has been explained that the clinically relevant aspects of the reproducibility of a method of measurement can be assessed even without comparisons with a reference method. Differences in bias between observers and the total random error can be estimated by performing serial measurements in patients. Even the various components of the total random error of a measurement can be separated to a certain extent. With the results of this study one can answer clinically relevant questions as 'How many measurements are needed in one patient to reduce the confidence limits of the mean value to a preset level ?', or 'What

method of measurement	random errors	
	Grubbs sd(e) (ml)	direct sd(e) (ml)
echo disc biplane	7.2	14.7
echo disc A	19.4	21.8
echo disc B	22.7	25.2
echo area-length biplane	9.0	14.9
echo area-length A	37.5	36.1
echo area-length B	35.2	34.1
echo ellipse biplane	19.5	23.6
echo ellipse A	46.9	48.7
echo ellipse B	37.7	39.4
angio disc biplane	11.7	12.3
angio disc A	37.9	35.5
angio disc B	17.4	17.9
angio area-length biplane	15.5	14.9
angio area-length A	40.5	37.3
angio area-length B	17.5	17.6
angio ellipse biplane	6.8	11.2
angio ellipse A	20.8	24.5
angio ellipse B	19.0	19.5

Table I-1. Comparison of the Grubbs' estimators applied on the left ventricular volume measurements by 18 different methods versus the standard deviations of the actual random errors. The abbreviations A and B refer to different two-dimensional single-plane cardiac cross-sections.

difference in measurement represents a significant change of the cardiac function of a patient ?'.

It should be noted that differences in observer bias and the extent of the observer random error are observer dependent. Every self-respecting echocardiographic laboratory should assess their own values for these contributions to the measurement error. Otherwise the above mentioned questions can never be answered.

REFERENCES

1. Huang H, Scheffers M, Rijsterborgh H and Roelandt J. Does echocardiography allow the monitoring of the cardiac effects of nitrates? Eur Heart J 9, 1988: 51-55.
2. Armitage P. Statistical methods in medical research. Blackwell Scientific Publications. Oxford 1973: 189-202.
3. Grubbs FE. On estimating precision of measuring instruments and product variability. JASA 43, 1948: 243-264.
4. Lubsen J, Roelandt J, Rijsterborgh H and van Domburg R. Quantitative aspects of measurement error in echocardiography. In: Roelandt J (ed) The practice of M-mode and two-dimensional echocardiography. Martinus Nijhoff Publishers, The Hague/Boston/London 1983: 74-89.
5. Van Eekelen P. Het schatten van juistheid en precisie bij enkelvoudige gepaarde metingen met twee of drie instrumenten (in Dutch). Afstudeerverslag Technische Hogeschool Delft (afd. Wiskunde en Informatica, Vakgroep Statistiek) 1984.
6. Maloney CJ. Significance test for Grubbs' estimators. Biometrics 26, 1970: 671-676.
7. Erbel R, Krebs W, Henn G, Schweizer P, Richter HA, Meyer J. and Effert S. Comparison of single-plane and biplane volume determination by two-dimensional echocardiography. Eur Heart J 3, 1982: 469-480.

CHAPTER II

THE REPRODUCIBILITY OF CONTINUOUS WAVE DOPPLER MEASUREMENTS IN THE ASSESSMENT OF MITRAL STENOSIS OR MITRAL PROSTHETIC FUNCTION: THE RELATIVE CONTRIBUTIONS OF HEART RATE, RESPIRATION, OBSERVER VARIABILITY AND THEIR CLINICAL RELEVANCE

Hans Rijsterborgh, Anselmo Mayala, Tamas Forster, Wim Vletter, Bas van der Borden, George R. Sutherland and Jos Roelandt

Interuniversity Cardiology Institute of The Netherlands and Thoraxcentre, Erasmus University Rotterdam, The Netherlands

ABSTRACT

The reproducibility of continuous wave Doppler echocardiographic measurements of transmitral diastolic flow velocity were studied in terms of bias and random error in forty patients with either mitral stenosis or a Björk-Shiley mitral valve prosthesis. Twenty-seven patients were in sinus rhythm; thirteen patients had atrial fibrillation. Intra-observer and interobserver differences in bias were small for the Doppler parameters studied i.e. early peak velocity (0.6% vs 3.6%), mean diastolic velocity (1.1% vs 8.6%), mean temporal velocity (2.3% vs 14.5%) and pressure half-time (2.7% vs 4.8%).

The overall random error of the measurements (in terms of twice the standard deviation) was estimated separately in patients in sinus rhythm and atrial fibrillation: early peak velocity 5.6% and 9.2%, respectively, mean diastolic velocity 9.4% and 22%, mean temporal velocity 8.6% and 19% and pressure half-time 34% and 46%.

The relative contributions to the overall random error of observer variation, heart rate dependency and respiratory variation were also studied. Heart rate dependency was demonstrated for both the mean diastolic velocity and the pressure half-time. Respiratory variation was found in the early peak velocity.

From the results of this study the number of measurements to reduce the random error of the final average could be determined. Our results indicate that for the measurements in which a respiratory effect is present it is advisable to average the measurements taken over complete respiratory cycles.

European Heart Journal 11, 1990: 592-600

INTRODUCTION

Since no 'gold standard' for measuring blood velocities *in vivo* is available, the accuracy and precision of Doppler echocardiographic measurements cannot be assessed. However, the accuracy of the Doppler method is only of theoretical importance if the method can be proved reproducible.

The reproducibility of any method is determined by the consistent error (bias) and the random error of the measurement. The bias itself cannot be measured, since the true value of the measurement is unknown, but estimates of differences in bias due to different observers can be made in order to determine the reproducibility of the measurement. Differences in bias can be measured with intraobserver and interobserver tests.

Another determinant of the reproducibility of the method is the overall random variation of the measurements. Since this type of error is random, its influence will be diminished if more measurements are taken to obtain a mean value. Information about the extent of the overall random variation is essential to determine the number of measurements needed to reduce the random error of the final result to a desirable level. Overall random error can be estimated by taking consecutive measurements in a number of patients and applying the one way analysis of variance [1].

Several components of the overall random variation can be distinguished. The beat-to-beat variation of the measurements due to variation in heart rate is one. A second is due to variation caused by respiration. A third component is introduced by the observer measuring the Doppler recordings. The latter represents the non-consistent component of the measurement error made by the observer. Knowledge about the contribution of each of these components to the total variation of the measurements is essential to optimize the measurement procedure in an individual patient.

Most of the pre-existing literature [2-11] on the reproducibility of Doppler measurements deals with aortic flow velocity measurements. No previous study has either defined the reproducibility of continuous wave Doppler measurements of mitral flow velocity or determined the threshold values which constitute a significant difference in Doppler measurements over the clinical spectrum of abnormal diastolic mitral flow velocity.

The purpose of this study is to investigate the differences in bias, the overall random variation and its various components (heart rate, respiration and observer) in patients undergoing continuous wave Doppler evaluation of mitral valve function. Four basic Doppler parameters were studied for each diastole: the early (peak) diastolic velocity, the mean temporal velocity, the mean diastolic velocity and the

pressure half-time. Derived parameters such as the pressure drop and the orifice area were not included since they are directly related to the basic parameters which were being measured. The bias and random variation of the derived parameters could then easily be calculated from the results of this study. Since the pressure drop is calculated by squaring the velocity, the relative error of the pressure drop is twice the relative error of the velocity.

MATERIAL AND METHODS

To determine the reproducibility of continuous wave Doppler measurements for patients with mitral valve disease two groups of twenty patients each were studied.

The first group was randomly selected from a larger group of outpatients attending routine follow-up at the Thoraxcentre and consisted of ten patients with mitral stenosis and ten patients with a Björk-Shiley mitral valve prosthesis. Seven patients were in sinus rhythm and thirteen patients had atrial fibrillation. The second group consisted of ten consecutive outpatients with mitral stenosis and ten consecutive patients with a Björk-Shiley mitral valve prosthesis. Only patients in sinus rhythm were included in this study group. None of the patients were dyspnoeic.

Patients were examined in the left lateral decubitus position with the transducer in the apical position. Continuous wave Doppler recordings were made using a Toshiba SSH-65A with the lowest possible wall filter settings (400 Hz) at a paper speed of 50 mm/s. In patients of the second group an additional respiratory curve was recorded. The Doppler velocity recordings were traced and analysed using a personal computer (Olivetti M 24), a digitizing tablet (Summagraphics MM961) and dedicated software [12].

Doppler recordings of patients of the first group were analysed by measuring a number of consecutive cardiac cycles: ten cycles in patients in sinus rhythm and fifteen in cases of atrial fibrillation. All 263 recorded cardiac cycles were analysed three times: twice by a first investigator who was blinded to his previous results by a two-month interval and once by another investigator who did not know the results of the first observer. One observer had an experience of several years in tracing Doppler recordings; the other had a training of six months.

In recordings of the patients of the second group ten cardiac cycles were measured at end expiration and ten cycles at end inspiration. A total number of 400 cardiac cycles was analysed by one observer.

By tracing on a digitizing tablet the envelope of the Doppler velocity curve, the early- and mean diastolic and mean temporal velocities were derived [12]. The diastolic filling period and the QQ-interval were taken as the integration interval

to calculate the mean diastolic velocity and mean temporal velocity, respectively. The pressure half-time was defined as the time interval between the instant of peak velocity and the moment where the Doppler velocity curve had decreased to $1/\sqrt{2}$ times the peak velocity [4,8]. Smoothing of the velocity curve was done by a least square fit of a straight line through the initial part of the downslope of the curve. QQ-intervals were measured by indicating the Q-points of the QRS-complex of the ECG.

Statistical analysis

Differences in observer bias, observer random error, overall random error and heart rate dependency of the measurements were estimated from the data of the first group of patients in which consecutive measurements were made. Intra-observer and interobserver differences in bias were estimated by calculating the mean values, and standard deviations of the paired differences of every parameter.

The observer random error was estimated from the standard deviations obtained in the intraobserver test. The standard deviation of the paired differences represents the combination of two random errors introduced by the observer at both instances. Assuming that the random error had the same variance at both instances and the paired random errors did not correlate (their covariance is zero), the standard deviation of the random error produced by the observer was estimated by dividing the standard deviation of the paired differences by the square root of two.

The estimation of the overall random variation was done by calculating the mean values of the parameters in every patient and by analysing the differences between the consecutive measurements and their respective mean values using one way analysis of variance [1].

This was done separately for data obtained from patients in sinus rhythm and measurements made in patients with atrial fibrillation. The variation in the individual patient was expressed as a standard deviation and comparisons of variation in sinus rhythm and variation during atrial fibrillation were made by an F-ratio test. In order to compare the overall random variation of the different measurements, the range of the overall random error was calculated in terms of plus or minus twice the standard deviation expressed as a percentage with respect to the mean group value. Approximately 90% of the consecutive measurements in a patient will fall within this range.

The effects of heart rate

Investigation of the heart rate dependency of the measurements was performed by analysing the data of the group as well as the data obtained in every single patient. For the group analysis the relative differences of the consecutive measurements with respect to the mean value of the measurement in every patient were calculated for every parameter studied. Simple linear regression analysis was performed between the relative differences of the QQ-interval and the relative differences of the other parameters. This was done separately for the subgroup of patients with atrial fibrillation and the subgroup of patients in sinus rhythm.

The heart rate dependency was also studied in every patient separately. Simple linear regression analysis was performed between the consecutive measurements and the QQ-intervals of the corresponding cardiac cycles. Regression coefficients were tested against zero using a Student's t-distribution.

The effects of respiration

The influence of respiration on the measurements was studied in the second group of patients in which measurements were obtained at end inspiration and at end expiration only. Mean values of the ten measurements during end expiration and mean values of the ten measurements at end inspiration were calculated. A paired t-test was applied to investigate whether the differences were statistically significant. Scatterplots were made of the absolute differences and relative differences versus the mean value of the measurement at end inspiration to investigate whether the differences had a relationship with the magnitude of the measurement at end inspiration.

RESULTS

The results of the analysis of intra- and interobserver differences are listed in Table II-1 together with the mean values, standard deviations and ranges of the measurements obtained in the first group. Small, but significant differences ($p < 0.01$) were found in the Doppler parameters studied, except for the intraobserver difference in the measurement of pressure half-time. Scatterplots of the paired data of the early diastolic velocity, the mean diastolic velocity and the pressure half-time are shown in Figs. II-1 to II-3. Visual inspection of the scattergrams showed that the observer random variation of pressure half-time appeared to increase with increasing pressure half-time (Fig. II-3), whereas the random variation of the other measurements appeared to be constant within the ranges measured.

Doppler parameter		univariate statistics			intraobserver differences		interobserver differences	
		mean	sd	range	mean	sd	mean	sd
Early peak velocity	(m/s)	1.81	0.43	1.05-3.29	0.010*	0.052	0.065*	0.060
Mean diastolic velocity	(m/s)	1.21	0.45	0.48-2.66	0.013*	0.070	0.104*	0.079
Mean temporal velocity	(m/s)	0.64	0.28	0.29-1.66	0.015*	0.035	0.093*	0.037
Pressure half-time	(ms)	141	94.5	23-550	3.79	35.7	6.78*	41.2
Diastolic filling period	(ms)	438	112	151-788	4.75*	17.4	26.4*	18.7
QQ-interval	(ms)	817	135	457-1173	0.82	11.3	0.20	9.9

Table II-1. Mean values, standard deviation (sd) and ranges of 263 measurements in 20 patients (first group). Results of the intra- and interobserver test are shown on the right-hand side (= $p < 0.01$).*

Doppler parameter		observer variation		overall variation			
				sinus		a.f.	
		sd	(2xsd%)	sd	(2xsd%)	sd	(2xsd%)
Early peak velocity	(m/s)	0.037	(4.1%)	0.051	(5.6%)	0.083	(9.2%)
Mean diastolic velocity	(m/s)	0.050	(8.3%)	0.057	(9.4%)	0.134	(22%)
Mean temporal velocity	(m/s)	0.025	(7.8%)	0.028	(8.6%)	0.060	(19%)
Pressure half-time	(ms)	25.2	(36%)	23.8	(34%)	32.6	(46%)
Diastolic filling period	(ms)	12.3	(5.6%)	21.7	(9.9%)	100.8	(46%)
QQ-interval	(ms)	8.0	(2.0%)	25.2	(6.2%)	106.0	(26%)

Table II-2. The observer variation and the overall variation of the measurements in the patients of the first group. The percent ranges in terms of twice the standard deviation with respect to the mean group value are shown between brackets (2xsd%). Abbreviations: sinus = sinus rhythm; a.f. = atrial fibrillation.

The standard deviations of the estimated random errors introduced by the observer are listed in Table II-2, together with the standard deviations of the overall random error in sinus rhythm and in atrial fibrillation.

The overall random variation in sinus rhythm was significantly ($p < 0.01$) larger than the observer random variation in the measurements of the early peak velocity and the diastolic filling period (and the QQ-interval). The overall variation of the measurements obtained in atrial fibrillation proved to be significantly larger than the overall variation estimated in patients in sinus rhythm, except for pressure half-time.

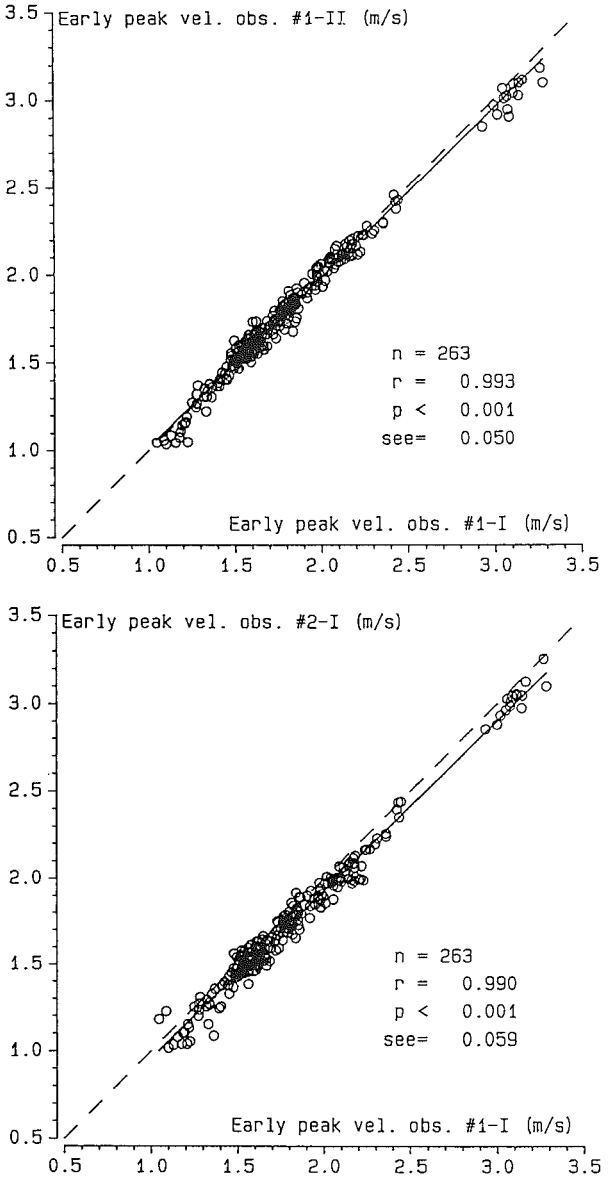

Fig. II-1. Scatterplots of the data obtained in the intra- and interobserver test of the measurement of the early peak velocity. Top: the first measurements of the first observer (horizontal) versus the second measurements of the first observer (vertical). Bottom: the first measurements of the first observer (horizontal) versus the measurements of the second observer (vertical). The solid line is the line of regression; the dashed line is the line of identity.

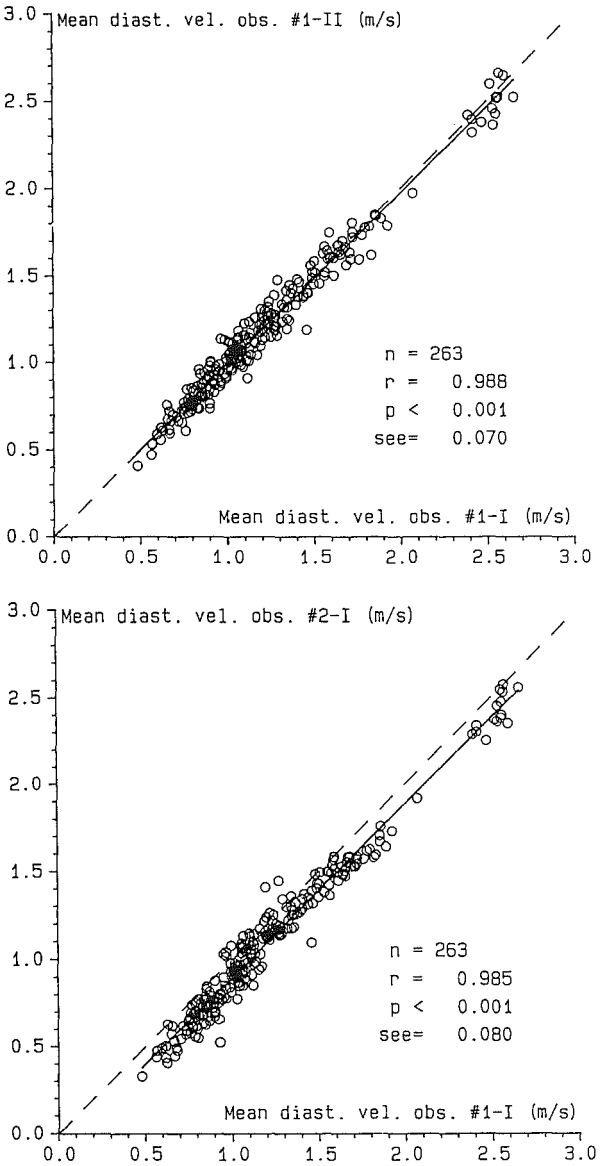

Fig. II-2. Scatterplots of the data obtained in the intra- and interobserver test of the measurement of the mean diastolic velocity. Top: the first measurements of the first observer (horizontal) versus the second measurements of the first observer (vertical). Bottom: the first measurements of the first observer (horizontal) versus the measurements of the second observer (vertical). The solid line is the line of regression; the dashed line is the line of identity.

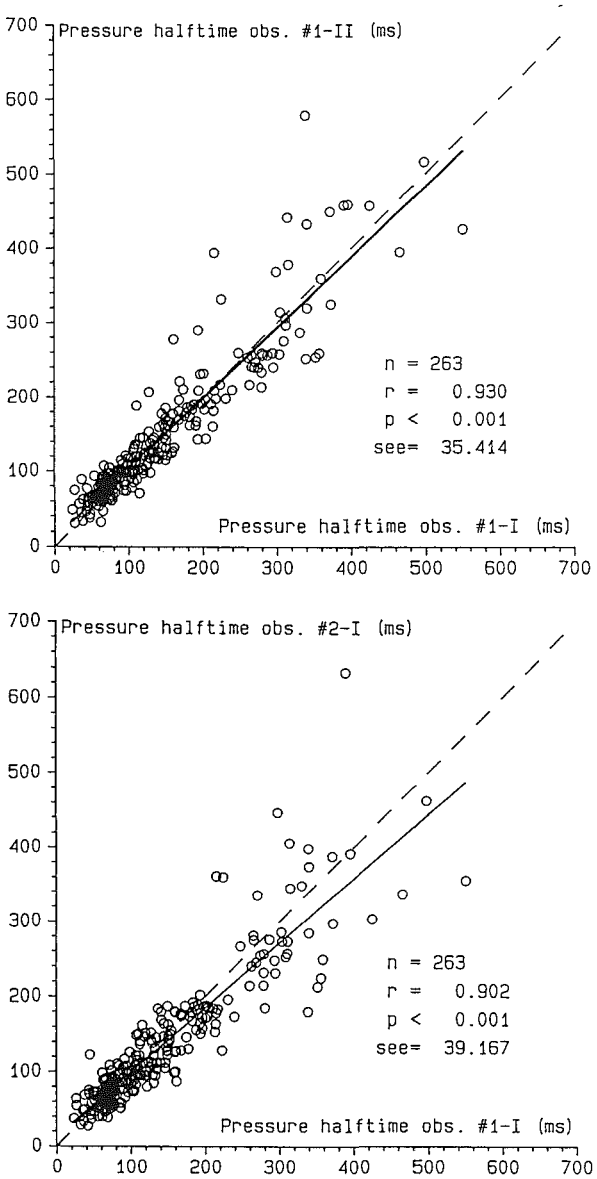

Fig. II-3. Scatterplots of the data obtained in the intra- and interobserver test of the measurement of the pressure half-time. Top: the first measurements of the first observer (horizontal) versus the second measurements of the first observer (vertical). Bottom: the first measurements of the first observer (horizontal) versus the measurements of the second observer (vertical). The solid line is the line of regression; the dashed line is the line of identity.

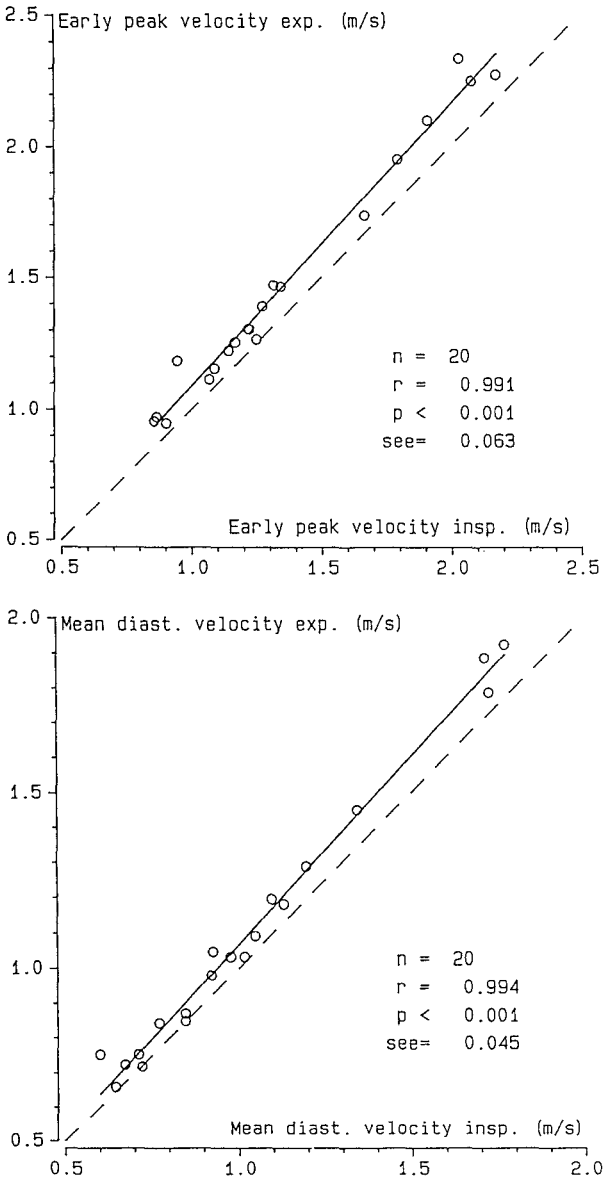

Fig. II-4. Scatterplots of the comparison of measurements taken at end inspiration (horizontal) and at end expiration (vertical). Top: early peak velocity. Bottom: mean diastolic velocity. Each data point is based on an averaged value of 10 measurements in every patient. The solid line is the line of regression; the dashed line is the line of identity.

Visual inspection of the scatterplots showed that the random variation expressed as a percentage relative to the mean value can be regarded as constant within the ranges measured.

Analysis of the heart rate dependency of the measurements in the subgroup of 13 patients with atrial fibrillation showed a significant correlation ($p < 0.01$) of three parameters with the QQ-interval. An increase of 10% in QQ-interval produced, on the average, a decrease of 7.6% in the mean diastolic velocity, an increase of 3.6% in pressure half-time and an increase of 17% in diastolic filling period. In the subgroup of 7 patients in sinus rhythm a significant increase of 11% in the diastolic filling period was found with an increase of 10% of the QQ-interval. For the other parameters no heart rate dependency could be proven.

Investigation of the heart rate dependency of the measurements in individual patients showed that in 8 of the 13 patients in atrial fibrillation a significant correlation ($p < 0.01$) was found between the mean diastolic velocity and the QQ-interval. For the mean temporal velocity and pressure half-time a significant correlation was found in four and one patient, respectively, out of the 13 atrial fibrillation patients. No relationship between heart rate and early peak velocity could be proven in any of these patients. In only one of the seven patients in sinus rhythm was a significant correlation with the QQ-interval found in the measurements of the mean diastolic velocity and mean temporal velocity. The results of the analysis of the paired differences of the measurements taken at end inspiration and the measurements at end expiration (second patient group) showed a statistically significant difference ($p < 0.01$) for the early peak velocity, mean diastolic velocity and mean temporal velocity. Scatterplots of the mean values of the measurements taken at end inspiration versus end expiration are shown in Fig. II-4.

Visual inspection of the scattergrams of the paired differences versus the measurements at end inspiration showed that the relative differences could be interpreted as being constant within the ranges measured. Compared with the end-inspiration measurements, the end-expiration measurements of the early peak velocity, the mean diastolic velocity and mean temporal velocity were 8.6%, 6.7% and 7.8% larger, respectively. No significant respiratory differences were found in the measurements of pressure half-time, diastolic filling period and QQ-interval.

DISCUSSION

Consistent error (bias)

The results of the intraobserver test showed that intraobserver differences for the four Doppler parameters studied are very small. The largest difference was found

in the measurement of pressure half-time: only 2.7% with respect to the mean group value.

Interobserver differences in bias were less than 5% in the measurements of the early peak velocity and the pressure half-time. Differences in bias of the measurements of the mean diastolic velocity (8.6%) and mean temporal velocity (14.5%) were larger.

It is noteworthy that the second observer underestimated all the velocity measurements compared with the first observer. The second observer must have indicated the envelope of the velocity curves somewhat lower. Another explanation for the relatively large differences in the measurements of the mean velocities may be found in the bad definition of the ending of the velocity curve due to the wall filter of the Doppler instrument, especially during the long QQ-intervals in atrial fibrillation. Therefore, Doppler tracings must be made with the lowest possible wall filter settings, if mean velocities are to be measured.

Observer differences in bias are not a real problem in quantitative Doppler. The difference between observers can easily be estimated and by analysing the possible causes they may be reduced. If this proves to be unsuccessful, the differences in bias can be taken into account.

Overall random variation

One of the goals of this study was to estimate the overall variation of the measurements in order to evaluate the influence of random errors on an average value of consecutive measurements in a single patient. The overall random variation of a mean value can be calculated by dividing the random variation of the measurement by the square root of the number of consecutive measurements. If we apply this to the measurements taken in patients in sinus rhythm (10 measurements) the overall variation of the mean value, in terms of twice the standard deviation, the result would be 1.8% for the early peak velocity, 3.0% for the mean diastolic velocity, 2.7% for the mean temporal velocity, 11% for pressure half-time and 3.1% for the diastolic filling period. These values are reasonably small, except for the pressure half-time measurements.

The overall random variation of the mean values of the 15 consecutive measurements taken in patients with atrial fibrillation in terms of twice the standard deviation can be calculated to be 2.4% for the early peak velocity, 5.7% for the mean diastolic velocity, 4.9% for the mean temporal velocity, 12% for the pressure half-time and 12% for the diastolic filling period. Most of these ranges are wider than those obtained in the mean values of the measurements made in patients in sinus rhythm. Thanks to the results of this study we can calculate the

number of measurements needed to achieve the same variation of the mean value as that obtained in sinus rhythm. For instance, it can easily be calculated that for the peak velocity we should have taken 26 consecutive measurements in patients with atrial fibrillation to obtain a variation of the mean value with the same range as for patients in sinus rhythm.

Components of the overall random error

From the comparisons of the standard deviation of the overall variation of the measurements of the patients in sinus rhythm and the observer variation, it can be concluded that the overall random errors of the mean diastolic velocity, the mean temporal velocity and the pressure half-time are mainly determined by the random error of the observer. The overall random variations of the other variables are only partly determined observer variation.

The fact that the overall random variation as estimated in patients with atrial fibrillation is larger than that found in sinus rhythm is no proof that the larger variation was caused by the changes in heart rate, but merely an indication. The final proof was found in the correlation of the changes of QQ-interval with the changes of the measurements.

The measurements of the mean diastolic velocity, the pressure half-time and the diastolic filling period showed a dependency on heart rate in the patients with atrial fibrillation. It should be noted that the (weak) relationship between the pressure half-time and QQ-interval is in contradiction with the literature [4].

The heart rate dependency of the mean diastolic velocity could not be shown in every single patient, perhaps because the basic heart rate dependency is covered by other influences such as observer variation or differences due to respiration. Another factor may be the small range of variation of the QQ-interval as in patients with sinus rhythm. In both cases one needs more than 10 or 15 measurements to prove a relationship.

For the investigation of the influence of the respiration on the measurements only patients in sinus rhythm were considered. Therefore, the measurements were supposed to be independent of changes in heart rate. By taking measurements at end inspiration and end expiration we intended to estimate only the maximum (plus or minus) value of the contribution to the variation due to respiration. Respiratory dependency was shown in the measurements of the early peak velocity, the mean diastolic velocity and mean temporal velocity. If we translate the observed differences into a range of maximum variation (plus or minus half the observed differences in the comparison of expiration versus inspiration), this would be $\pm 4.3\%$ for the early peak velocity, $\pm 3.4\%$ for the mean diastolic velocity and $\pm 3.9\%$ for the

mean temporal velocity. Concerning the mean velocities these values are small compared with the range of observer variation, in terms of twice the standard deviation. This is consistent with the previous conclusion that the overall random variation of the mean diastolic velocity, the mean temporal velocity in sinus rhythm is mainly determined by the observer random variation.

No respiration dependency was found in the measurement of pressure half-time. This finding is also in accordance with the previous conclusion that the overall random variation of this measurement depends mainly on observer random variation. In the measurement of the early peak velocity the range of variation in terms of twice the standard deviation is approximately equal to the range of respiratory variation. This explains the previous finding that the overall variation of this measurement is not determined by the observer random variation alone. Therefore, it must be concluded that the overall variation is determined by a combination of random observer variation and respiratory variation.

Clinical implications of this study

In order to diminish the influence of random variation, one has to take an average value of a number of consecutive measurements. Since some of the measurements are influenced by respiration it would be advisable to measure the consecutive cardiac cycles over a complete respiratory cycle. With the results of this study, one can, as explained above, estimate the random error of a mean value given the number of measurements.

Another clinically relevant application is to calculate the minimum difference needed to decide whether a difference seen during follow-up is indeed significant. The threshold differences are dependent on the number of measurements and the significance level. In Table II-3 these values have been calculated for 10 measurements (patients in sinus rhythm) and 15 measurements (patients in atrial fibrillation) at two different levels of significance. Since the relative random variation can be regarded as constant within the measured ranges, the threshold differences are expressed as percentages. It should be noted that these differences are caused by random variation only. In order to determine the actual difference needed to obtain a significant difference, one should add the inter- or intraobserver differences in bias as measured. Since these differences will depend on the respective observers, every echocardiographic laboratory should assess its own inter- and intraobserver differences in bias after standardization of the method of measurement.

From the above findings the clinical value of precise pressure half-time measurements is in doubt, owing to random variability. In the clinical setting our

	sinus (n=10)		a.f. (n=15)	
Doppler parameter	p<0.05	p<0.01	p<0.05	p<0.01
Early peak velocity	2.6%	3.6%	3.4%	4.6%
Mean diastolic velocity	4.4%	6.1%	8.2%	11%
Mean temporal velocity	4.0%	5.5%	7.1%	9.6%
Pressure half-time	16%	22%	17%	23%
Diastolic filling period	4.7%	6.4%	17%	23%
QQ-interval	2.9%	4.0%	9.7%	13%

Table II-3. Threshold values needed in a single patient for a significant difference in Doppler measurements due to random variation only. Abbreviations: sinus = sinus rhythm; a.f. = atrial fibrillation; n = number of measurements; p = level of significance.

findings suggest that changes in pressure half-time smaller than 15% may not be significant and may be caused by random variation.

The use of pressure half-time in the evaluation of native mitral valve stenosis or prosthetic valve stenosis must be approached with caution. Only changes in pressure half-time greater than 15% should be viewed as being significant. The value of ±15% is constant over the whole spectrum of pressure half-time values. This value obviously improves, if more measurements are taken to obtain a mean value due to the random nature of the variability.

CONCLUSIONS

In patients with mitral stenosis or mitral prostheses the overall random variation of the measurements of mean diastolic velocity and mean temporal velocity are mainly determined by observer random variation with a small, but significant contribution of respiratory variation. The random variation of the measurements of the early peak velocity is determined by a combination of random observer variation and respiratory variation.

REFERENCES

1. Armitage P. Statistical methods in medical research. Oxford: Blackwell Scientific Publications, 1973: 189-202.
2. Gardin JM, Burn CS, Childs WJ and Henry WL. Evaluation of blood velocity in the ascending aorta and main pulmonary artery of normal subjects by Doppler echocardiography. Am Heart J 107, 1984: 310-319.

3. Gardin JM, Dabastani A, Matin K, Allfie A, Rusell D and Henry WL. Reproducibility of Doppler aortic blood flow measurements: Studies on intra-observer, inter-observer and day-to-day variability in normal subjects. Am J Cardiol 54, 1984: 1092-1098.
4. Hatle L, Angelson B and Tromsdal A. Noninvasive assessment of atrioventricular pressure half-time by Doppler ultrasound. Circulation 60, 1979: 1096-1104.
5. Krafchek J, Robertson JH, Radford M, Adams D and Kisslo J. A reconsideration of Doppler assessed gradients in suspected aortic stenosis. Am Heart J 110, 1985: 765-773.
6. Meijboom EJ, Rijsterborgh H, Bot H, De Boo JAJ, Roelandt JRTC and Bom N. Limits of reproducibility of blood flow measurements by Doppler echocardiography. Am J Cardiol 55, 1987: 133-137.
7. Ramirez ML and Wong M. Reproducibility of standalone continuous wave Doppler recordings of aortic flow velocity across bioprosthetic valves. Am J Cardiol 55, 1985: 1197-1199.
8. Smith MD, Handshoe R, Handshoe S, Kwan OL and DeMaria AN. Comparative accuracy of two-dimensional echocardiography and Doppler pressure half-time methods in assessing severity of mitral stenosis in patients with and without prior commissurotomy. Circulation 73, 1986: 100-107.
9. Stewart WJ, Jiang L, Mich R, Pandian N, Guerrero JL and Weyman AE. Variable effects of changes in flow rate through the aortic, pulmonary and mitral valves on valve area and flow velocity. Impact on quantitative Doppler flow calculations. J Am Coll Cardiol 6, 1985: 653-662.
10. Wallerson DC and Devereux RB. Reproducibility of quantitative echocardiography: factors affecting variability of imaging and Doppler measurements. Echocardiography 3, 1986: 219-235.
11. Williams GA and Labovitz AJ. Doppler hemodynamic evaluation of prosthetic (Starr-Edwards and Björk-Shiley) and bioprosthetic (Hancock and Carpentier-Edwards) cardiac valves. Am J Cardiol 56, 1985: 325-32.
12. Van der Borden SG, Roelandt J and Rijsterborgh H. Computer-aided analysis of Doppler echocardiograms. In: Roelandt J, ed. Color flow imaging and other advances in Doppler echocardiogaphy. Dordrecht: Martinus Nijhoff Publishers, 1986: 39-49.

CHAPTER III

DOPPLER ASSESSMENT OF AORTIC STENOSIS: BERNOULLI REVISITED

Hans Rijsterborgh and Jos Roelandt

Thoraxcenter, Erasmus University, Rotterdam and
Interuniversity Cardiology Institute, The Netherlands

ABSTRACT

The application of Bernoulli's law allows the non-invasive estimation of pressure drops across stenotic valves from Doppler velocity measurements. However, several assumptions are to be made, which influence the accuracy of the pressure drop estimation. Energy losses, non-uniform velocity profiles, pressure recovery, unsteady flow and omission of the upstream velocity measurement affect accuracy and are critically reviewed.

In vitro experiments, published in the literature, show good correlations between estimated and actual pressure drop. In only one of these studies does the data allow a comparison between theory and practice and to study the relationship between the pressure drop at the inlet of the obstruction and the Doppler velocity measurement. It appears that this relationship is not completely described by the simplified Bernoulli equation.

In vivo verifications of Bernoulli's law show favorable correlations. However, the expected differences between peak pressure drops measured by cardiac catheterization and the pressure drops estimated by Doppler echocardiography may be as high as 25 mmHg.

Ultrasound in Medicine and Biology 13, 1987: 241-248

INTRODUCTION

More than three decades ago Gorlin and Gorlin [1] used the relationship between the pressure drop across a stenotic valve and the blood flow velocity which was already formulated by Bernoulli [2], a Dutch born mathematician in the 18th century, for the calculation of the orifice area. In their approach they estimated the blood flow velocity at the stenotic orifice by an invasive pressure drop measurement.

The application of Bernoulli's law has received renewed interest because a direct and accurate blood velocity measurement can be obtained by Doppler echocardiography allowing pressure drops across stenotic valves to be estimated. The Bernoulli equation relates the pressure drop across the inlet of an obstruction in a flow channel to the flow rate through it. Although this relationship is based on fundamental physical laws, a number of assumptions are to be made when applied for calculating pressure drops across stenotic valves.

We will critically review these assumptions and assess their influence on the accuracy of the pressure drop estimation from Doppler velocity recordings in valvular aortic stenosis.

THE THEORY OF BERNOULLI'S LAW

Let us consider a circular flow channel which includes a narrow segment as depicted in Fig. III-1. In this simple model, steady flow is assumed and energy losses within the fluid are neglected. The relationship between flow rate, fluid velocity and pressure at the inlet of the flow obstruction is basically described by two laws: the law of mass conservation and the law of energy conservation which are both integrated in Bernoulli's law.

The law of conservation of mass describes the fact that the flow rate is constant at every cross-sectional area of the model and is represented by the equation:

$$Q = A_1 \cdot v_1 = A_2 \cdot v_2 \qquad (1)$$

in which Q is the flow rate (cm^3/s), A_1 and A_2 the respective cross-sectional areas (cm^2), v_1 and v_2 the respective fluid velocities (cm/s) at pressure taps I and II. This relationship is called the continuity equation; it relates the fluid velocity with the cross-sectional area of flow at a given volume flow (Fig. III-1). As the fluid enters the obstruction in the flow channel its velocity must increase.

Bernoulli's law is represented by the equation:

$$p_1 - p_2 = \tfrac{1}{2} \cdot \rho \cdot (v_2^2 - v_1^2) \qquad (2)$$

in which ρ is the density of the fluid, p_1-p_2 represents the pressure drop between taps I and II, v_1 and v_2 are the respective fluid velocities. The right-hand term of eqn (2) represents the increase in kinetic energy (velocity) of the fluid as it passes from tap I to tap II, whereas the left-hand term is the difference in potential energy (pressure) of the fluid. The result is a low pressure at tap II as compared to the pressure at tap I. The kinetic energy of the fluid is increased at the expense of the

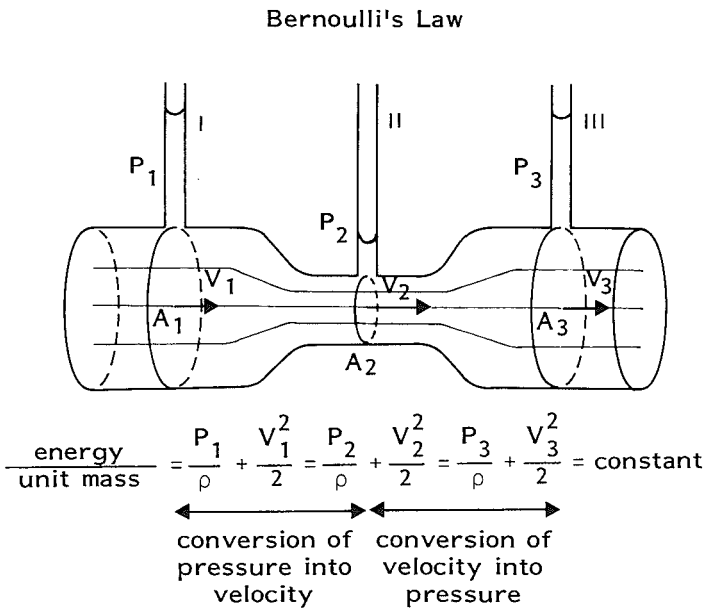

Fig. III-1. Model of a flow channel including a narrow segment. At every cross-sectional flow area the total energy per unit mass is constant. Without energy losses the pressure drop between tap I and tap II is fully recovered as the fluid passes from tap II to tap III and there is no pressure difference between tap I and tap III.

potential energy, keeping total energy constant. The formal derivation of eqn (2) can be found elsewhere [3].

If the upstream fluid velocity is assumed to be low as compared to the velocity at the site of the obstruction, and the density of the blood (1024 kg/m^3) entered into the Bernoulli equation it is reduced into its most simple form:

$$p_1\text{-}p_2 = 4 \cdot v_2^2 \qquad (3)$$

in which p_1-p_2 is the pressure drop between taps I and II in mmHg, and v_2 is the fluid velocity at tap II in m/s. It should be noted that eqns (2) and (3), describe the relationship between the pressure drop and velocity at the inlet of the obstruction. In order to assess the pressure drop across the obstruction (p_1-p_3) one must include the effects of flow dynamics which occur in the downstream or outlet region and which will be discussed later. The application of Bernoulli's law in the non-invasive estimation of a pressure drop is simple and straightforward. The blood velocity at the site of the obstruction can be accurately measured with an ultrasound Doppler

instrument and in clinical practice the pressure drop is calculated using eqn (3).

However, several assumptions are to be made. Firstly, we must assume that the conversion of potential energy into kinetic energy takes place without energy losses. Otherwise the pressure drop will be larger as obtained by the Bernoulli equation at a given flow rate.

Secondly, we must assume a uniform velocity distribution at the cross-sectional flow area in the obstruction. Otherwise the right-hand term of eqn (3) does not represent the increase of kinetic energy of the fluid.

In case of non-uniform velocity profiles, Bernoulli's law still holds but the solution of eqn (2) becomes more complicated. In order to calculate the increase in kinetic energy one has to average the squares of the spatial velocities over the cross-sectional area of the obstruction. Unfortunately, the distribution of flow velocities through an obstruction cannot be obtained by a standard Doppler instrument, which measures the maximum velocity at the site of the obstruction and not the actual spatial distribution of the velocities. Therefore in case of non-uniform velocity profiles a perfect relationship between the maximal velocity measured with a Doppler instrument and the actual pressure drop will not be obtained.

THE PRACTICE OF BERNOULLI'S LAW

In fluid mechanics a device similar to the model shown in Fig. III-2 is commonly used to measure flow rates [4]. Theoretically the combination of the continuity equation and Bernoulli's law relates the pressure drop at the inlet of the orifice with the flow rate through it. In practice, however, the observed pressure drops are larger as predicted by the theory. As a result, empirically derived correction factors are listed in handbooks known as 'coefficients of discharge' [4] in order to estimate the actual pressure drop for a given geometry of the flow channel at a given flow rate. The discrepancy between theory and practice is in part explained by the assumption that the actual volume flow is contracted to a smaller cross-sectional area of flow at some distance from the orifice (the 'vena contracta') which is dependent upon the shape of the inlet. Thus the effective cross-sectional flow area is as compared to the actual orifice area, resulting in a larger pressure drop. A further increase in pressure drop may be caused by energy losses.

The magnitude of the discrepancy between theory and practice may be illustrated by the following example. Given a steady flow rate of 300 cm^3/s and cross-sectional flow areas of 3 cm^2 and 1 cm^2, respectively, the application of the continuity eqn (1) results in velocities of v_1 = 100 cm/s and v_2 = 300 cm/s. If the test fluid is water (ρ = 1000 kg/m^3), the Bernoulli eqn (2) gives 30 mmHg for the

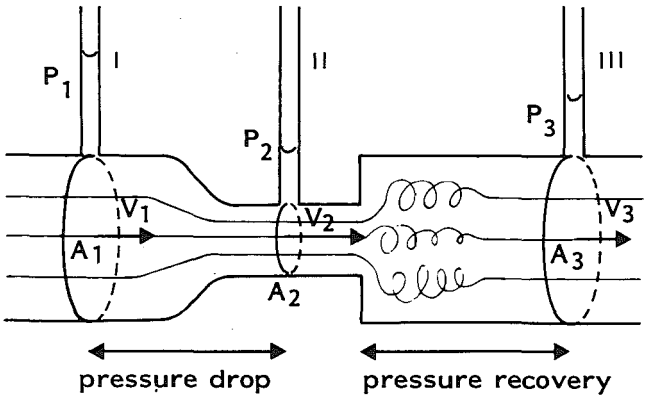

Fig. III-2. Model of a valvular stenosis. Between tap II and tap III most of the kinetic energy is lost due to turbulence and the pressure is prevented from being fully recovered.

theoretical pressure drop. In order to estimate the actual pressure drop, corrections have to be made by the empirically determined coefficient of discharge [5]. In this situation (square edged orifice, pipe Reynolds $\sim 10^4$, area ratio $A_2/A_1 = 0.33$) the coefficient of discharge is 0.64, corresponding to an actual pressure drop of 73 mmHg, which is more than twice the theoretical value. Following the theory of the 'vena contracta', the measured velocities are higher as predicted by the continuity equation at a given flow rate and occur at some distance downstream from the stenosis. One may question whether the excess in measured velocity is in accordance with the surplus of the actual pressure drop as calculated in the example above.

In most studies on the verification of Bernoulli's law *in vitro* [5-11] no reference is made to the flow rates at which the experiments were conducted. 'Look-alike models' as depicted in Fig. III-2 were used in all of these studies, but comparisons were only made between the pressure drops calculated by the simplified Bernoulli eqn (3) and the actual pressure drops. Therefore no conclusions can be drawn on the differences between the theoretical velocities and pressures (given by the continuity equation and Bernoulli's law) and the measured velocities and pressures.

In recent literature the only experiments in which velocity and pressure measurements were accompanied by independent flow rate measurements were published by Requarth et al. [5]. Their data offer the opportunity to directly compare theory and practice. In Table III-1 the theoretical velocities calculated from the continuity eqn (1) on the basis of the geometry of the model and the flow rates are listed together with the theoretical pressures calculated by eqn (2) and the

actual measurements. From these data it is obvious that the measured pressure drops are much higher than the theoretical pressure drops. The same conclusions may be drawn with regard to the velocity at the site of the orifice. However, despite these large differences between theory and practice, the differences between the actual pressure drops and the estimation of the pressure drops by the simplified Bernoulli eqn (3) are within reasonable limits, except for trials 6-8. Thus, the Requarth study indicates that the velocities measured were always higher than expected from the theory assuming a uniform flow velocity profile.

This suggests a non-uniform velocity profile. In case of a non-uniform velocity profile the pressure drops are always higher as compared to the pressure differences of a uniform velocity distribution at the same volume flow rate. Therefore the existence of a non-uniform velocity profile would explain both the higher velocities and the higher pressure drops. But the relationship between pressure drop and velocities is not described by the Bernoulli equation, since the right-hand term of eqn (2) does not represent the increase in kinetic energy of the fluid.

CAN THE VELOCITY PROXIMAL TO THE OBSTRUCTION BE NEGLECTED ?

In order to apply the simplified Bernoulli equation for the estimation of pressure drops, the assumption has to be made that the proximal velocity is small as compared to the velocity at the site of the obstruction. The relationship between the upstream velocity v_1 and velocity v_2 is determined by the continuity equation: $v_1 = v_2 \cdot A_2/A_1$. Substituting this relationship into eqn (2) gives:

$$p_1 - p_2 = \tfrac{1}{2} \cdot \rho \cdot v_2^2 \cdot (1-(A_2/A_1)^2) \qquad (4)$$

The bracketed term is a constant multiplicative factor, determined by the respective flow areas and independent of the measured velocity v_2. This means that the commonly made assumption that v_1 can be neglected when v_2 is relatively high does not hold since v_1 changes proportionately with v_2. The result of the omission of the proximal velocity is an overestimation of the pressure drop. Thus it should be realized that the extent of the overestimation is determined by the ratio of the cross-sectional flow areas and not by the measured velocity v_2.

In clinical practice the ratio of the cross-sectional flow areas is unknown. Therefore the application of the simplified Bernoulli equation is influenced by an unknown factor that may be different in any given patient. It becomes relatively less important when the stenotic orifice becomes smaller. An estimate of the upstream velocity can be made by a pulsed Doppler instrument.

trial no	flow area A_2 cm^2	flow rate cm^3/s	Bernoulli's theory v_1 cm/s	Bernoulli's theory v_2 cm/s	Bernoulli's theory p drop mmHg	measurements v_2 cm/s	measurements p drop mmHg	calc. p drop mmHg
1	0.842	140	36	166	9.9	240	20.0	21.6
2	0.842	130	34	154	8.5	225	18.0	19.0
3	0.842	113	29	135	6.5	195	13.0	14.3
4	0.842	92	24	109	4.2	160	8.0	9.6
5	0.842	83	22	99	3.5	150	7.0	8.4
6	0.568	102	26	179	11.8	280	21.1	29.4
7	0.568	90	23	158	9.2	240	14.0	21.6
8	0.568	82	21	144	7.6	230	13.0	19.8
9	0.568	70	18	123	5.6	180	12.0	12.2
10	0.568	50	13	88	2.8	130	4.2	6.3
11	0.294	102	26	346	44.6	400	55.0	60.0
12	0.294	87	22	295	32.4	340	44.9	43.4
13	0.294	78	20	266	26.5	300	35.1	33.8
14	0.294	63	16	215	17.3	260	25.0	25.3
15	0.294	50	13	170	10.8	210	19.0	16.5

Table III-1. Theoretical values and measurements of in vitro flow experiments (measurements derived from Requarth et al. [5]). The theoretical velocities v_1 and v_2 were calculated using the continuity equation (A_1 = 3.87 cm^2). The theoretical pressure drops were calculated by eqn (2). The calculated pressure drops (last column) were based on the simplified Bernoulli eqn (3). Since the test fluid was water the multiplicative factor 3.75 was used in the pressure drop calculations.

ENERGY LOSSES AT THE INLET OF THE STENOSIS

Clark [12] formulated a theory relating the pressure difference across a stenosis to the flow through it considering the processes that occur at the inlet region and at the area distal to the obstruction. In a laboratory model the theory was tested. Several nozzle designs were used to represent different degrees in severity of obstruction. When energy losses at the inlet were taken into account the following relationship could be derived when the fluid passes from tap I to tap II (Fig. III-2):

$$p_1 - p_2 = \tfrac{1}{2} \cdot \rho \cdot v_2^2 / C_d^2 \qquad (5)$$

Flow velocity proximal to the obstruction is assumed to be relative to the velocity at the site of the obstruction. The energy losses at the inlet region are represented by the nozzle coefficient C_d. The value of the nozzle coefficient varies from one to zero with increasing energy losses. A nozzle coefficient which equals one corresponds to a situation without energy losses. In that theoretical situation the

eqns (4) and (2) are then identical.

The nozzle coefficients of various nozzle shapes were determined. The optimal nozzle shape with regard to minimal energy losses reached a nozzle coefficient close to 0.9. This means that an increase in pressure drop of 24% as compared to the ideal situation without energy losses. Therefore, even when using water as a flowing fluid and a conical tapered inlet of the obstruction, the differences should not be neglected. If in the experiments the velocity at the site of the obstruction would have been accurately measured by a Doppler instrument and used in eqn (3) the actual pressure drop would be underestimated by 19%.

The question arises whether these results would have been different if blood had been used as a test fluid instead of water. Blood is a mixture of fluid and small particles and its viscosity is higher as compared to water. Yoganathan [13] published data on pressure drops across prosthetic valves measured in a laboratory set-up. Two different test fluids were used: water and a polyol solution, which has approximately the same viscosity as blood. Their results indicate that the error of the pressure drops obtained with the polyol solution and water were similar (± 0.5 mmHg). Thus, it appears that the viscosity of the fluid has little influence on the energy losses within the physiologic range of viscosities.

Two conclusions can be made here. Firstly, even under optimal conditions (geometrical shape of the inlet of the obstruction), the energy losses are considerable. Secondly, the energy losses vary with the size and shape of the obstruction in the flow channel. In clinical practice the size and the shape of the stenosis will vary from patient to patient. The extent of the underestimation of the pressure drop by Doppler echocardiography across the inlet of the stenosis resulting from different sizes and shapes of the obstruction remains an uncertain factor.

PRESSURE RECOVERY DISTAL TO THE STENOSIS

As already mentioned, the Bernoulli equation describes the relationship between the pressure drop across the inlet of the obstruction (p_1-p_2 in Fig. III-2) and the velocity at the site of the obstruction. In order to calculate pressure drops across an obstruction (p_1-p_3 in Fig. III-2) from blood velocity measurements, the assumption is made that distal to the stenosis the pressure remains constant with distance (p_3-p_2=0). However, the sudden expansion of the cross-sectional flow area produces flow turbulence in which part of the kinetic energy is lost and the pressure is prevented from being fully recovered. However, part of the pressure will be recovered, resulting in a gradual pressure increase from tap II to tap III.

Both Clark [12] and Yoganathan [13] have studied the area distal to the obstruction in their respective laboratory models. In the model of aortic stenosis

as well as in the prosthetic valve model considerable quantities of pressure recovery were observed. In the region distal to the obstruction the pressure gradually increases. With the model of aortic stenosis the pressure recovery extended to 9 cm downstream of the nozzle. The results of the prosthetic valve study indicate that the region of pressure recovery can extend as far as 15 to 27.5 cm downstream of the valve.

Clark [12] derived an equation for the total pressure recovery:

$$p_3 - p_2 = \rho \cdot v_2^2 \cdot ((A_2/A_3) - (A_2/A_3)^2) \qquad (6)$$

It appears that the pressure recovery is determined by the velocity at the site of the obstruction and by the ratio between the cross-sectional flow areas at the obstruction and that of the flow channel distal to the obstruction.

In clinical practice, the maximal blood velocity at the orifice of a stenotic valve can be accurately measured by Doppler echocardiography. The ratio of the stenotic area to the anatomical cross-sectional area distal to the valve, however cannot be accurately measured.

Pressure recovery is therefore another uncertain factor in the calculation of the pressure drop across a stenotic valve and will introduce some uncertainty when pressure drops across stenotic valves are calculated from Doppler velocity traces and compared with those obtained by catheter. To obtain a good pressure recording the catheter must be positioned somewhere outside the turbulent area in the region of pressure recovery which is at a variable distance from the stenotic valve. It is therefore unknown which part of the pressure recovery is included in the pressure drop measurement. A direct comparison between pressure drops calculated from Doppler velocities and catheter measurements therefore has limitations.

BERNOULLI'S LAW AND UNSTEADY FLOW

Basically the blood velocity within the heart is pulsatile and varies in time. Due to inertia of the blood it will take some time until the blood elements reach the velocity dictated by the pressure difference. Therefore in case of acceleration or deceleration of the blood, the maximal velocity will be delayed with respect to the instantaneous pressure difference.

The differences between instantaneous maximal velocities and pressure differences in case of unsteady flow are mathematically described by the acceleration term. The acceleration term is related to the change in velocity of the fluid versus time, that is, the slope of the velocity curve on the Doppler recording. Steep slopes, either negative or positive, will result in larger errors in the

relationship between velocities and pressures estimated by the simplified Bernoulli equation.

In Hatle et al. [14] an estimate is given of the acceleration term in patients with mitral stenosis. Slopes up to 1 m/s are related to negligible errors (< 0.2 mmHg) in the calculation of the pressure drop. Similar estimations with the same results can be made in aortic stenosis.

ACTUAL PRESSURE DROP ACROSS A STENOTIC VALVE

It appears that the uncertain factors influencing the accuracy of the pressure drop calculation from Doppler velocities are the energy losses at the inlet of the stenosis and the pressure recovery in the region distal to the obstruction. By combining eqn (5), describing the pressure drop across the inlet considering energy losses, and eqn (6), which describes the pressure recovery, a formal expression regarding the actual pressure drop across the stenotic valve can be derived:

$$p_1 - p_3 = \tfrac{1}{2} \cdot \rho \cdot v_2^2 \cdot [(1/C_d^2) - 2 \cdot (A_2/A_3) + 2 \cdot (A_2/A_3)^2] \qquad (7)$$

The square bracketed term is independent of both the flow rate and the velocity at the site of the obstruction. In practice it turns out that the underestimation caused by neglecting the energy losses is partly compensated by the overestimation due to pressure recovery. This may be illustrated by the following examples.

Mild aortic stenosis with an orifice area of 1.2 cm^2. We assume a nozzle coefficient of 0.9 and a cross-sectional flow area of 4.9 cm^2 in the aorta. The term between square brackets in eqn (7) is 0.84. Thus the simplified Bernoulli equation will overestimate the actual pressure drop across the stenotic valve by 16%.

Severe aortic stenosis with an orifice area of 0.5 cm^2. We assume the same values of the nozzle coefficient and cross-sectional flow area of the aorta. The term in eqn (7) is now 1.05. This results in an underestimation by 5% when the simplified Bernoulli equation is applied.

Thus when the simplified Bernoulli equation is applied the bracketed term of eqn (7) is assumed to equal one. This will result in underestimation of severe aortic stenosis and overestimation of the pressure drop in mild to moderate disease.

LITERATURE REVIEW

So far, more than ten studies have been published [15-25] comparing pressure drops obtained from Doppler velocity traces by the simplified Bernoulli equation with those measured by catheter during cardiac catheterization in patients with aortic stenosis. Favorable correlations were found in most studies. However, normal

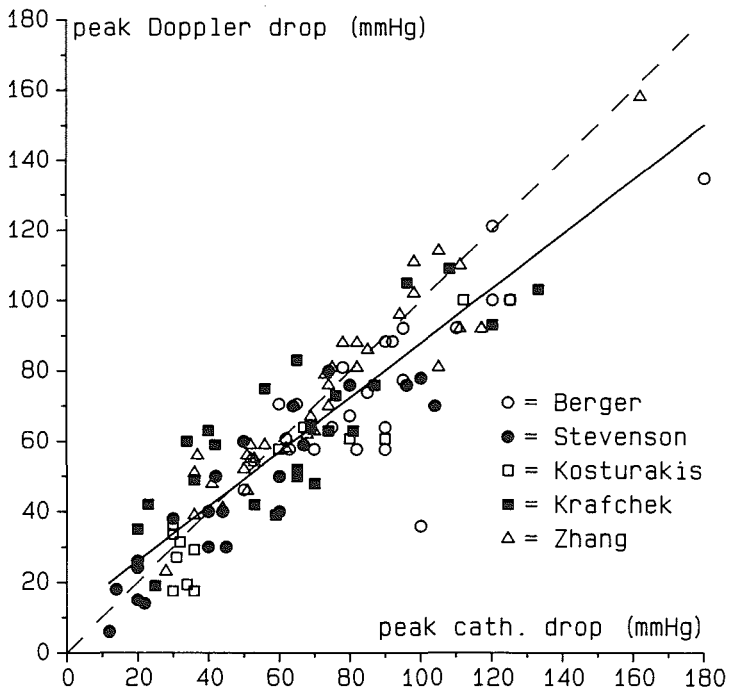

Fig. III-3. Comparison of peak pressure drops at catheterization versus peak pressure drops estimated by Doppler echocardiography. Shown are 118 paired data points obtained in five different studies published in the literature. The dashed line is the line of identity. The continuous line is the regression line. The different symbols refer to the different investigators.

subjects and patients without pressure differences across the aortic valve were included in some of these studies. It is clear from eqn (4) that in these conditions the Bernoulli equation cannot be applied in its simplified form.

From the studies which reported their original data, 118 paired data of peak Doppler pressure drop and peak pressure drop by catheter can be obtained excluding those with a peak pressure drop at catheterization less than 10 mmHg. Fig. III-3 shows the plot of these paired data. A good correlation is found ($r = 0.89$). However, individual differences are up to 25 mmHg and independent of the magnitude of pressure drop found during cardiac catheterization (Fig. III-4). This means that the percentage or relative differences increase when the pressure differences across the stenotic valve becomes smaller indicating that the simplified Bernoulli equation becomes unreliable when applied in patients with mild aortic

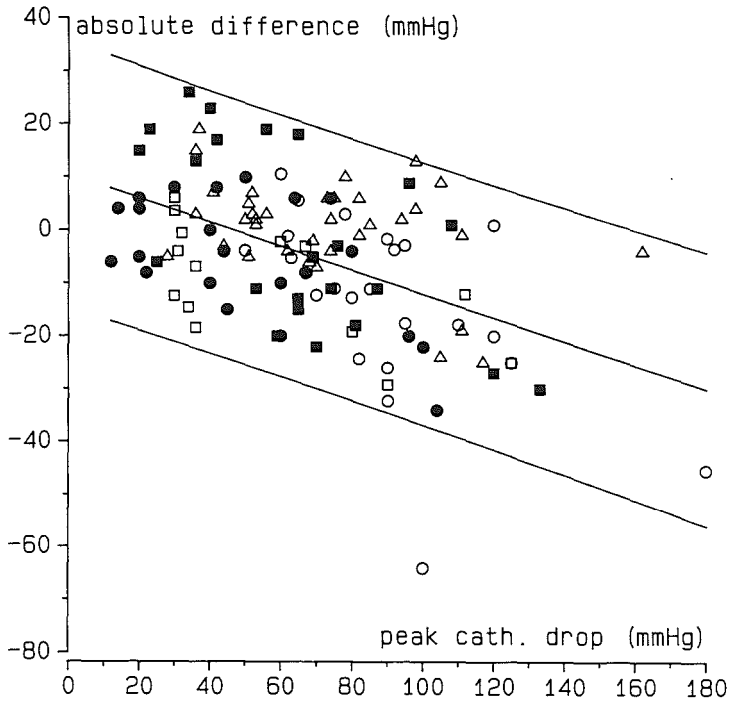

Fig. III-4. Plot of the absolute pressure drop differences (Doppler measurements minus catheterization measurements) versus the peak catheterization pressure drops. Shown are the regression line and the 5th-95th percent confidence limits.

stenosis (Fig. III-5).

From Fig. III-4 it becomes clear that below a peak catheterization pressure drop of 50 mmHg the pressure difference obtained by the simplified Bernoulli equation tends to be overestimated while above a pressure difference of 50 mmHg the Bernoulli equation underestimates the pressure difference obtained by catheter. This is in agreement with the theory as predicted by eqn (7). In Fig. III-4 the 5%-95% confidence limits are indicated. If the differences between Doppler and catheter derived pressure drops are assumed to be random, we expect a normal distribution of the data points. Thus 10% of the data are expected to fall outside the 5%-95% confidence limits. This is only the case for one patient. There is no obvious reason for this observation. One explanation may be whenever an extreme difference between Doppler and catheterization is found, the data are critically reviewed by the investigators and then eventually rejected for a variety of reasons. On the other hand, the data which fall within the confidence limits are accepted

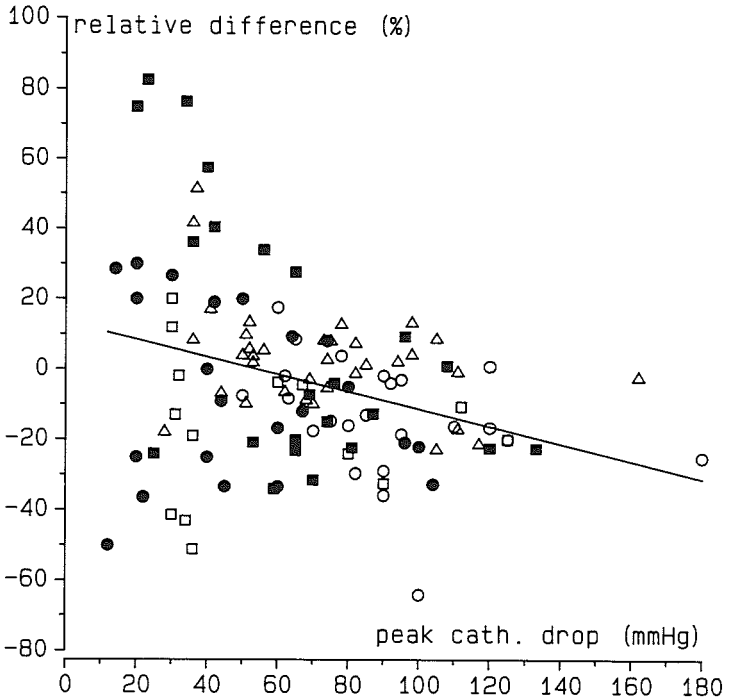

Fig. III-5. Plot of the percent relative differences of the pressure drop versus the peak catheterization drop. The continuous line is the regression line.

without further scrutiny. It is also conceivable that some investigators may omit some patients from their statistical analysis when the data show an extreme discrepancy.

The variability of the Doppler results as shown can be explained on the basis of different inflow characteristics which vary from patient to patient (eqn 4). Furthermore, it should be noted that the paired measurements were made non-simultaneously, introducing another uncertain difference.

DISCUSSION

A number of assumptions are made when the pressure drop across the stenotic aortic valve is estimated by Doppler echocardiography using the simplified Bernoulli equation. Neglecting upstream or proximal velocities in eqn (2) leads to an overestimation of the pressure drop. This overestimation will decrease with smaller orifice areas. Upstream blood velocity can be measured with pulsed Doppler technique. In patients with mild to moderate stenosis eqn (2) should be

considered for the calculation of the pressure drop. The simplified Bernoulli equation should only be applied in cases of a flow obstruction in the sense that the cross-sectional area of the obstruction is small as compared to the upstream flow area. It would make no sense to apply simplified Bernoulli in a person with a normal valve.

The energy losses at the inlet of the stenotic aortic valve cannot be quantified in the individual patient as it is dependent upon the actual geometry of the inlet region and the shape of the valve. The pressure recovery in the region downstream of the stenotic valve depends mainly on the orifice area of the stenotic valve and the cross-sectional area of the aorta. A non-uniform velocity distribution will lead to overestimation of the pressure drop since the measured velocity is higher as compared to the theoretical velocity when a uniform velocity distribution is assumed. Fortunately, these unknown factors seem to compensate one another. The spread of the measurements can mainly be explained by different inflow characteristics which vary from one patient to another.

It should be realized that when pressure drops are measured by catheter and compared with pressure drops estimated by Doppler echocardiography, an additional variability in the catheter data is introduced by the pressure recovery. To obtain a good pressure recording in the aorta, a catheter must be positioned somewhere in the region of pressure recovery. This will be outside the area of turbulence at a variable distance from the stenotic valve. No standardization seems possible and it is therefore unknown which part of the pressure recovery is included in the pressure drop measurement by catheter. Theoretically, the most accurate estimate of the severity of a stenosis is represented by the velocity in the stenotic orifice, that is, the pressure difference calculated from eqn (2). Thus, in the future we may think of expressing the severity of aortic valve stenosis in terms of velocities rather than a pressure drop. At present, the reproducibility of the measurement within individual patients has to be evaluated.

CONCLUSIONS

Although in most studies a good agreement between Doppler and catheter measured pressure drops across stenotic aortic valve has been reported, the use of the Bernoulli equation is not always straightforward. A large variability is found in mild to moderate stenosis and clinical results should therefore be interpreted with caution. In severe aortic stenosis, the Doppler pressure drop underestimates the severity of the lesion. Pressure recovery within the aorta introduces variability and inaccuracy of the catheter pressure drop measurement. The latter can therefore not be considered as a 'gold standard' for comparison.

REFERENCES

1. Gorlin R and Gorlin SG. Hydraulic formula for calculation of the area of the stenotic mitral valve, other cardiac valves and central circulatory shunts I. Am Heart J 41, 1951: 1-29.
2. Bernoulli D. Hydrodynamics (1738); Bernoulli J. Hydraulics (1743). Translated from Latin. Dover Publications, New York 1968.
3. Klip W. Theoretical foundations of medical physics II. An introduction into medical physics. University of Alabama Press, Alabama 1969: 776-780.
4. Streeter VL and Wylie BE. Fluid mechanics. McGraw-Hill, New York, 1975: 471-473.
5. Requarth JA, Goldberg SJ, Vasko SD and Allen HD. In vitro verification of Doppler prediction of transvalve pressure gradient and orifice area in stenosis. Am J Cardiol 53, 1984: 1369-1373.
6. Holen J, Waag RC, Gramiak R, Violante MR and Roe SA. Doppler ultrasound in orifice flow. In vitro studies of the relationship between pressure difference and fluid velocity. Ultrasound In Med & Biol 11, 1985: 261-266.
7. Wong M, Vijayaraghaven G, Bae JH and Shah PM. In-vitro study of the pressure-velocity relation across stenotic orifices. Am J Cardiol 56, 1985: 465-469.
8. Cannon SR, Richards KL and Morgann RG. Comparison of Doppler echocardiographic peak frequency and turbulence parameters in the Quantification of aortic stenosis in a pulsatile flow model. Circulation 71, 1985: 129-135.
9. Teirstein PS, Yock PG and Popp RL. The accuracy of Doppler ultrasound measurement of pressure gradients across irregular, dual, and tunnellike obstructions to blood flow. Circulation 72, 1985: 577-584.
10. Smith MD, Dawson PL, Elion JL, Booth DC, Handshoe R, Kwan OL, Earle GF and DeMaria AN. Correlation of continuous wave Doppler velocities with cardiac catheterization gradients: an experimental model of aortic stenosis. J Am Coll Cardiology 6, 1985: 1306-1314.
11. Valdes-Cruz LM, Yoganathan AP, Tamura T, Tomizuka F, Woo YR and Sahn DJ. Studies in vitro of the relationship between ultrasound laser Doppler velocimetry and applicability of the simplified Bernoulli relationship. Circulation 73, 1986: 300-308.
12. Clark C. The fluid mechanics of aortic stenosis: I. Theory and steady flow experiments. J Biomechanics 9, 1976: 521-528.
13. Yoganathan AP and Corcoran WH. Pressure drops across prosthetic heart valves under steady and pulsatile flow: in vitro measurements. J Biomechanics 12, 1979: 153-164.
14. Hatle L, Brubakk A, Tromsdal A and Angelsen B. Noninvasive assessment of pressure drop in mitral stenosis by Doppler ultrasound. Br Heart J 40, 1978: 131-140.
15. Hatle L, Angelsen B and Tromsdal A. Noninvasive assessment of aortic stenosis by Doppler ultrasound. Br Heart J 43, 1980: 284-292.
16. Stamm R and Martin RP. Quantification of pressure gradients across stenotic valves by Doppler ultrasound. J Am Coll Cardiol 2, 1983: 707-718.
17. Berger M, Berdorf RL, Galterstein PE and Goldberg E. Evaluation of aortic stenosis by continuous wave Doppler ultrasound. J Am Coll Cardiol 3, 1984: 150-156.
18. Stevenson JG and Kawabori I. Noninvasive determination of pressure gradients in children: two methods employing pulsed Doppler echocardiography. J Am Coll Cardiol 3, 1984: 179-192.
19. Kosturakis D, Allen HD, Goldberg SJ, Sahn DJ and Valdes-Cruz LM. Noninvasive quantification of stenotic semilunar valve areas by Doppler echography. J Am Coll Cardiol 3, 1984: 1256-1262.

20. Currie PJ, Seward JB, Reeder GS, Vlietstra RE, Bresnahan DR, Bresnahan JF, Smith HC, Hagler DJ and Tajik AJ. Continuous wave Doppler echocardiographic assessment of severity of calcific aortic stenosis: a simultaneous Doppler-catheter correlative study in 100 adult patients. Circulation 71, 1985: 1162-1169.
21. Krafchek J, Robertson JH, Radfort M, Adams D and Kisslo J. A reconsideration of Doppler assessed gradients in suspected aortic stenosis. Am Heart J 110, 1985: 765-773.
22. Hegrenas L and Hatle L. Aortic stenosis in adults. Non-invasive estimation of pressure differences by continuous wave Doppler echocardiography. Br Heart J 54, 1985: 396-404.
23. Smith MD, Dawson PL, Elion JL, Wisenbaugh T, Kwan OL, Handshoe S and DeMaria AN. Systematic correlation of continuous wave Doppler and hemodynamic measurements in patients with aortic stenosis. Am Heart J 111, 1986: 245-252.
24. Zhang Y, Ihlen H and Nitter-Hauge S. Estimation of peak-to-peak pressure gradient in aortic stenosis by Doppler echocardiography. Int J Cardiol 10, 1986: 197-212.
25. Yeager M, Yock PG and Popp RL. Comparison of Doppler derived pressure gradient to that determined at cardiac catheterization in adults with aortic valve stenosis: implications for management. Am J Cardiol 57, 1986: 644-648.

CHAPTER IV

THE REPRODUCIBILITY OF LEFT VENTRICULAR VOLUME AND EJECTION FRACTION MEASUREMENTS FROM CROSS-SECTIONAL ECHOCARDIOGRAMS

Hans Rijsterborgh, Conny Tirtaman, Stefano Domenicucci and Jos Roelandt

Thoraxcenter, Erasmus University Rotterdam and
Interuniversity Cardiology Institute of the Netherlands

ABSTRACT

The random variation (random observer variation and beat-to-beat variation) of left ventricular cross-sectional echocardiographical measurements was estimated by performing serial measurements of sequential images of 108 patients. The left ventricular volumes and ejection fractions were calculated by the modified Simpson's method and the single plane area-length method from images obtained in the apical four-chamber view as well as in the apical long-axis view.

The percent standard deviation of the random variation of left ventricular volumes and ejection fractions calculated by the modified Simpson's method (7.5% resp. 11.8%) was significantly smaller as compared to the percent standard deviation obtained with the area-length method (10.5% resp. 18.8%).

Detailed analysis showed that the random variation of left ventricular volume measurements increased significantly with the magnitude of the volume itself. A small, non-significant decrease of the random variation of ejection fraction measurements with increasing ejection fractions was found. With the results of this study the number of measurements in a single patient may be determined in order to reduce the random variation of the obtained mean value to a desired level. Plots were made of the threshold differences, due to random variation, representing significant differences in a single patient using an unpaired t-test.

Submitted for publication

INTRODUCTION

Clinical decision making in cardiology most often requires quantitative information on left ventricular function. Echocardiography offers the advantage that it is non-invasive and that the examination can be repeated as many times as needed.

Measurements of left ventricular volumes and ejection fraction from cross-sectional echocardiograms are subject to various types of errors. First of all the error made during the interpretation of the cross-sectional images. Two types of observer errors can be distinguished: the constant type of observer error (bias) and the random observer error. The magnitude of these errors can be assessed by analyzing paired measurements made by two observers interpreting the same echocardiographic images. The mean value of the paired differences gives the difference in bias of the two observers; the standard deviation of the paired differences is caused by the sum of the two random observer errors made at both instances.

The second source of error is the beat-to-beat variation of the measurement. I.e. the left ventricular volume of the patient may not be regarded as being constant, but random variations may be caused by changes in heart rate and by respiratory effects. The total random variation is the sum of the random observer error and the random beat-to-beat variation. Due to the random nature of this variation, its influence on the final result will decrease if more measurements are taken to obtain a mean value.

Knowledge about the magnitude of the total random variation has important clinical relevance. Every measurement taken from a cross-sectional echocardiographical image has a random component caused by beat-to-beat variation and random observer error. The magnitude of the random component determines the number of measurements to be taken in a single patient in order to reduce the random variation of the mean value to a preset level. Another important clinical application is the estimation of a minimum difference in measurements representing a significant difference in an individual patient during a follow-up study.

The reliability of cross-sectional echocardiography for the detection of cardiac disease and assessment of its severity depends upon the reproducibility of the measurements. In the literature data has been reported on inter-observer and intra-observer variability of left ventricular volume and ejection fraction measurements [1-7] and on the temporal variability of these measurements [3,4,7,8]. Beat-to-beat variation [3,4] has not been studied extensively in a large group of routine patients.

The purpose of this study was to estimate the magnitude of the random variation of the left ventricular volume and ejection fraction measurements due to random observer error and beat-to-beat variation. Another goal was to study the relationship between the extent of this variation and the magnitude of the measurement itself. The total random variation was assessed by performing a series of measurements of sequential images in a large number of patients. Variance

analysis allows to estimate the variation of the measurements within patients.

Two methods of left ventricular volume measurement were evaluated. The area-length method (single plane) using four-chamber and apical long-axis views and the modified Simpson's rule using short-axis views.

METHODS

Subjects

The study group consisted of 108 outpatients who had various cardiac disorders and underwent two-dimensional echocardiographic imaging. The group included 86 males and 22 females ranging in age from 18 to 77 years (mean 51 years). Fifty-one patients had clinically stable coronary artery disease, 5 of them had coronary artery bypass grafts; 14 patients had valvular heart disease; 3 patients received cytostatic treatment for extra cardiac malignancies and showed no cardiac pathology; 7 patients had cardiomyopathy and 7 patients had various other cardiac diseases. All patients were in sinus rhythm at the time of the investigation.

Two-dimensional echocardiography

Cross-sectional images were obtained with a Hewlett-Packard phased array sector scanner HP77020A using a 3.5 MHz transducer. Patients were examined in supine or 30 degrees left lateral decubitus position. Studies were recorded for subsequent analysis onto video tape with a Panasonic VHS video recorder (NV8500). Four standard echocardiographic views were recorded [10]: (1) parasternal short axis at the level of the mitral valve; (2) parasternal short axis at the level of the papillary muscles; (3) apical long axis; (4) apical four chamber. Technically unsatisfactory two-dimensional images were excluded from further analysis.

Two-dimensional analysis

A commercially available microcomputer system (Digisonics) was used to analyze the two-dimensional echocardiograms. End-diastolic and end-systolic endocardial contours were traced manually on the video screen with the aid of a digitizing tablet interfaced with the computer. End diastole was identified as the beginning or the peak of the R-wave of the QRS complex and end systole as the peak of the T-wave.

Of every echocardiographic view six successive beats were traced in end diastole as well as in end systole. Of every cardiac cycle the ejection fraction was calculated using two different models of the human left ventricle.

Area-length method (single plane). With the assumption that the ventricle has the shape of a prolate ellipsoid the volume calculation can be performed with the measurements of the cross-sectional area and the long axis of the left ventricle [11,12]. This method was applied on both the apical long-axis and the four-chamber cross-sectional images. The linear long-axis dimension was measured as the distance from the apex to the intersecting point of the aorta and the mitral valve at the base for the apical long-axis view. For the four-chamber cross-section the distance from apex to the midpoint of the mitral valve at the base of the ventricle was taken as the long-axis dimension.

Modified Simpson's method. This model of the left ventricle is based on a combination of three geometrical shapes: the basal third of the ventricle is cylindrical, the mid-third of the ventricle is a truncated cone and the apical third is conical. The volume calculation is based on the measurements of the cross-sectional area at the base of the ventricle, the area at the level of the papillary muscles and a linear long-axis dimension [12].

Statistical analysis

Variance analysis allows to estimate the variation of the measurements within patients [9]. The measurements obtained from apical long-axis views, four-chamber views and short-axis views were analyzed separately. A fourth set of measurements was formed by combining the measurements taken from the images of both apical views. The variation within patients was calculated both as an absolute value as well as a percentage variation with respect to the mean value of the measurement obtained in a patient. Differences in variation were analyzed using a variance ratio test (F-test).

In order to explore the relationship between the random variation and the magnitude of the measurement itself, subgroups were formed on the basis of individual mean values. In each patient the mean value of the consecutive measurements was calculated. Data was classified and subgroups were formed of measurements having 22 adjacent consecutive mean values (approximately 130 measurements per subgroup). Variance analysis was performed of each subgroup separately. This analysis was done of the short-axis data and the combined data obtained in the apical views.

The variation of the left ventricular volume measurements within patients was expressed as a standard deviation and plots were made of twice the standard deviation (approximately 90% confidence limits) versus the mean value of the subgroup. Interpolation of the confidence limits between subgroups was done by simple linear regression of the confidence limits and the mean values of the

subgroups. If the results of the regression reached the $p=0.01$ level of significance it was concluded that the variation of the measurements was related to the value of the measurement itself.

RESULTS

Results of the variance analysis of the four sets of measurements are shown in Table IV-1 (volume) and Table IV-2 (ejection fraction). The variation of the measurements of left ventricular volume and ejection fraction obtained from long-axis views were significantly larger ($p<0.01$) as compared to the variation of the measurements calculated by the short-axis method. Comparison of the percentage variation of the measurements taken from the two different apical views showed no significant difference between the methods. The absolute variation of the measurements obtained from apical long-axis views was significantly larger as compared to the results of the four-chamber views.

Analysis of the relationship between the random variation and the value of the volume measurements showed a statistically significant increase of the variation of the measurement with the increase of the mean volume for both the short-axis method as well as the long-axis method.

The variation of the volume measurements obtained from the apical views in terms of twice the standard deviation increased from ± 13.5 ml at a mean volume of 50 ml to ± 40.0 ml at a mean volume of 250 ml (Fig. IV-1). For the measurements calculated by the short-axis method these values measured ± 10.7 ml and ± 26.3 ml respectively. The variation of the left ventricular ejection fraction measurements showed a slight decrease with increasing mean ejection fraction for both methods (Fig. IV-2). This decrease did not prove to be statistically significant in both cases. Therefore the variation in terms of twice the standard deviation of the ejection fraction measurements are ± 14% for the long-axis method and ± 9% for the short-axis method expressed in units of percentage ejection fraction.

DISCUSSION

The assessment of the total random variation of cross-sectional echocardiographic measurements is of clinical relevance since every measurement of left-ventricular size will be influenced by a combination of beat-to-beat variation and the random component of the observer error. Although the reproducibility of cross-sectional measurements may depend strongly on the magnitude of this random variation, it seems that it has not been studied extensively.

Gordon et al. [4] analyzed cross-sectional measurements taken in 20 normal subjects and 10 patients with coronary heart disease. Reported values for beat-to-

	volume measurements				
view	no of beats	no of mean values	mean (ml)	sd(e) (ml)	sd(e%) (%)
SAX	682	130	134	9.07	7.46
LAX	1686	294	129	12.59	10.47
LAX 4-C	736	126	125	10.71	9.96
LAX API	950	168	131	13.88	10.84

Table IV-1. Random variation of volume measurements expressed as an absolute standard deviation sd(e) and as a percentage standard deviation sd(e%). Abbreviations: SAX = measurements obtained from short-axis views by the modified Simpson method; LAX = measurements obtained by the area-length method from apical views; LAX 4-C = measurements obtained by the area-length method from apical four-chamber views; LAX API = measurements obtained by the area-length method from apical long-axis views.

	ejection fraction measurements				
view	no of beats	no of mean values	mean (%)	sd(e) (%)	sd(e%) (%)
SAX	331	65	47.1	4.52	11.82
LAX	829	147	44.8	6.99	18.83
LAX 4-C	361	63	44.8	6.57	18.72
LAX API	468	84	44.8	7.31	18.91

Table IV-2. Random variation of ejection fraction measurements expressed as an absolute standard deviation sd(e) and as a percentage standard deviation sd(e%). For abbreviations see Table IV-1.

beat variation in terms of twice the standard deviation was 10% for end-diastolic volumes and 15% for end-systolic volumes. Our values would be considerably higher: resp. 19% and 25%. In order to make this comparison we had to assume that their reported values were estimated in all the subjects studied and that their mean end-diastolic and end-systolic volumes studied measured 108 ml and 59 ml respectively. This difference in beat-to-beat variation might be explained by the fact that the study group was different and would stress the recommendation that these type of measurements must be averaged.

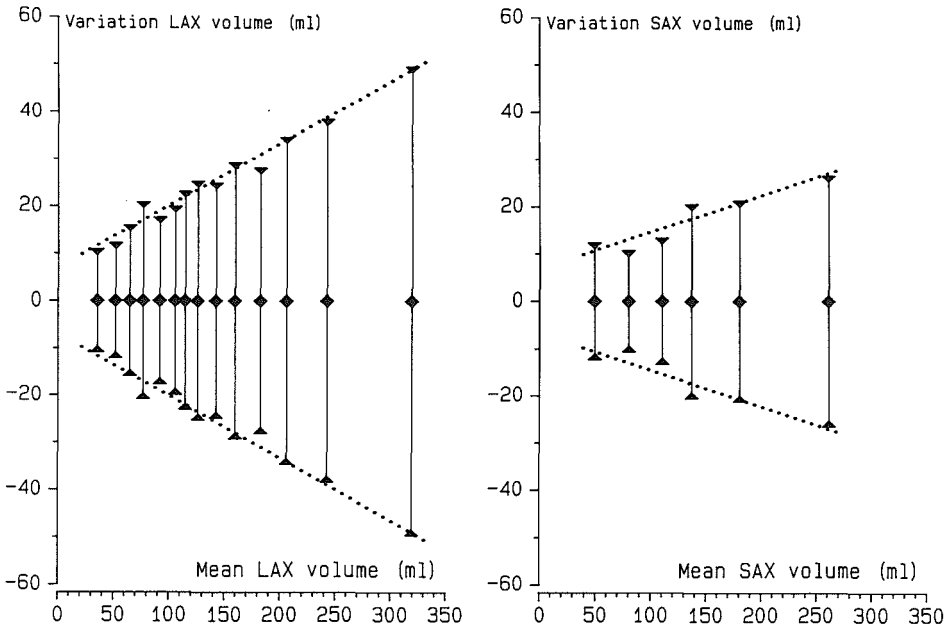

Fig. IV-1. Random variation of volume measurements in terms of twice the standard deviation versus the volume measurement. Left-hand side the area-length volumes calculated from apical views (LAX). Right-hand side the modified Simpson's volumes calculated from short-axis views (SAX). The dotted lines are obtained by linear interpolation.

Erbel et al. [3] assessed beat-to-beat variability in an alternative fashion. The explained variance was calculated using factor analysis. Since no specific values for beat-to-beat variation were reported and a different method was used, no comparison with our results can be made here.

The variation of the measurements of left ventricular volumes and ejection fraction was significantly smaller if the left ventricular volume measurements are obtained from short-axis images as compared to the long-axis determinations. The variation of the volumes measurements was smaller by a factor of 0.72 whereas the variation of the ejection fraction was smaller by a factor of 0.65. This does not mean that short-axis images would be better than long-axis images if one wants to obtain measurements from them. The calculation of a volume with the area-length method is based on one cross-sectional area, squared, whereas in the calculation of a volume with the modified Simpson's rule two independent cross-sectional areas are involved: the area at mitral valve and the area at the papillary muscles.

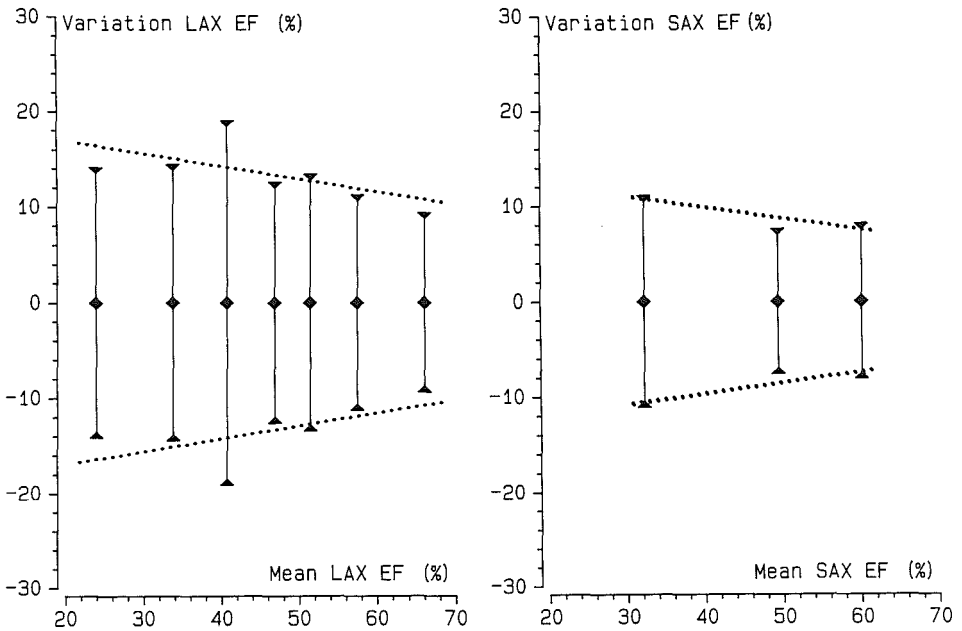

Fig. IV-2. Random variation of ejection fraction (EF) measurements in terms of twice the standard deviation versus the ejection fraction measurement. Left-hand side the ejection fraction measurements calculated by the area-length method from apical views (LAX). Right-hand side the ejection fraction measurements calculated by the modified Simpson's rule from short-axis views (SAX). The dotted lines are obtained by linear interpolation.

In the Simpson's rule calculation an extra independent area measurement is included. Therefore this method is expected to have a smaller random error, assumed that the magnitude of the random error of an area measurement does not depend on the type of cross-section (long axis or short axis).

If the variation of the volume measurement is determined by the random error of the cross-sectional area measurement, the variation of Simpson's volumes would be a factor of 0.71 smaller as compared to the long-axis method. This factor is equal to the square root of two, representing the fact that two independent cross-sectional areas were included in the measurement. Taking the average of two independent long-axis cross-sectional areas (i.e. area-length, biplane) would give comparable results in terms of random error as compared to a single Simpson volume measurement.

The observation that the variation of a volume measurement increases with the magnitude of the volume itself has a number of consequences. The variation of measuring an end-diastolic volume is larger as compared to the variation of the end-systolic volume measurement in absolute units. It can be appreciated from Fig. IV-1 that the 90% confidence limits of the variation of an end-diastolic long-axis volume of 160 ml are ± 28 ml whereas an end-systolic volume of 90 ml has a variation of ± 19 ml. In relative terms these values are ± 18% and ± 21% respectively. The relative random errors are of the same order of magnitude and will have equal contribution to the random error of the ejection fraction calculation. If we take the volumes of a normal adult male person as an example (end-diastolic volume of 110 ml and end-systolic volume of 45 ml [13]) the percentage random error of the end-systolic volume (29%) is larger as compared to that of the end-diastolic volume (20%). In this case the random error of the end-systolic volume will have a larger contribution to the random error of the ejection fraction calculation. In general, small volumes have a comparatively large percentage random error. This should be realized if decisions are to be made on the number of measurements to obtain a mean value.

The clinical applications of this study

In Figs. IV-1 and IV-2 the 90% confidence limits are given of the variation of the measurements of left ventricular volume and ejection fraction respectively. These confidence limits depict the average random variation of one measurement versus the mean value. The clinical relevance of the data shown on these figures is that one can estimate the random error of a mean value for a given number of consecutive measurements. The standard error of a mean value is inversely related to the square root of the number of measurements. Therefore the resulting variation of a mean value may be estimated by dividing the estimated variation of one measurement by the square root of the number of measurements.

Two examples are given here. The variation of a long-axis volume of 100 ml is estimated to be ± 20 ml according to Fig. IV-1. If three measurements are taken to obtain a mean value, the resulting variation of the mean value would be approximately ± 12 ml. With six measurements this variation would be reduced to ± 8 ml. On the other hand, one can calculate the number of measurements given a preset level of variation of the mean value. For instance if one wants to estimate a mean ejection fraction with a variation of ± 5% one has to average 7 long-axis or 3 short-axis measurements.

The results of this study can also be applied if comparisons are to be made within a single patient. For instance in a follow-up or interventional

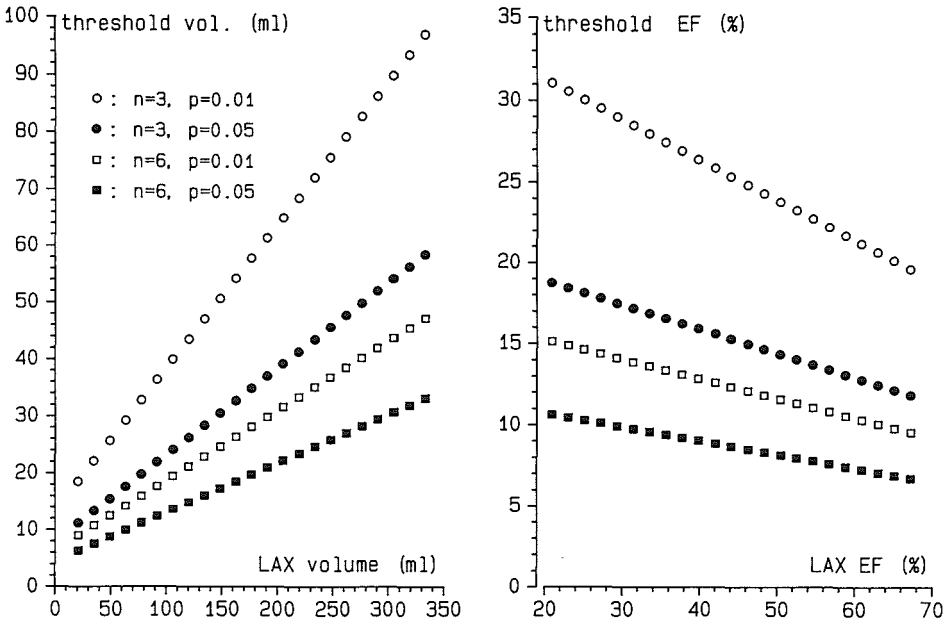

Fig. IV-3. Threshold values, due to random variation, needed for a statistically significant difference in a single patient using an unpaired t-test. Shown are the values for cross-sectional echocardiographic measurements obtained from long-axis images (LAX). Left-hand side: echocardiographic volumes. Right-hand side: ejection fractions (EF). The different symbols refer to the respective combinations of number of measurements (n) and significance level (p).

echocardiographic study [14]. Based on the magnitude of the random variation within patients one can calculate the 'threshold' difference needed for a statistically significant difference using an unpaired t-test, given the number of measurements and the desired level of significance. An example of these threshold values of volume and ejection fraction measurements based on long-axis cross-sectional images is presented in Fig. IV-3. As explained above, the threshold values for the measurements taken from short-axis images are expected to be relatively smaller by the square root of two.

It should be noted that the depicted threshold values are due to random variation only. In order to make realistic comparisons one has to take into account the differences in bias produced by the respective observers, which have to be established separately.

CONCLUSIONS

Random variation, a combination of random observer variation and beat-to-beat variation, of left ventricular volume measurements determined by cross-sectional echocardiography was estimated to be 7.5% (in percentage standard deviation) in images obtained in short-axis views and 10.5% in images obtained in apical views. The random variation of ejection fraction measurements was estimated to be 11.8% and 18.8% respectively.

The observed differences of the random variation between the long-axis method and short-axis method can be explained by the fact that an extra independent cross-sectional area measurement is included in the short-axis determination of the measurement as compared to the long-axis method. The random variation of echocardiographically determined volume measurements increases with increasing volumes, whereas the random variation of ejection fraction measurements can be regarded as independent from the magnitude of the ejection fraction itself.

REFERENCES

1. Schiller NB, Acquatella H, Ports ThA, Drew D, Goerke J, Ringertz H, Silverman NH, Brundage B, Botvinick E, Boswell R, Carlsson E and Parmley WW. Left ventricular volume from paired biplane two-dimensional echocardiography. Circulation 60, 1979: 547-555.
2. Stamm RB, Carabello BR, Mayers DL and Martin RP. Two-dimensional echocardiographic measurement of left ventricular ejection fraction: prospective analysis of what constitutes an adequate determination. Am Heart J 104, 1982: 136-144.
3. Erbel R, Schweizer P, Meyer J, Krebs W and Effert S. Quantification of left ventricular function by two-dimensional echocardiography. In: Advances in noninvasive cardiology. Meyer J, Schweizer P and Erbel R (eds.), Martinus Nijhoff Publishers, Boston, 1983: 67-80.
4. Gordon EP, Schnittger I, Fitzgerald PJ, Williams P and Popp RL. Reproducibility of left ventricular volumes by two-dimensional echocardiography. J Am Coll Cardiol 2, 1983: 506-513.
5. Tortoledo FA, Quinones MA, Fernandez GC, Waggoner AD and Winters WL. Quantification of left ventricular volumes by two-dimensional echocardiography. A simplified and accurate approach. Circulation 67, 1983: 579-584.
6. Murray PP, Murray RG and Litter WA. A simple semi-automated technique for estimating left ventricular volumes by two-dimensional echocardiography. J Card Ultrasonography 3, 1984: 333-338.
7. Wallerson DC and Devereux RB. Reproducibility of quantitative echocardiography: factors affecting variability of imaging and Doppler measurements. Echocardiography 3, 1986: 219-235.
8. Conetta DA, Geiser EA, Oliver LH, Miller AB and Conti R. Reproducibility of left ventricular area and volume measurements using a computer endocardial edge detection algorithm in normal subjects. Am J Cardiol 56, 1985: 947-952.
9. Armitage P. Statistical methods in medical research. Blackwell Scientific Publications, Oxford 1973: 189-202.

10. Henry WL, DeMaria A, Gramiak R, King DL, Kisslo JA, Popp RL, Sahn DJ, Schiller NB, Tajik A, Teichholz LE and Weyman AE. Report of the American Society of Echocardiography Committee on nomenclature and standards in two-dimensional echocardiography. Circulation 62, 1980: 212-217.
11. Dodge HT, Sandler H, Ballew DW and Lord JD. Use of biplane angiography for measurement of left ventricular volume in man. Am Heart J 60, 1960: 762-776.
12. Folland ED, Parisi AF, Moynihan PF, Jones DR, Feldman CL and Tow DE. Assessment of left ventricular ejection fraction and volumes by real-time, two-dimensional echocardiography. A comparison of cineangiographic and radionuclide techniques. Circulation 60, 1979: 760-766.
13. Rijsterborgh H, Romdoni R, Vletter W, Bom N and Roelandt J. Reference ranges of left ventricular cross-sectional echocardiographic measurements in the adult male population. J Am Soc Echo 2, 1989, 415-418.
14. Huang H, Scheffers M, Rijsterborgh H and Roelandt J. Does echocardiography allow the monitoring of the cardiac effects of nitrates? Eur Heart J 9, 1988: 51-55.

CHAPTER V

REFERENCE RANGES OF ECHOCARDIOGRAPHIC MEASUREMENTS IN THE DUTCH POPULATION

P.J. Voogd, H. Rijsterborgh, J. Lubsen, A.C. Arntzenius, L.K. Monsjou and E.H. Godijn

Department of Cardiology, University Hospital Leiden and Interuniversity Institute of Cardiology, the Netherlands

ABSTRACT

Reference ranges for echocardiographic measurements were determined in 609 healthy Dutch subjects, using height, weight, age, sex, RR-interval and blood pressure (in adults only) as determinants. End-systolic as well as end-diastolic measurements of the aortic root as well as left ventricular inner diameter, posterior and septal thickness were taken, as was the left atrial end-systolic diameter. After logarithmic transformation of all the variables multiple linear regression was performed, using height, weight, age, RR-interval and sex as independent variables. The residuals were calculated in order to determine the percentile limits by means of linear interpolation. Sex and weight were significant determinants in all the echocardiographic parameters studied.

The results were presented twofold, with a simple version for males and females separately, using only weight as a determinant and allowing graphical presentation, and secondly a complex version taking into account all determinants, which can only be solved with the help of a calculator.

European Heart Journal 5, 1984: 762-770

INTRODUCTION

The usefulness of echocardiographic measurements in clinical medical practice depends to an extent on the availability of reference ranges of these measurements in healthy people. The size of the heart and therefore the reference ranges is influenced by a number of determinants. In children the size of the heart clearly depends on body size [1]. Almost all studies dealing with reference ranges for echocardiographic measurements therefore have used body surface area (estimated from weight and height) as determinant [2-8]. In addition age, independently of body size, has also been shown to influence echo dimensions. For instance, an

increase in aortic diameter, wall thickness and left atrial size all have been observed with aging [6,8]. The influence of sex does not appear to have been investigated thoroughly. As shown by DeMaria et al. [9], heart rate in normal subjects influences left ventricular and left atrial echo dimensions during atrial pacing. Although hypertension is associated echocardiographically with an increase in left ventricular wall thickness [10], blood pressure as a determinant of reference ranges has not been studied in healthy people.

The purpose of this study was to establish reference ranges for echocardiographic measurements in a health Dutch population group (age range 3-60 years) using age, sex, weight, height and heart rate as determinants. In addition blood pressure was also used but in adults only.

METHODS

The population studied consisted of two groups: 432 children and adolescents aged from 4-17 years and 177 adults aged 20-64 years. Children and adolescents (208 boys and 224 girls) were investigated within the framework of the Youth Health Care system in the Netherlands. This system provides for a medical check-up every two years. The check-up consists of notes on the medical history, a physical examination and determination of height and weight. Haemoglobin is measured when indicated, e.g. when history reveals tiredness or fatigue or when pallor is noted on physical examination. In our study, children with any physical or mental disorder were excluded. However, children who were considered to have an innocent heart murmur were included.

The 177 adults (95 males and 82 females) studied were either employees of the town of Leiden who were to receive a regular medical check-up anyway or, alternatively, people who applied for governmental jobs and needed medical examination to apply. These check-ups consist of a medical history and a physical examination. The blood pressure was measured using the random zero technique as described by the London School of Hygiene [11]. Erythrocyte sedimentation rate, measurement of haemoglobin and urine examination for glucose and albumen were assessed routinely. From this study, people with any physical or mental disorder were excluded as were known hypertensives on medication. Those who were considered to have an innocent cardiac murmur were, however, included in the study.

Echocardiographic measurements

M-mode echocardiograms were taken in the left sided recumbency position by means of an Organon Technika 003 echocardiograph equipped with a two-dimensional linear array system. After visualisation of the cross section through the long axis, the M-mode transducer was positioned on the chest wall in the third, fourth, or fifth intercostal space and complete M-mode scans were performed. For children and adolescents up to the age of 17 years, a 5 MHz transducer was used whereas a 2.5 MHz transducer was used for the adult population. The M-mode transducer was positioned in such a fashion that in the M-mode scan the anterior wall of the aorta was more or less on the same horizontal level as the endocardium of the right side of the intraventricular septum. The recordings were made on a Honeywell LS6 fibre-optic recorder, at a paper speed of 50 mm/s, using the lowest gain setting possible. In addition the gain was set in such way that the epicardial-lung interface could just be visualised as separate lines. Calibration dots, derived from a stable crystal controlled oscillator, were half a second apart and indicated steps of 10 mm in depth.

Measurements were taken as follows, aortic root diameter in end diastole was measured at the level where the aortic valve was visualised using the onset of the QRS complex as the indicator for end diastole. Left atrial dimension was taken at end systole at the site where the largest left atrial diameter was seen to occur. At this level, the aortic root diameter was measured in end systole. Left ventricular end-diastolic diameter, as well as end-diastolic septal and posterior wall thickness were also measured at the onset of the QRS complex. Left ventricular end-systolic diameter, posterior wall and septal thicknesses were measured at the peak inward motion of the posterior wall.

All measurements were made at the leading edge of the echoes. Care was taken not to incorporate the septal tricuspid leaflet into the septal thickness; the endocardium of the left ventricular posterior wall was differentiated from the chordae tendineae by taking the thinnest line with the highest motion velocity as the line of the endocardium.

Measurements were performed by means of a digitising tablet connected to a PDP 11/10 minicomputer. The above defined measurements points on the recordings were indicated with the digitising pen and the appropriate dimensions were computed. The results were stored on disc together with the other data (subject number, sex, age, height, weight, heart rate, and blood pressure in adults only) for statistical analysis.

Statistical methods

Statistical analysis was carried out using the multiple regression technique. The procedure 'regression' of the Statistical Package for the Social Sciences was employed [12]. Two regression models were fitted.

The first model was designed to evaluate the relationships between the average echocardiographic measurements of the respective dimensions and the determinants 'weight', 'height', 'sex', 'age' and 'RR-interval'.

Logarithmic transformation of the echocardiographic measurements and the independent variables 'weight', 'height', 'age' and 'RR-interval' were made. 'Sex' was entered as an indicator variable. After inverse logarithmic transformation, for each echocardiographic dimension a regression equation was obtained of the form:

$$M = A \cdot (\text{age})^B \cdot (\text{height})^C \cdot (\text{weight})^D \cdot (\text{RR-interval})^E \cdot (\text{sex})^F$$

in which M is the average of the respective echo dimensions given its determinants.

The residuals i.e. the differences between M and the individual measurements, were calculated from the cumulative distribution of the residuals. Three values of A were determined by linear interpolation which describe the 5th, 50th and 95th percentiles respectively of the individual measurements given its determinants.

To determine whether in adults blood pressure is an independent determinant given weight, height, age, RR-interval and sex, the residuals as defined above were correlated with systolic and diastolic blood pressures. Multiple linear regression analyses were made between these residuals and the systolic and diastolic blood pressure measurements.

Based on the results of the analysis described above reduced regression models were fitted of the form:

$$M = a \cdot (d)^b$$

The determinant (d) was chosen as the one which was shown to account for the largest amount of variability of the individual measurements (i.e. had the largest 'F to remove' in the full model).

If 'sex' was a significant determinant in the full model, such reduced models were obtained for males and females separately. From these reduced models, the 5th and 95th percentile of residuals were again obtained and simple nomograms were drawn. Conventional 90 percent confidence hyperbolae [13] were obtained also and compared with the method described above.

attribute		males (n = 303)	females (n = 306)
age	(years)	4 - 64	3 - 59
height	(cm)	101 - 198	95 - 186
weight	(kg)	15 - 120	14 - 82
RR-interval	(ms)	450 -1325	457 -1287

Table V-1. The ranges of age, height, weight and RR-interval of the population studied.

RESULTS

Full model

Characteristics of the population studied are summarised in Table V-1. Table V-2 shows the results of the statistical analysis using the full model, including all the available independent variables.

When 'weight', 'age', 'RR-interval' and 'sex' were considered together, 'weight' and 'sex' were statistically significant determinants ($p<0.01$) for each of the echocardiographic measurements studied.

'Age' and 'height' did not contribute significantly to the prediction of the left ventricular inner dimension in end diastole. 'Height' was also significant in the equations of both the end-systolic and end-diastolic diameter of the aorta, but in all other cases 'age' and 'height' did contribute significantly at the $p<0.01$ level of significance.

Notice that the exponent of 'height' in the regression formulas (Table V-2) of the end-systolic and end-diastolic dimension of the left ventricular septum and posterior wall was negative, suggesting an inverse relationship between these dimensions of wall thickness and the height of a person. The dimension of the left atrium proved to be inversely related to 'height'. The left ventricular dimension in end systole was to decrease with advancing age.

The RR-interval proved to contribute significantly to the prediction equations of five of the derived regression equations: the end-diastolic ventricular inner dimension, both the left ventricular wall thickness measurements in end systole, and the dimensions of the aorta and left atrium in end systole.

From the results given in Table V-2, the 5th, 50th and 95th percentiles of the reference range of an echocardiographic dimension may be calculated for a given person whose attributes are within the ranges of the population studied.

echo dimension	exponents B age	C height	D weight	E RR-int.	F sex	constants A_5	A_{50}	A_{95}
IVS (ED)	0.131*	-0.317*	0.374*	0.0337	-0.105*	4.44	5.74	7.37
LVID (ED)	0.007	0.105	0.197*	0.0552*	-0.055*	7.99	8.90	9.91
LVPW(ED)	0.060*	-0.466*	0.494*	0.0459	-0.103*	5.96	7.40	9.49
IVS (ES)	0.153*	-0.405*	0.284*	0.0866*	-0.147*	9.55	12.07	15.11
LVID (ES)	-0.067*	0.393*	0.184*	0.0300	-0.033*	1.65	1.97	2.30
LVPW(ES)	0.124*	-0.483*	0.409*	0.0712*	-0.065*	10.59	13.55	16.84
Ao (ED)	0.096*	-0.115	0.282*	-0.0104	-0.094*	10.58	12.34	14.48
Ao (ES)	0.126*	-0.033	0.212*	0.0485*	-0.073*	6.54	7.64	8.94
LA (ES)	0.086*	-0.756*	0.404*	0.1260*	-0.069*	91.14	108.57	129.27

*Table V-2. Derivation of the 5th, 50th and 95th percentile of echocardiographic dimensions. Exponents and constants for the regression equation of the full model. In order to obtain the percentiles of the echocardiographic dimensions in mm units, age should be in years, height in cm, weight in kg, and RR-interval in ms. Sex has to be entered as an indicator variable: sex = 1 for males, sex = 2 for females. To calculate the 5th, 50th and 95th percentile the constant A of the regression equation has to be replaced by A_5, A_{50} and A_{95} respectively. Abbreviations: IVS = interventricular septum; LVID = left ventricular inner dimension; LVPW = left ventricular posterior wall; Ao = aorta diameter; LA = dimension of the left atrium; ES = end systole; ED = end diastole. *=statistically significant ($p<0.05$).*

For instance a 16.4 year old female with height = 177 cm, weight = 57.7 kg and RR-interval = 826 ms, the 95th percentile of the left ventricular inner dimension in end systole follows from

$$P_{95} = 9.91 \cdot (16.4)^{0.007} \cdot (177)^{0.105} \cdot (57.7)^{0.197} \cdot (826)^{0.0552} \cdot (2)^{-0.055} = 53.95 \text{ mm}$$

Analysis of residuals versus blood pressure

The result of the multiple regression analysis between the residuals of the echocardiographic measurements and the systolic and diastolic blood pressure, showed no significant relationship between echocardiographic dimensions and blood pressure, except for the diameter of the aorta in end systole and end diastole. The diastolic blood pressure proved to be a significant determinant ($p<0.05$) of the residuals of the end-systolic aortic diameter. Whereas both diastolic and systolic blood pressure showed to be significant determinants of the residuals of the end-diastolic dimension of the aorta. The regression formulas, obtained for the residuals of the dimension of the aorta are shown in Table V-3. The ranges of blood pressures and mean values in the adult population (n = 177) were found to be:

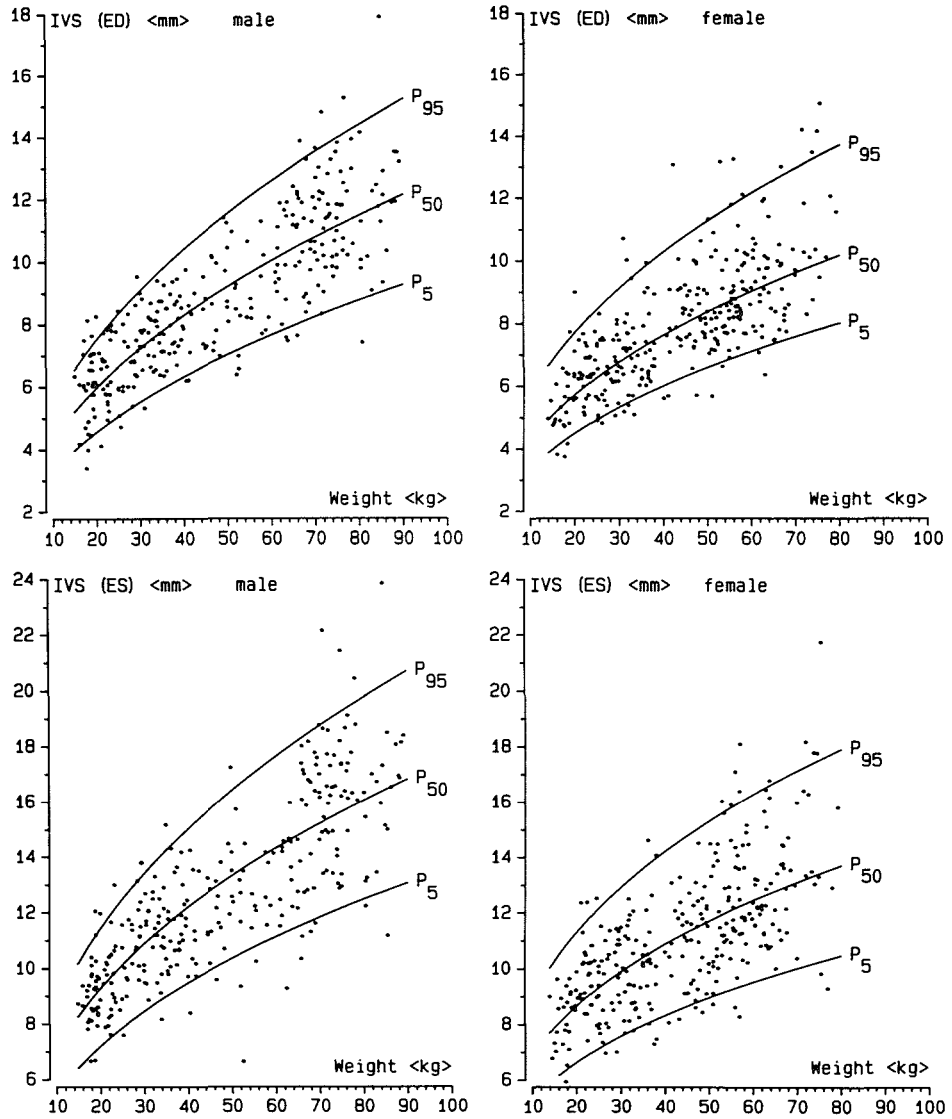

Fig. V-1. Nomograms of the sex-specific percentiles of the interventricular septum (IVS) in end diastole (ED) and end systole (ES) plotted versus weight. The individual measurements appear as dots.

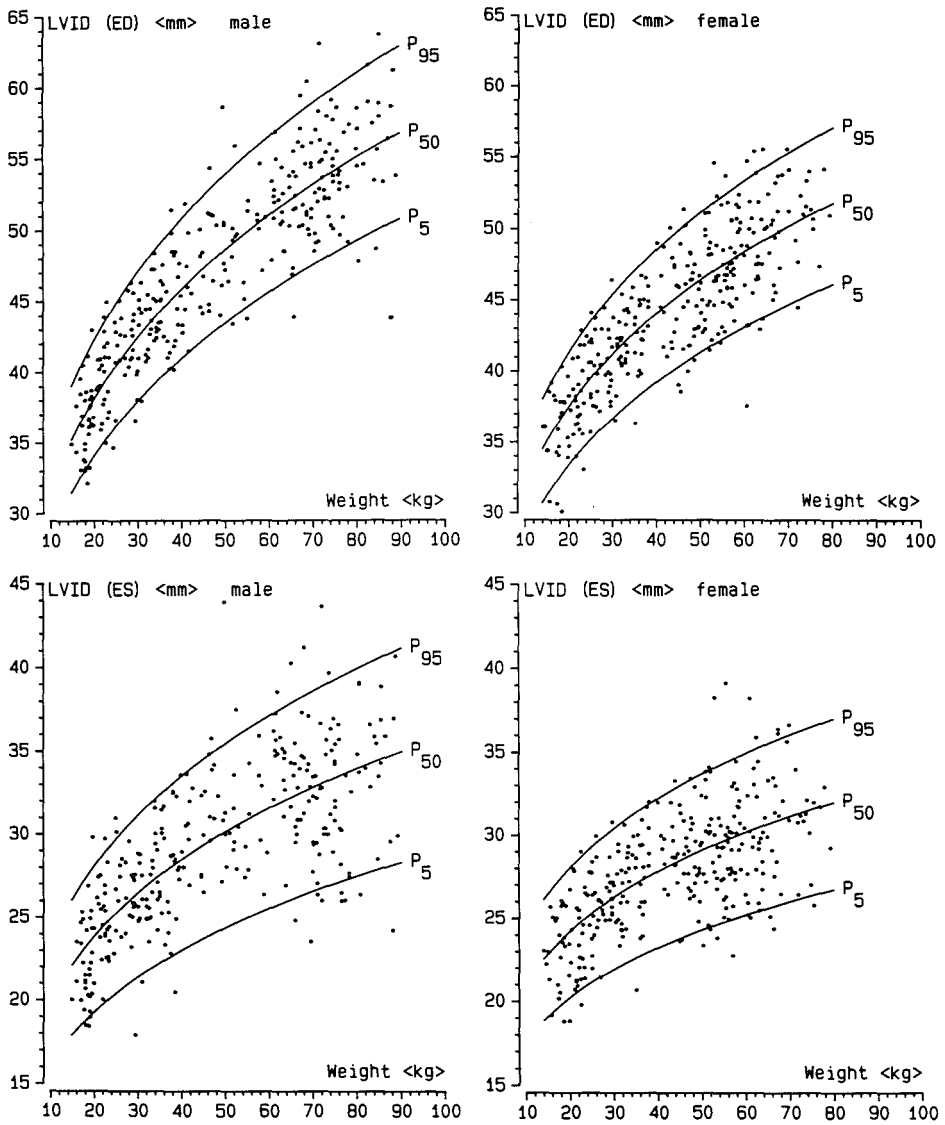

Fig. V-2. Nomograms of the sex-specific percentiles of the left ventricular inner dimension (LVID) in end diastole (ED) and end systole (ES) plotted versus weight. The individual measurements appear as dots.

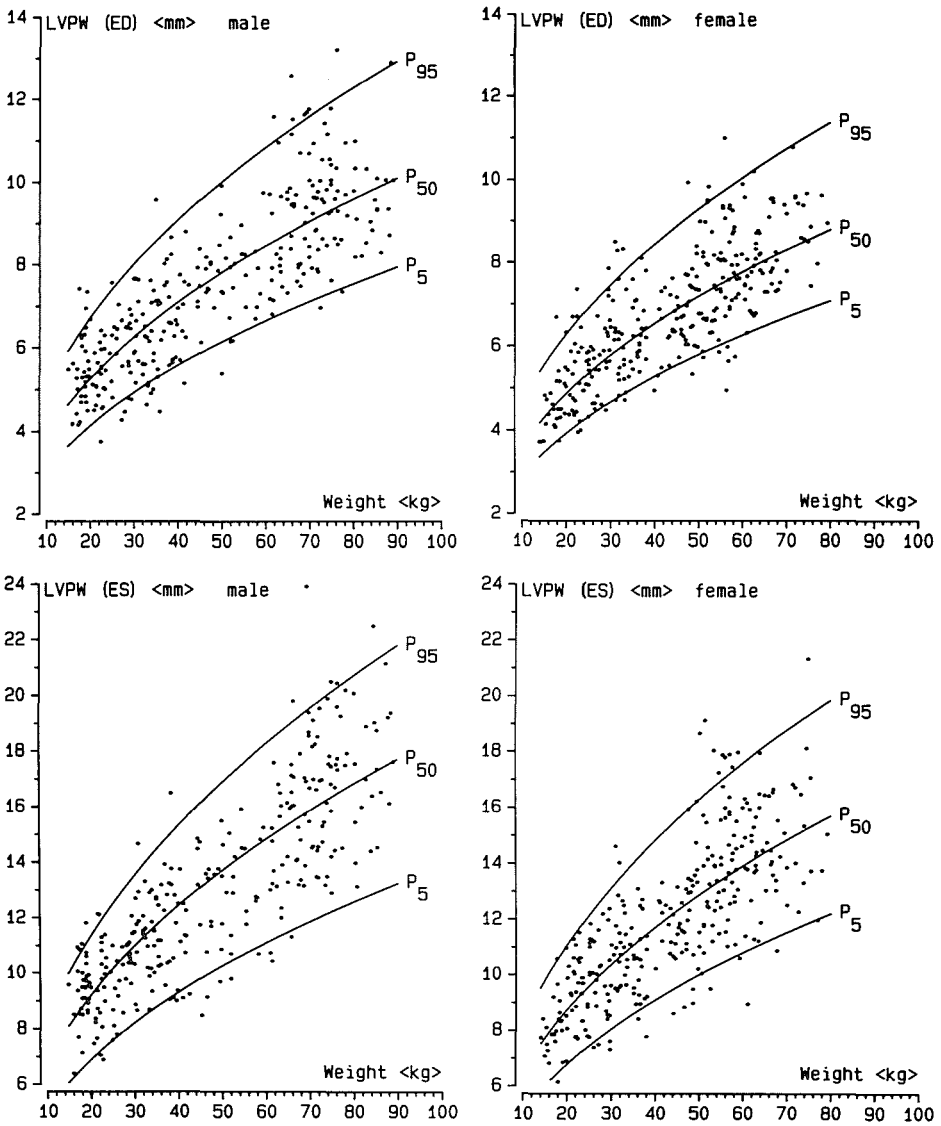

Fig. V-3. Nomograms of the sex-specific percentiles of the left ventricular posterior wall (LVPW) in end diastole (ED) and end systole (ES) plotted versus weight. The individual measurements appear as dots.

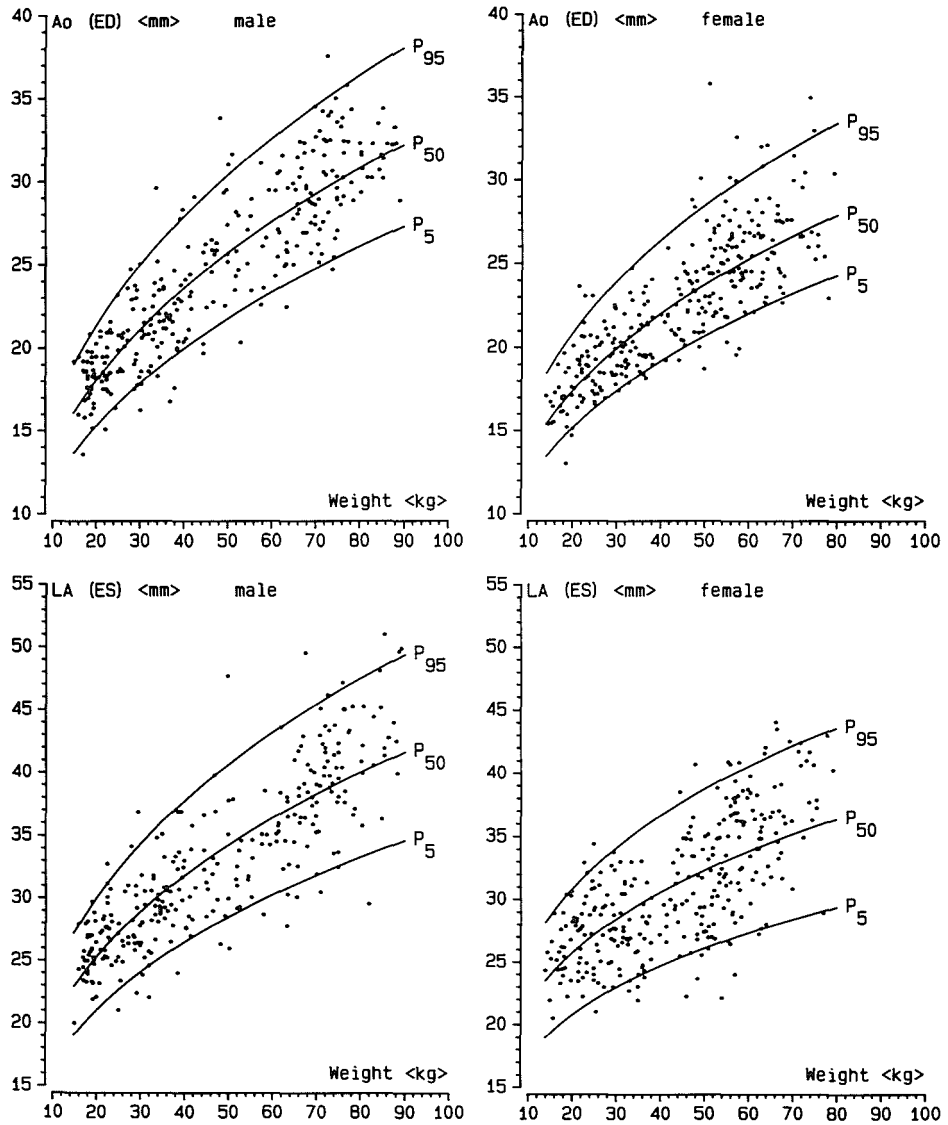

Fig. V-4. Nomograms of the sex-specific percentiles of the diameter of the aorta (Ao) in end diastole (ED) and left atrium (LA) in end systole (ES) plotted versus weight. The individual measurements appear as dots.

	regression equation
Residuals Ao (ES)	= -0.016 · (BPS) + 0.103* · (BPD) - 5.8 mm
Residuals Ao (ED)	= -0.034* · (BPS) + 0.084* · (BPD) - 1.9 mm

Table V-3. Relationship between residuals of aortic dimension and blood pressure. The residuals are in mm units, the blood pressures in mmHg. Abbreviations: Ao = diameter of the aorta; BPS = systolic blood pressure; BPD = diastolic blood pressure; ES = end systole; ED = end diastole.

systolic blood pressure 90-210 mmHg (mean 133 mmHg) and diastolic blood pressure 55-127 mmHg (mean 80 mmHg).

Simple model

Since the complex model had shown 'sex' and 'weight' to be the best determinants of the echocardiographic dimensions, the simple model was based on only these two determinants. According to the methods described above, plots were made of the 5th, 50th and 95th percentiles of the echocardiographic dimensions versus 'weight', for males and females separately. In the nomograms, shown in Figs. V-1 to V-4, the individual measurements appear as dots.

DISCUSSION

Most previous studies have used $(\text{weight})^2$, body surface area [2,4,6,7] or a metameter of body surface area [8,5,3], as a determinant of echocardiographic dimensions in the analyses. Body surface area (BSA) using the formula of Dubois and Dubois [14] is determined as follows:

$$\text{BSA} = 71.84 \cdot (\text{weight})^{0.425} \cdot (\text{height})^{0.725}$$

Studies using body surface area so calculated assume a linear relationship between body surface area and the echo dimensions. The relationship being of the form:

$$M = a \cdot (\text{BSA}) + c$$

Or alternatively if a metameter of body surface area was used:

$$M = a \cdot (\text{BSA})^{\text{exponent}} + c$$

where a and c are the usual least square regression constants and M the average of the respective echo dimensions given their determinants.

Since in this study the regression equations were fitted on a logarithmic scale for echo dimensions and for weight and height, we assumed a similar relationship but without fixed exponents weight and height, these exponents being determinants on the basis of 'goodness of fit' and not on Dubois and Dubois' investigation. Notice that, for example, when the regression equation is employed to predict posterior and septal wall thickness it can easily be shown that two persons of identical weight and of difference in height, the taller person has thinner posterior and septal wall thickness when compared with the shorter person. Apparently increase in height exerts a negative influence on wall thickness. Clearly, if the formula of Dubois and Dubois or a metameter of this formula had been used, this finding would not have shown up. By use of the regression equation (Table V-2), it can be demonstrated that the relative contribution of height as a determinant of the echo dimension is small taking into account the contribution of weight.

Several studies have not analysed their data with respect to sex differences [2,4,5,8]. In other studies, sex differences were noted, but these differences lost significance when body surface area [7] or weight [1] were taken into account. Only Henry et al. [3] found significant echo measurement differences between male and female children which, however, they considered too small to take into account for reference ranges.

The main finding of our study is that the difference in echo dimensions observed between males and females could not be explained by differences in weight and height since the exponent of sex is statistically significant in all multivariate analyses. As appears from Fig. V-2 the difference may be quite substantial: up to 4 mm for the left ventricular dimension in end diastole. From this we conclude that differences between male and female should not be neglected in the determination of reference ranges of echocardiographic measurements.

As shown in the full model, the heart rate significantly influences the left ventricular end-diastolic diameter, the end-systolic septal thickness, the end-systolic posterior wall thickness, the aortic root diameter at end systole and the left atrial dimension. An increase in heart rate results in a decrease of left ventricular inner dimension at end diastole. These results agree with DeMaria's findings [9] but the influence of heart rate in our study is of a much smaller magnitude. For example the regression equation of the left ventricular inner dimension in end diastole from Table V-2 (full model), it can be shown that increases in heart rate from 60 to 120 beats/min is associated with a sizable decrease of the 55 mm by 2 mm. If alternatively DeMaria's regression equation were to be used this would account

for a decrease in left ventricular end-diastolic dimension down to 8.9 mm associated with a concomitant increase in end-diastolic septal and posterior wall thickness. This latter tendency is also present in our study but it did not reach significance.

One explanation for the differences may be the fact that atrial pacing of normal subjects, as employed by DeMaria, reflects a non physiologic increase of heart rate. Another would be that DeMaria investigated intra-patient variability in heart rate whereas our study dealt with inter-patient variability.

As described, the influence of blood pressure on measurements of echo dimensions were analysed in adult participants only, as no blood pressure readings in children were available. As shown in Table V-3, an increase in diastolic blood pressure significantly affected both end-systolic and end-diastolic aortic root diameter but did not influence the other echo dimensions. It should be noted that systolic blood pressure, in contrast to diastolic blood pressure, exerted a decreasing tendency on the end-diastolic aortic root diameter and also did not affect the other dimensions. Although we cannot easily explain these small but significant opposing influences of systolic and diastolic blood pressure on the end-diastolic aortic root diameter, our results clearly demonstrate that the presence or absence of high blood pressure in our population did not exert any influence on the other echo dimensions except for the aortic root diameter.

Whether these findings are due to the fact that our study population did not show sufficient variability of blood pressure, or alternatively that one blood pressure reading is not sufficient to assess the variability of blood pressure over the day, is not clear and beyond the scope of our study.

We presented our study twofold: with a full model, employing the rather complicated regression equation which in spite of its complicated nature can easily be used with help of a simple programmable commercially available pocket calculator and secondly a simple version which allows graphical presentation and, therefore, easily clinical application.

The region of the percentile limits of both versions are almost similar. The difference between the full model and the simple version is determined by a difference in predictor or, in other words, the 50th percentile for a given echo measurement is different in both versions. So, a given echo dimension determined by weight and sex alone will enter the reference region on a different level compared with an echo measurement which also takes heart rate, height and age as a reference. For this very reason also, the 5th and 95th percentile limits will be different. The full model allows for a more accurate prediction. The difference between the two versions will be up to 1-4 mm, depending on the measurement involved.

Finally, these reference ranges for echocardiographic dimensions only apply to the ranges of predictor variables studied and their use should be restricted to the well-nourished population.

ACKNOWLEDGEMENTS

The authors thank Mrs M.G. Zwart-Feenstra MD, head of the GG and GD, who provided the opportunity to take echocardiographic measurements at the municipal health center of Leiden and for her encouragement and continuous support.

We wish to express our gratitude to I.M. Tjoa-Tan MD, E.S. Oonk-Groen MD, R.C. Schweizer-Lindeman MD, H.P.A. van de Water MD, C.T. Herweyer MD, A.W. Gorlee-Kroon and A. Cameraat for their kind and efficient cooperation during the study at the municipal health center.

REFERENCES

1. Lundström NR. Clinical application of echocardiography in infants and children. Acta Paediatr Scand 63, 1974: 23-32.
2. Epstein ML, Goldberg SJ, Allen HD, Konecke L and Wood J. Great vessel, cardiac chamber and wall growth patterns in normal children. Circulation 51, 1975: 1124-1129.
3. Henry WL, Ware J, Gardin JM, Hepner SI, Mckay J and Weiner M. Echocordiographic measurements in normal subjects. Growth related changes that occur between infancy and early adulthood. Circulation 57, 1978: 278-285.
4. Rogé CLL, Silverman NH, Hart PA and Ray RM. Cardiac structure growth pattern determined by echocardiography. Circulation 57, 1978: 285-290.
5. Gutgesell HP, Paquet M, Duff DF and McNamara DG. Evaluation of left ventricular size and function by echocardiography. Results in normal children. Circulation 56, 1977: 457-462.
6. Gerstenblith C, Frederiksen J, Yin FCP, Fortuin NJ, Lakatta EG and Weisfeldt ML. Echocardiographic assessment of a normal adult aging population. Circulation 56, 1977: 273-278.
7. Valdez RS, Motta JA and London E. Evaluation of the echocardiograms as an epidemiologic tool in an asymptomatic population. Circulation 60, 1979: 921-928.
8. Henry WL, Gardin JM and Ware JH. Echocardiographic measurements in normal subjects from infancy to old age. Circulation 62, 1980: 1054-1061.
9. DeMaria AN, Neumann A, Schubart PJ, Lee G and Mason DT. Systematic correlation of cardiac chamber size and ventricular performance determined with echocardiography and alteration in heart rate in normal persons. Am J Cardiol 43, 1979, 1-9.
10. Savage DD, Drayer JIM and Henry WL. Echocardiographic assessment of cardiac anatomy and function in hypertensive subjects. Circulation 59, 1979: 623-631.
11. Rose GA, Holland WW and Crowley EA. A sphygmonameter for epidemiologist. Lancet 1964: 296-300.
12. Nie NH, Hull H, Jenkins JG, Steinbrenner F and Bent DH. Statistical package for the social sciences (2nd edition). McGraw-Hill, 1975: 320-323.
13. Woolf CH. Principles of biometry. Van Nostrand, 1968: 199-202.
14. Dubois D and Dubois EF. A formula to estimate the approximate surface area if height and weight be known. Arch Intern Med 17, 1916: 863-871.

CHAPTER VI

REFERENCE RANGES OF LEFT VENTRICULAR CROSS-SECTIONAL ECHOCARDIOGRAPHIC MEASUREMENTS IN ADULT MEN

Hans Rijsterborgh, Rochmad Romdoni, Wim Vletter, Nicolaas Bom and Jos Roelandt

The Interuniversity Cardiology Institute of the Netherlands and Thoraxcenter, Erasmus University Rotterdam, the Netherlands

ABSTRACT

Reference ranges for left ventricular cross-sectional echocardiographic measurements were determined in 67 healthy Dutch male subjects using age, weight, height and heart rate as determinants. The images were made using the apical long-axis view and the calculations were done utilizing the area-length method. The end-diastolic volume, end-systolic volume, stroke volume and ejection fraction were measured for six consecutive cardiac cycles in every subject and averaged. Data were analyzed using both simple linear regression as well as multiple linear regression after logarithmic transformation of all measurements.

Weight proved to be the best predictor of the echocardiographic end-diastolic volume, end-systolic volume and stroke volume. The left ventricular ejection fraction could be regarded as independent of the determinants studied. Nomograms of the 5th, 50th and 95th percentile limits were made of the echocardiographic parameters versus weight.

Journal of the American Society of Echocardiography 2, 1989: 415-418

INTRODUCTION

The usefulness of quantitative two-dimensional echocardiography of the left ventricle in clinical practice depends to an extent on the availability of appropriate reference ranges of these measurements established from healthy people. In order to make a precise distinction between normal and abnormal measurements, the characteristics of healthy subjects must be known.

The previous reference ranges of published cross-sectional echocardiographic left ventricular parameters have been based on small groups of males and females [1-4]. The variations in these measurements with the subject's attributes such as age, height, weight and heart rate have not been sufficiently investigated.

The purpose of this study was to establish reference ranges for cross-sectional echocardiographic left ventricular measurements in a healthy Dutch adult male population group (age range 22-64 years) using age, weight, height and heart rate as determinants.

MATERIAL AND METHODS

The study group consisted of 67 healthy adult male subjects without history of cardiac disease. All subjects had a normal ECG.

The mean values, standard deviations and ranges of the attributes of the subjects measured to be respectively: age 45.9 ± 11.2, 22-64 years; weight 77.4 ± 10.2, 52-102 kg; height 178 ± 37.1, 162-193 cm and heart rate 67.9 ± 10.2, 46.9-91.9 beats/min.

Two-dimensional echocardiographic examinations were performed with a Hewlett Packard (HP 77020 A) sector scanner using a 3.5 MHz transducer. The subjects were examined in the 30 degrees left lateral decubitus position. Apical long-axis views including a lead II electrocardiogram and video frame counter with time increments of 20 ms were recorded on a standard VHS video tape recorder.

Quantitative analysis of the images was done on a commercially available analysis system (Digisonics) based on an Apple II computer using a Panasonic NV 8500 video tape recorder with stop frame and search facilities. End diastole was identified at the onset of the QRS-complex of the ECG and end systole was defined at the peak of the T-wave.

End-diastolic and end-systolic contours were traced manually using the digitizer tablet and the video overlay of the computer system. The left ventricular long axis was measured as the distance from the apex to the intersection of the aortic valve and the mitral valve.

Cardiac volumes were calculated by the computer system using the single plane area-length method [5,6]. Stroke volume and ejection fraction were derived in the usual manner. Heart rate was calculated on a beat-to-beat basis, using the RR-intervals derived from the video frame counter.

In every subject six consecutive cardiac cycles were analyzed. The measurements were averaged to obtain a mean value of the respective echocardiographic parameters in every subject. Altogether a total number of 804 frames were analyzed for this study.

STATISTICAL METHODS

Statistical analysis was carried out using the simple linear regression technique as well as multiple linear regression analysis.

Logarithmic transformation of the echocardiographic measurements and the independent variables 'age', 'weight', 'height' and 'heart rate' were made. Simple linear regression analysis was done between the echocardiographic measurements and the respective independent variables to determine the best predictor of the measurements. The best determinant of the echocardiographic measurements was defined as having the smallest standard error of the estimate in the simple regression model. Standard errors were compared using an F-ratio test and a significance level of $p<0.01$. After inverse logarithmic transformation for each echocardiographic parameter a regression equation was obtained of the form:

$$M = A \cdot (d)^B$$

in which M is the average of the respective echo parameter, given its determinant d. The regression coefficients A and B are to be determined.

Nomograms were made of the results of the simple linear regression on linear scales including the conventional 90 percent confidence hyperbolae based on the Student's t-distribution (65 degrees of freedom, $t = 1.99$).

Multiple linear regression was performed between the respective echocardiographic measurements and the independent variables 'age', 'weight', 'height' and 'heart rate', after logarithmic transformation of all the variables [7]. The technique of stepwise exclusion was applied: independent variables without a significant contribution ($p>0.01$) were subsequently removed from the regression equation on the basis of the lowest 'F-to-remove' value, until a regression equation was obtained consisting of significant contributors only.

RESULTS

The mean values, standard deviations and ranges of the cross-sectional echocardiographic measurements are listed in Table VI-1.

Simple linear regression analysis showed a statistically significant correlation ($p<0.01$) between the echocardiographic parameters and the respective independent variables 'weight' or 'height' except for the measurement of the ejection fraction ($p = 0.03$). The standard error of the regression with 'weight' was smaller as compared to that of the regression with 'height'. The ratio of the respective standard errors showed not to be significant ($p>0.01$) using the F-ratio test.

Parameter		univariate statistics		
		mean	sd	range
Volume ED	(ml)	111	25.3	70.4 - 181
Volume ES	(ml)	43.6	12.5	26.8 - 83.4
Ejection fraction	(%)	61.0	4.0	53.4 - 68.8
Stroke volume	(ml)	67.4	14.4	43.4 - 104.9

Table VI-1. Mean values, standard deviations (sd) and ranges of the cross-sectional measurements of the study group. Abbreviations: ED = end diastole; ES = end systole.

Parameter		simple regression	
		regression equation	p-value
Volume ED	(ml)	$= 3.01 \cdot (\text{weight})^{0.862}$	<0.001
Volume ES	(ml)	$= 0.52 \cdot (\text{weight})^{1.013}$	<0.001
Ejection fraction	(%)	$= 107 \cdot (\text{weight})^{-0.130}$	0.031
Stroke volume	(ml)	$= 3.21 \cdot (\text{weight})^{0.697}$	<0.001

Table VI-2. The regression equations and the respective p-values obtained with the simple regression analysis versus weight (in kg). Abbreviations: ED = end diastole; ES = end systole.

No significant relationships were found in the simple regression analysis with 'age'. The nomograms of the echocardiographic parameters versus 'weight' including the 90 percent confidence hyperbolae are shown in Figs. VI-1 and VI-2. The respective regression equations are given in Table VI-2.

Multivariate analysis showed, when 'age', 'weight', 'height' and 'heart rate' were considered together, 'weight' to be a statistically significant determinant ($p < 0.01$) of the echocardiographic measurements studied except for the measurement of the ejection fraction ($p = 0.03$).

After subsequent removal of the non-significant determinants only 'weight' remained in the regression equation of the measurements of the end-diastolic, end-systolic, and stroke volume. None of the independent variables proved to be a significant determinant of the ejection fraction at a significance level of $p = 0.01$.

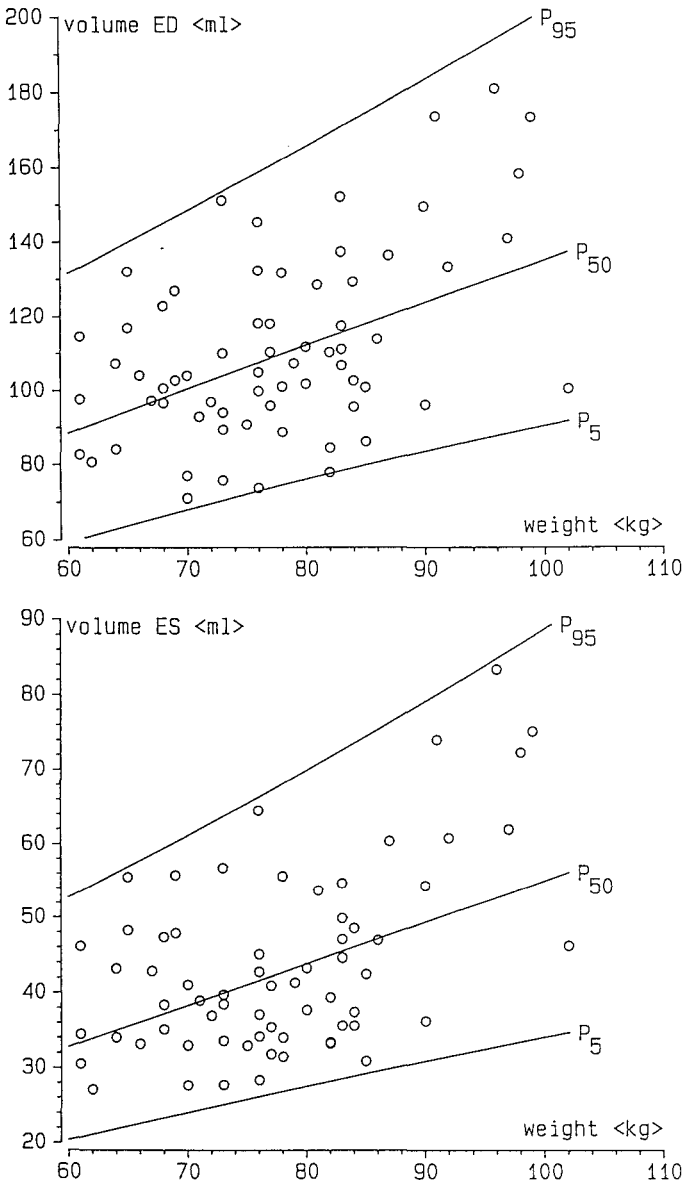

Fig. VI-1. Nomograms of the percentiles of left ventricular cross-sectional echocardiographic measurements plotted versus weight. Top: end-diastolic volume (ED). Bottom: end-systolic volume (ES). The open circles indicate the individual measurements. The solid lines represent the 5th, the 50th and the 95th percentile, respectively.

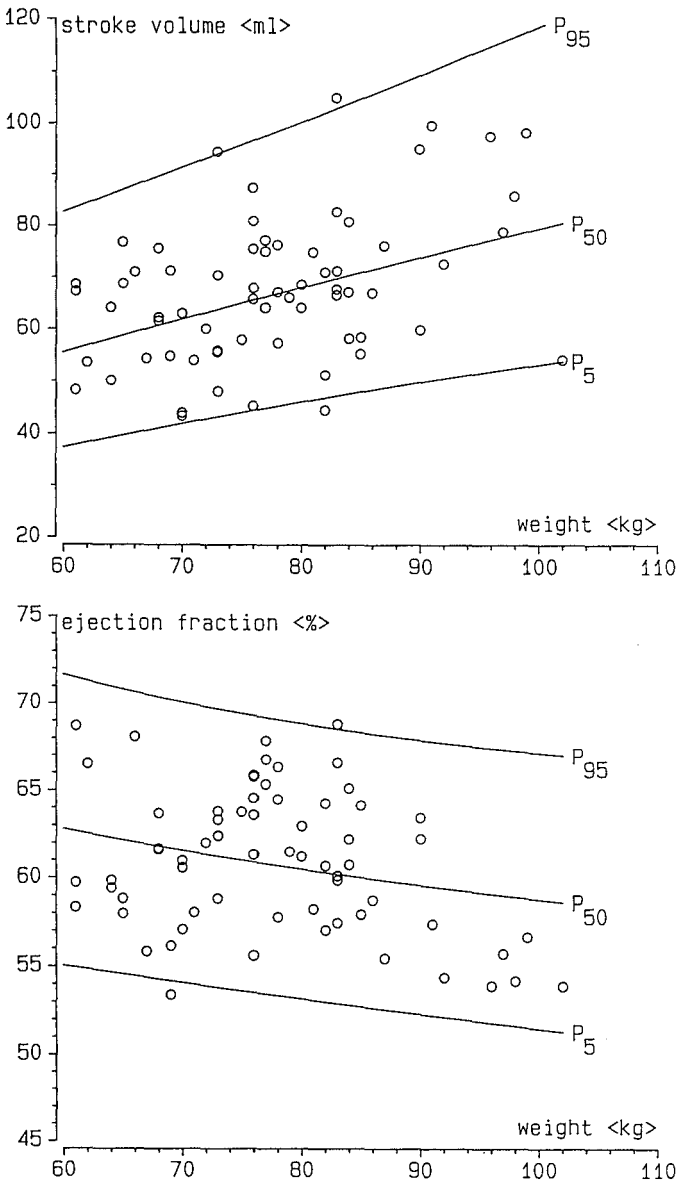

Fig. VI-2. Nomograms of the percentiles of left ventricular cross-sectional echocardiographic measurements plotted versus weight. Top: stroke volume. Bottom: ejection fraction. The open circles indicate the individual measurements. The solid lines represent the 5th, the 50th and the 95th percentile, respectively.

DISCUSSION

The results of this study showed 'weight' or 'height' to be good predictors of most of the cross-sectional echocardiographic measurements. Since the height and the weight of a subject are not independent of one another (in our study the correlation coefficient measured 0.7), simple linear regression with 'weight' produced comparable results as regression with 'height' in terms of goodness of fit.

There are two reasons to justify the choice of 'weight' as the best determinant of the echocardiographic measurements. In the simple regression model 'weight' produced the narrowest confidence limits (smallest standard error of the estimate) as compared to 'height'. Secondly, if in the multivariate model 'weight' and 'height' were considered together, 'height' proved to be a non-significant contributor to the regression equation.

From this last observation it may also be concluded that 'body surface area' would not be better than 'weight' as determinant of the echocardiographic measurements. Body surface area is based on a combination of 'weight' and 'height' with fixed exponents as determined by Dubois and Dubois [8]. Since in this study the regression equations were fitted on a logarithmic scale for echo parameters and for 'weight' and 'height', we assumed a similar relationship but without fixed exponents of 'weight' and 'height'. Using multivariate analysis the exponents are determined on the basis of 'goodness of fit' rather than on Dubois and Dubois' investigation. Since 'height' proved not to be a significant contributor to the regression equation with 'weight' and 'height' as independent variables, it must be concluded that body surface area would not be a better predictor as compared to 'weight' alone.

A (weak) relationship was found between the left ventricular stroke volume and the end-diastolic left ventricular volume. Both the stroke volume and the end-diastolic volume had a similar relationship with 'weight'. Therefore, their ratio appears to be independent of 'weight'. The normal range in terms of the 90% confidence limits of the echocardiographically measured ejection fraction of a healthy adult male person can simply be given by 53-69%.

Since the study group only consisted of adult subjects, the ranges of the independent variables are not very wide. Statistical analysis on linear scales, without prior logarithmic transformation of the variables, would have given comparable results in terms of goodness of fit. However, the echocardiographic variables appear to have a tendency to an asymmetric distribution as can be appreciated from the nomograms. The logarithmic transformation provided for asymmetric percentile limits on linear scales.

Finally, with the application of the nomograms, it should be realized that they are based on the mean value of six consecutive measurements taken in apical long-axis views using the area-length method. The reference ranges only apply to the ranges of the predictor variables studied.

CONCLUSIONS

In the derivation of reference values of two-dimensional echocardiographic left ventricular measurements in adult male subjects, 'weight' has proven to be the best predictor, except for the measurements of ejection fraction. The ejection fraction can be regarded as being independent of the subject attributes 'weight', 'height', 'age' and 'heart rate'.

REFERENCES

1. Erbel R, Schweizer P, Henn G, Meyer J and Effert S. Apikale zweidimensionale Echokardiographie. Normalwerte für die monoplane und biplane Bestimmung der Volumina und der Ejektionsfraktion des linken Ventrikels. Deut Med Wochenschr 107, 1982: 1872-1877.
2. Wahr DW, Wang YS and Schiller NB. Left ventricular volumes determined by two-dimensional echocardiography in a normal adult population. J Am Coll Cardiol 1, 1983: 863-868.
3. Triulzi MO, Wilkins GT, Gillam LD, Gentile F and Weyman AE. Normal adult cross-sectional echocardiographic values: left ventricular volumes. Echocardiography 2, 1985: 153-169.
4. Byrd BF, Wahr D, Wang YS, Bouchard A and Schiller NB. Left ventricular mass and volume/mass ratio determined by two-dimensional echocardiography in normal adults. J Am Coll Cardiol 6, 1985: 1021-1025.
5. Dodge NT, Sandler H, Ballew AM and Lord JA Jr. Use of biplane angiography for the measurement of left ventricular volume in man. Am Heart J 60, 1960: 762-776.
6. Folland ED, Parisi AF, Moynihan PF, Jones DR, Feldman CL and Tow DE. Assessment of left ventricular ejection fraction and volumes by real-time, two-dimensional echocardiography. Circulation 60, 1979: 760-766.
7. Dixon WJ. BMDP Statistical software manual. University of California Press, 1985.
8. Dubois D and Dubois EF. A formula to estimate the approximate surface area if height and weight be known. Arch Intern Med 17, 1916: 863-871.

CHAPTER VII

ULTRASONIC MYOCARDIAL INTEGRATED BACKSCATTER AND MYOCARDIAL WALL THICKNESS IN ANIMAL EXPERIMENTS

Hans Rijsterborgh, Frits Mastik, Charles Lancée, Ton van der Steen, Loes Sassen, Piet Verdouw, Jos Roelandt and Klaas Bom

Thoraxcentre, Erasmus University Rotterdam and
Interuniversity Cardiology Institute of the Netherlands

ABSTRACT

The purpose of this study was to distinguish between normal and ischemic myocardium using ultrasonic integrated backscatter (IB) measurements and to relate IB with myocardial wall thickness. IB was measured in 9 open chested Yorkshire pigs (24-30 kg) before, after 30 minutes of partial occlusion of the proximal left anterior descending coronary artery (LADCA) and after 60 minutes of subsequent reperfusion. The ultrasound transducer (4.6 MHz) was sutured onto the epicardial surface perfused by the LADCA. IB measurements were made with a repetition rate of 50 times per heart rate simultaneously with a left ventricular pressure signal. Myocardial wall thickness was measured off-line. The measurements of integrated backscatter, left ventricular pressure and wall thickness were based on mean values of ten subsequent cardiac cycles.

End-systolic IB measurements were 5.3 dB higher during occlusion as compared to the reference measurements (7.1 ± 3.2 dB versus 1.8 ± 2.6 dB, $p = 0.002$). No statistically significant differences were found in end-systolic IB measurements.

End-systolic wall thickness was 5 mm smaller during occlusion as compared to the reference measurements (7.2 ± 1.4 mm versus 12.2 ± 1.2 mm, $p < 0.001$). Simple linear regression analysis showed a statistically significant inverse relationship between IB measurements and wall thickness in 21 out of the 23 sequences in which wall thickness could be measured. End-systolic IB measurements are favorable to distinguish acute ischemic myocardium from normal myocardium. There is a distinct inverse relationship between IB and myocardial wall thickness.

Ultrasound in Medicine and Biology 16, 1990, 29-36

INTRODUCTION

Integrated backscatter is a relative measure of the ultrasonic energy backscattered by a small volume of tissue. Integrated backscatter provides a potentially useful index for quantitative ultrasonic characterization of myocardial tissue. In animal studies it has been shown that integrated backscatter is increased in acute ischemic myocardium as compared to normal myocardium [1-3].

Another promising application of integrated backscatter measurements is the assessment of the myocardial contractile function. Variation of integrated backscatter during the cardiac cycle has been reported in normal and ischemic myocardial tissue of dogs [4-6] and in human subjects [7,8]. In normal myocardium the diastolic value of integrated backscatter is higher as compared to the systolic value, whereas during acute ischemia this cyclic variation changes. Comparisons were made between the amplitude of cyclic variation of integrated backscatter and end-systolic percent myocardial wall thickening [9].

The goal of this study was to explore the possibilities of integrated backscatter measurements to distinguish normal myocardium and acute ischemic myocardium in animal experiments. Given the cyclic variation of backscatter we wanted to know which instant of the cardiac cycle is optimal to make the distinction. Another question was whether the relationship between integrated backscatter and wall thickening is maintained throughout the cardiac cycle. For that purpose a computer-based measurement system was developed to record the broadband high frequency signals offering the possibility to analyze dynamic integrated backscatter and simultaneously acquired myocardial wall thicknesses.

METHODS

General

Animal experiments were performed on Yorkshire pigs according to the guiding principles in the care and use of animals [10]. In order to provide adequate depth of anaesthesia, the animals (n=9, 24-30 kg) were sedated with an intramuscular injection of 120 mg azaperone and subsequently, anaesthetized with 150 mg of metomidate which was administered via a dorsal ear vein. After intubation the animals were connected to a respirator for artificial ventilation with a mixture of 30% oxygen and 70% nitrous oxide. Anaesthesia was maintained with 160 mg/kg alpha-chloralose (Merck, Darmstadt, FRG) followed by an infusion of 5 mg/kg/h pentobarbitone sodium (Sonofi, Paris, France) via a catheter placed in the superior vena cava by way of a jugular vein. Respiratory rate and tidal volume were adjusted to maintain physiological arterial blood gas values (ABL-3, Radiometer, Copenhagen, Denmark). During the course of the present study, these values were

7.39 ± 0.01 (pH), 45 ± 3 mmHg (pCO_2), 154 ± 4 mmHg (pO_2) and 93 ± 2% (O_2 saturation), respectively.

Sodium bicarbonate (8.4%) and haemaccel (Behringwerke, Marburg, FRG) were infused to correct base deficit and blood loss. Left ventricular and central aortic pressure were monitored with microtipped catheters (Honeywell-Philips, Best, the Netherlands). After the heart was exposed using a midsternal split, a hydraulic occluder (RE Jones, Silver Spring, MD, USA) was placed around the proximal left anterior descending coronary artery (LADCA) distal and connected to a 1 ml syringe (Hamilton Bonaduz, Bonaduz, Switzerland) which was driven by a micrometer. During the study the chest of the pig was retracted [11].

At the onset of occlusion regional myocardial function was estimated from myocardial wall thickness recordings obtained with the aid of a 4.6 MHz ultrasonic transducer (Krautkramer-Branson, Lewistown, PA, USA) sutured into a part of the epicardial surface perfused by the LADCA which was connected to an echocardiographic ultrasound system (Organon Teknika, Oss, the Netherlands).

Experimental protocols

After cardiovascular parameters had been stable for at least 30 minutes following completion of the surgical procedures, baseline values of systemic hemodynamics, regional myocardial function and arterial blood gases were obtained. LADCA flow was then reduced gradually by slowly inflating the balloon until regional wall thickening had decreased to less than 20% of its baseline value.

If necessary, minor adjustments in the degree of flow reduction were performed during the first 5 minutes of ischemia, but, thereafter, no further manipulations were allowed. We have shown that after these 5 minutes perfusion and function of the ischemic and non-ischemic myocardial do not change further during the following 30 minutes [11].

Data acquisition

Backscatter measurements were made at three episodes during the experiments: before the occlusion was applied, after 30 minutes of occlusion and after one hour of reperfusion in all pigs. The ultrasound transducer was connected to an in-house developed transmitter/wide band amplifier (input impedance 100 Ohm, fixed gain 29 dB, -6 dB cut-off frequencies 1.2 and 10 MHz). The amplified ultrasound signal was filtered by a 5th order 10 MHz low-pass Chebyshev filter and connected to one channel of a dual channel digital oscilloscope (LeCroy 9400).

Ultrasound signals from the time interval of interest (4.5 μsec - 24.5 μsec following the transmitter pulse) were digitized with 8-bit resolution at a sample

frequency of 25 MHz providing for a Nyquist frequency of 12.5 MHz. At the same time a left ventricular pressure signal was digitized by the oscilloscope. The digital oscilloscope was interfaced with an IBM computer system (AT-3) which was described extensively elsewhere [12].

Measurement sequences were generated by the computer system. The heart rate of the pig was entered into the computer and, starting at the onset of the pressure curve, 62 broadband transmitting pulses (approximately 1 μsec) were generated with the repetition rate of 50 times the heart rate. After the last transmitting pulse the accumulated digitized ultrasound and pressure signals of at least one cardiac cycle were transferred to the computer system and displayed on a video monitor. Measurements were rejected by the operator when the time interval of the cardiac cycle did not match with a preset value of ± 10%. This measurement procedure was repeated 10 times in order to obtain 10 useful cardiac cycles to be stored on disc. Usually this could be accomplished within 30-40 seconds. This procedure was repeated during the other episodes of the experiment.

Data processing

Data were processed off-line. Myocardial wall thickness was measured interactively using the computer system. The recorded ultrasound signals of the complete cardiac cycles were displayed on the video monitor of the computer system in brightness mode using a 30 dB logarithmic signal compression. The endocardial wall of every cycle was traced continuously by an observer using a mouse (of the computer system). Only the endocardial walls which were clearly visualized were processed for further analysis.

In the next step the 10 consecutive cycles of every measurement were matched in time on the basis of the pressure curve. The individual pressure curves were displayed on the monitor and the beginning and end of the cardiac cycle were indicated by the observer. All time scales of the cardiac cycles were converted to a linear scale expressed as a percentage of the cardiac cycle ranging from begin systole (0%) to end diastole (100%) with 2% increments. Ultrasound spectra were calculated by a Fourier transformation using the array processor (Data Translation 7020) of the computer system. A split cosine bell window [13] ($p=0.1$) of 5-8 μsec was chosen in order to exclude the endocardial wall signal from the spectra. Integrated backscatter was calculated by integrating the spectra from 2.5 to 7.5 MHz. The ultrasound data were not corrected for the frequency responses of the transducer and the wide band amplifier. All calculations were done using the same time window, resulting in the same, but arbitrary, 0 dB reference level for every backscatter measurement.

After completion of the data processing of the measurement sequences, the data of time dependent integrated ultrasound backscatter, wall thickness and left ventricular pressure at 2% increments of the cardiac cycle were available for each episode.
Mean values of the standard deviations of the integrated backscatter, wall thickness and left ventricular pressure were calculated for every 2% increment of the cardiac cycle in every pig. Plots were generated of the mean values and standard deviation versus time for visual inspection. End systole was defined as 30-46% cardiac cycle and end diastole as being 80-96%. End-systolic and end-diastolic measurements of integrated backscatter, wall thickness and left ventricular pressure were calculated by averaging the mean values of the parameters over the 9 time intervals within the ranges of the respective definitions. Hence this averaged data were based on 90 measurements (10 cardiac cycles times 9 time intervals). This procedure was repeated for each episode.

Finally, end-systolic and end-diastolic mean values and standard deviations of every parameter were calculated to obtain the group values in the population of 9 pigs at basal state (the reference values), during occlusion and during reperfusion.

Statistical analysis

To explore the differences of the measurements obtained during 3 episodes of the experiment, the end-systolic and end-diastolic group values of the measurements obtained during occlusion and reperfusion were compared to the respective group reference measurements. An unpaired t-test was applied and a p-value less than 0.01 was regarded as being significant.

The relationship between the integrated backscatter and myocardial wall thickness was explored in two ways. In order to investigate whether the inter-pig differences in integrated backscatter during the reference phase can be explained by differences in wall thickness, simple linear regression was performed between the reference measurements of integrated backscatter and the reference measurements of wall thickness. This was done for the end-systolic and end-diastolic mean values of the measurements separately.

Secondly, the dependency of the integrated backscatter on wall thickness was investigated in every separate measurement sequence. The 51 mean values of the time dependent integrated backscatter measurements were correlated with the mean values of the simultaneous wall thickness measurements. Simple linear regression analysis was applied using a significance level of $p < 0.01$.

RESULTS

Myocardial wall thickness could be measured in 8 pigs during the reference episode, in all pigs after 30 minutes occlusion, and in 6 pigs after 60 minutes reperfusion. In the other cases the endocardial wall was not clearly visualized.

A typical example of the time dependent measurements of integrated backscatter, myocardial wall thickness and left ventricular pressure during the reference episode and after 30 minutes of occlusion is shown in Fig. VII-1.

The obtained end-systolic and end-diastolic mean group values and the standard deviations during the three episodes are given in Table VII-1. The results of the integrated backscatter and wall thickness measurements obtained in end systole and end diastole during the three different episodes are graphically illustrated in Fig. VII-2.

End-systolic integrated backscatter during occlusion was significantly larger (5.3 dB) as compared to the end-systolic reference measurements. End-systolic wall thickness was significantly smaller (5 mm) during occlusion as compared to the end-systolic reference wall thickness. During reperfusion the left ventricular pressure was significantly lower (35 mmHg) as compared to the pressure during the reference episode.

No significant differences were found comparing the end-diastolic integrated backscatter measurements during occlusion and reperfusion with the respective reference measurements.

A small, but significant, difference (1.5 mm) was found comparing end-diastolic wall thickness measurement during occlusion with the reference measurement.

No significant relationship was found between the mean values of integrated backscatter and wall thickness measured during the reference episodes, both in end systole and end diastole. Therefore, the inter-pig differences in integrated backscatter could not be explained by differences in wall thickness.

The results of the simple linear regression analysis of the integrated backscatter and wall thickness of the 23 available sequences in which wall thickness could be measured showed significant inverse relationships between integrated backscatter and wall thickness in all but two cases. In one sequence measured during occlusion there was a non-significant inverse relationship and in one reference sequence the integrated backscatter increased significantly with increasing wall thickness. Typical examples of the scatterplots are shown in Fig. VII-3 (reference and after 30 minutes occlusion).

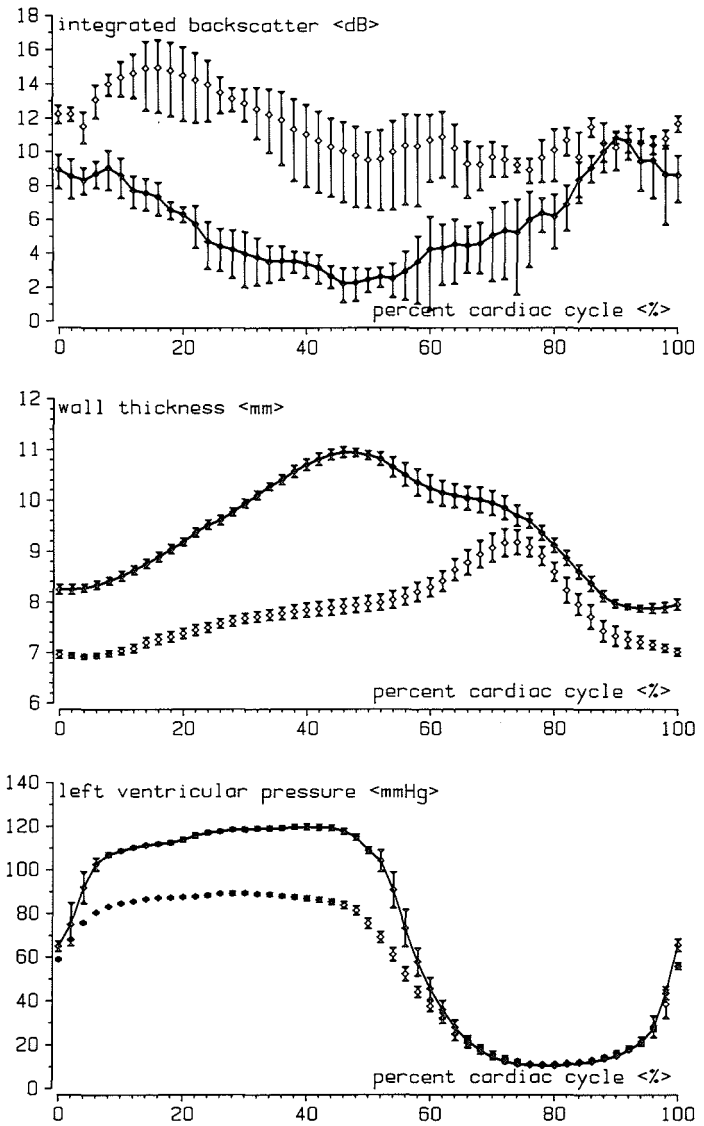

Fig. VII-1. The time dependent measurements of integrated backscatter (top), myocardial wall thickness (middle) and left ventricular pressure (bottom). Shown are the mean values (diamonds) and standard deviations (bars) of ten cardiac cycles in pig no. 8 during the reference episode (connected with a solid line) and after 30 minutes occlusion.

end systole		reference	occlusion	reperfusion
IB	(dB)	1.83 (2.62)	7.10 (3.15)*	3.51 (2.50)
Wall thickness	(mm)	12.2 (1.18)	7.22 (1.43)*	10.8 (2.29)
LV pressure	(mmHg)	5.0 (16.3)	73.3 (19.9)	59.8 (21.5)*
end diastole				
IB	(dB)	4.67 (3.57)	5.63 (4.25)	3.74 (4.05)
Wall thickness	(mm)	9.48 (1.24)	7.84 (1.05)*	10.2 (2.53)
LV pressure	(mmHg)	13.8 (6.12)	15.1 (4.83)	13.7 (6.14)

Table VII-1. Group mean values and standard deviations (between brackets) of integrated backscatter (IB), wall thickness and left ventricular (LV) pressure, in end systole (top) and end diastole (bottom) during the three different episodes of the experiments. An asterisk indicates a significant difference ($p < 0.01$, unpaired t-test) as compared to the respective reference measurements.

DISCUSSION

An important clinical application of myocardial ultrasonic integrated backscatter is the distinction between normal myocardium and ischemic myocardium. Our experiments show that end-diastolic integrated backscatter measurements could not distinguish between normal myocardium and acute ischemic myocardium.

End-systolic integrated backscatter measurements showed more favorable perspective for this application of cardiac tissue characterization. In our experiments the end-systolic integrated backscatter was 5.3 dB higher during occlusion as compared to the end-systolic reference measurements. However, due to the overlap of the measurements it would be difficult in some cases to distinguish normal myocardium from occluded myocardium.

An important observation is the large variation (range ± 5 dB) of the integrated backscatter of the different myocardia of the various pigs, which could not be explained, even in part, by the differences in wall thickness. At this stage this variation can only be labelled as inter-pig variation, which must be subject for further investigation. A fairly large inter-subject variation would be a serious drawback for the application of tissue characterization.

The other subject of the study was to explore the relationship between integrated backscatter and myocardial wall thickness throughout the cardiac cycle. Since 21 out of 23 measurement sequences showed a statistically significant inverse relationship, there is evidence that the integrated backscatter increases with decreasing wall thickness. These observations were made during the separate

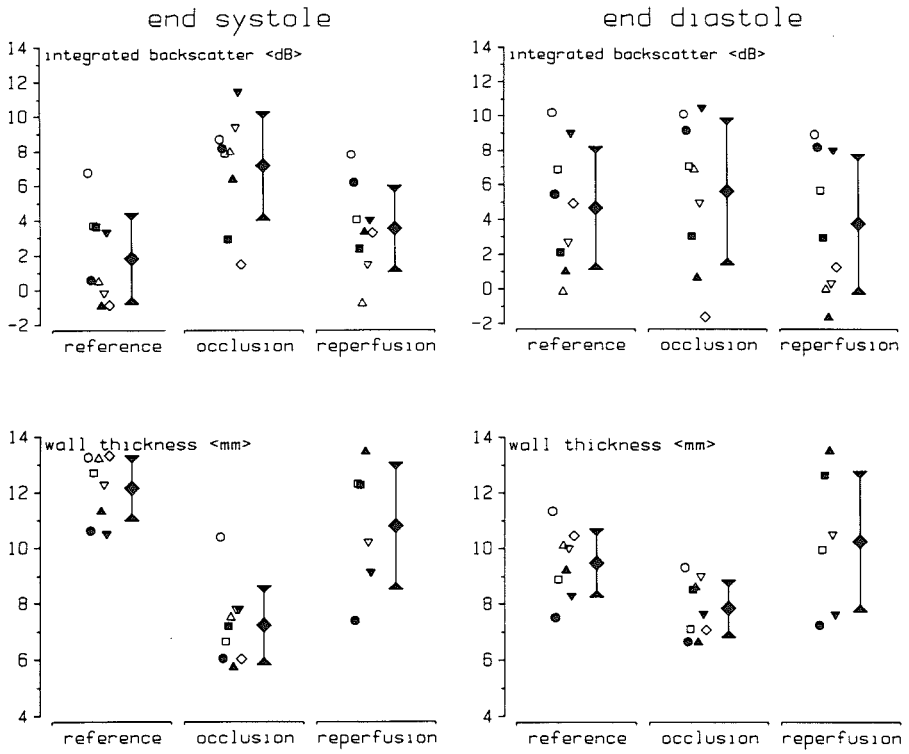

Fig. VII-2. End-systolic (left-hand side) and end-diastolic (right-hand side) mean values of integrated backscatter (top) and wall thickness (bottom) obtained during the different episodes of the experiment. Shown are the mean group values (solid diamonds) and standard deviations (bars). The different symbols indicate the measurements obtained in the different pigs.

episodes of the study and cannot be applied to explain the increase in end-systolic integrated backscatter by the decrease of end-systolic myocardial wall thickness after occlusion of a coronary artery. It should be realized that after the occlusion of a coronary artery two factors might influence the integrated backscatter change simultaneously: the myocardial wall thickness decreases and the myocardial tissue enters the state of acute ischemia. During ischemia the end-diastolic wall thickness was only 1.6 mm smaller as compared to the reference thickness, whereas end-systolic wall thickness was 5 mm smaller as compared to the baseline measurements.

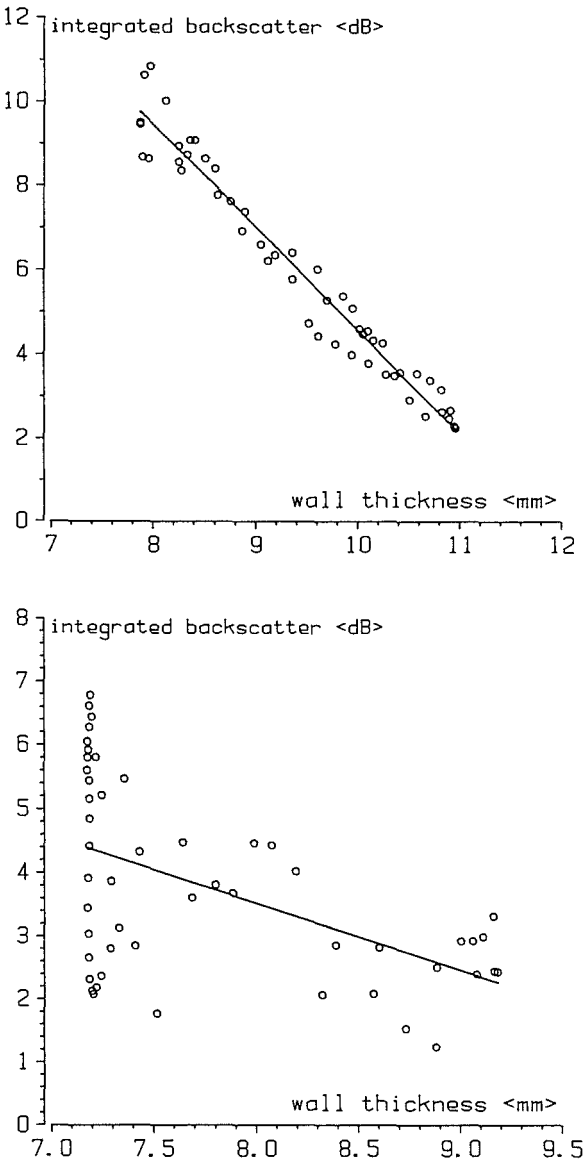

Fig. VII-3. Scatterplot of the integrated backscatter measurements versus myocardial wall thickness. The open circles depict the paired mean values of ten cardiac cycles during the reference episode (top, $R = 0.98$, $p < 0.01$) and after 30 minutes of acute ischemia (bottom, $R = 0.52$, $p < 0.01$).

This larger end-systolic difference in wall thickness might explain part of the end-systolic increase in backscatter measurements during ischemia, given the observed relationship between backscatter and wall thickness. Another part may be explained by the state of acute ischemia of the myocardium.

Since the variables 'ischemia' and 'wall thickness' are not dependent one cannot access their relative contribution to the increase in end-systolic integrated backscatter very accurately. To avoid this problem, one has to compare normal and ischemic myocardial tissue having the same ranges of wall thickness. Theoretically, this can be done by comparing reference measurements of backscatter and wall thickness with the reperfusion measurements in those pigs with persisting ischemia during reperfusion. Given the small number of available measurements (in our study only 5 pigs had measurable wall thicknesses during reperfusion and did not recover from ischemia) and the large inter-subject variation of the backscatter measurements the results in this study would be inconclusive.

An inverse relationship between integrated backscatter and myocardial wall thickness confirms the reported cyclic variation of myocardial integrated backscatter in dogs [4-6], although these measurements were obtained from the focal zone of the transducer at 5 cm distance. Our measurements were obtained in the near field of the transducer. In this situation phase cancellations at the aperture of the transducer may have influenced our measurements. It has been shown [14] that phase cancellations will cause fluctuations in the backscatter measurements and a lower absolute level of integrated backscatter as compared to phase insensitive measurements. Even though phase cancellations cannot be neglected, they do not seem to affect the cyclic variation of integrated backscatter measurements greatly. An essential point of this investigation is that all analysis was done with the same time-window at the same distance from the transducer, so that measurements could be compared and differences in measurements could not be explained by differences in phase cancellations.

CONCLUSION

On the average end-systolic integrated backscatter in acute ischemic pig myocardium measured to be 5 dB higher as compared to end-systolic backscatter measurements in normal myocardium. Therefore, end-systolic integrated backscatter measurements may be able to distinguish normal myocardium from acute ischemic myocardium contrary to end-diastolic backscatter measurements.

There is a significant inverse relationship between the myocardial integrated backscatter and the myocardial wall thickness throughout the cardiac cycles of the various episodes (references, occlusion and reperfusion) and shows integrated

backscatter measurements to be an indicator of myocardial contractile performance. Whether this relationship explains the increase in end-systolic integrated backscatter after an occlusion in full is still under investigation.

ACKNOWLEDGEMENT

These investigations are supported by the Netherlands Technology Foundation (STW).

REFERENCES

1. O'Donnell M, Bouwens M, Mimbs JW and Miller JG. Broadband integrated backscatter: an approach to spatially localized tissue characterization in vivo. Proc IEEE Ultrasonics Symp 79, 1979: 175-178.
2. Miller JG, Pérez JE and Sobel BE. Ultrasonic characterization of myocardium. Prog Cardiovasc Dis 28, 1985: 85-110.
3. Glueck RM, Mottley JG, Miller JG, Sobel BE and Pérez JE. Effects of coronary artery occlusion and reperfusion on cardiac cycle-dependent variation of myocardial ultrasonic backscatter. Circ Res 56, 1985: 683-689.
4. Mottley JG, Glueck RM, Pérez JE, Sobel BE and Miller JG. Regional differences in the cyclic variation of myocardial backscatter that parallel regional differences in contractile performance. J Acoust Soc Am 76, 1984: 1617-1623.
5. Wickline SA, Thomas LJ, Miller JG, Sobel BE and Pérez JE. The dependence of myocardial ultrasonic integrated backscatter on contractile performance. Circulation 72, 1985: 183-192.
6. Wickline SA, Thomas III LJ, Miller JG, Sobel BE and Pérez JE. A relationship between ultrasonic integrated backscatter and myocardial contractile function. J Clin Invest 76, 1985: 2151-2160.
7. Vered Z, Mohr GA, Barzilai B, Gressler CJ, Wickline SA, Wear KA, Shoup TA, Weiss AN, Sobel BE, Miller JG and Pérez JE. Ultrasound integrated backscatter tissue characterization of remote myocardial infarction in human subjects. J Am Coll Cardiol 13, 1989: 84-91.
8. Skorton DJ. Ultrasound tissue characterization: can the state of the myocardium be assessed directly yet noninvasively ? J Am Coll Cardiol 13, 1989: 92-94.
9. Wickline SA, Thomas III LJ, Miller JG, Sobel BE and Pérez JE. Sensitive detection of the effects of reperfusion on myocardium by ultrasonic tissue characterization with integrated backscatter. Circulation 74, 1986: 389-400.
10. Office of Science and Health Reports. Guide for the care and use of laboratory animals. DHEW Publication No. (NIH)80-23, DRR/NIH Bethesda, 1980.
11. Sassen LMA, Den Boer MO, Rensen RJ, Saxena PR and Verdouw PD. B-isoprolol improves perfusion of ischaemic myocardium in anaesthetized pigs. Br J Pharmacol 95, 1988: 361-370.
12. Lancée CT, Mastik F, Rijsterborgh H and Bom N. Myocardial backscatter analysis in animal experiments. Ultrasonics 26, 1988: 155-163.
13. Bloomfield P. Fourier analysis of time series: an introduction. John Wiley, New York, London, Sydney, Toronto, 1976: 80-85.
14. Johnston PH and Miller JG. Phase-insensitive detection for measurement of backscattered ultrasound. IEEE Transactions UFFC-33/6, 1986: 713-721.

CHAPTER VIII

THE RELATIVE CONTRIBUTIONS OF MYOCARDIAL WALL THICKNESS AND ISCHEMIA TO ULTRASONIC MYOCARDIAL INTEGRATED BACKSCATTER DURING EXPERIMENTAL ISCHEMIA

Hans Rijsterborgh, Frits Mastik, Charles Lancée, Loes Sassen,
Piet Verdouw, Jos Roelandt and Klaas Bom

Thoraxcentre, Erasmus University Rotterdam and
Interuniversity Cardiology Institute of the Netherlands

ABSTRACT

The purpose of this study was to assess the empirical relationship between myocardial integrated backscatter (IB) and myocardial wall thickness (WT) in normal myocardium. A second object was to estimate the additional contribution to acute ischemic integrated backscatter levels given this relationship.

Myocardial IB measurements and simultaneous myocardial WT measurements were made in 16 open chested pigs with intact coronary circulation (normal myocardium) and 10 minutes after the flow in the left anterior descending coronary artery had been reduced to 20% of its baseline value (ischemic myocardium). Measurements were made 50 times during one cardiac cycle and averaged over ten cardiac cycles. Changes in IB and WT measurements were normalized with respect to the non-ischemic end-diastolic values. The relationship between IB and WT in normal myocardium was estimated in every individual pig by simple linear regression. Estimates of IB during ischemia were calculated on the basis of this relationship and the ischemic WT measurements. Differences of the estimator and the actual measurement made during ischemia depict the actual contribution of the state of acute ischemia, without the influence of WT.

The slope of the relationship between IB and WT during normal myocardial contraction ranged from -0.16 to 0.03 dB/% (mean value = -0.036 dB/%, sd = 0.06 dB/%). The additional contribution of ischemia ranged from -3.84 to 5.56 dB (mean = 0.31 dB, sd = 2.72 dB).

It was concluded that the average contribution of ischemia to IB measurements is insignificant if the IB dependency on WT is removed from the data and that the higher level of ischemic IB measurements can be explained by the decrease in wall thickness during ischemia and not by the ischemia itself.

Accepted for publication in Ultrasound in Medicine and Biology 1990.

INTRODUCTION

A well-known feature of ultrasonic myocardial integrated backscatter is its cyclic variation during the cardiac cycle: in normal myocardium end-diastolic integrated backscatter is higher as compared to end-systolic backscatter levels [1-3]. The cyclic variation suggests an inverse relationship between integrated backscatter measurements and myocardial wall thickness. This relationship has been investigated in normal and ischemic myocardial tissue [4,5] in animal experiments.

It has been shown previously that integrated backscatter levels in acute ischemic myocardial tissue are higher as compared to integrated backscatter levels measured in normal myocardium [1,5,6,7].

After the occlusion of a coronary artery the two factors which might influence the magnitude of integrated backscatter measurements change simultaneously: the myocardial tissue becomes acutely ischemic and the myocardial wall thickness decreases. The intriguing question therefore remains whether the observed increased levels of integrated backscatter during acute ischemia is related to the decreased wall thickness or the state of ischemia itself.

It was the purpose of this study to assess the magnitude of the relationship between integrated backscatter and myocardial wall thickness and to investigate the relative contributions of wall thickness and the presence of acute ischemia to integrated backscatter levels in animal experiments. The relationship between ultrasonic integrated backscatter and myocardial wall thickness was assessed during normal myocardial performance. On the basis of this relationship and the wall thickness measurements taken during acute ischemia the level of integrated backscatter was estimated and compared with the actual measurements. A significant difference between the estimated backscatter and the actual measurements designates the contribution of the state of acute ischemia.

A computer based measurement system was applied [5,8] to obtain simultaneous measurements of myocardial integrated backscatter, myocardial wall thickness and left ventricular pressure.

METHODS

General

Animal experiments were performed on young Yorkshire pigs according to the guiding principles for the care and use of animals [9]. The animals (n=16, 24-30 kg) were sedated with 120 mg azaperone (i.m.) and subsequently anaesthetized with 150 mg of metomidate (i.v.). After intubation, the animals were respirated with a mixture of 30% oxygen and 70% nitrous oxide. Anaesthesia was maintained with 160 mg/kg/h alpha-chloralose (Merck, Darmstadt, FRG) followed

by an infusion of 5 mg/kg pentobarbitone sodium (Sonofi, Paris, France) via a catheter placed in the superior vena cava. Respiratory rate and tidal volume were adjusted to maintain physiological arterial blood gas values (mean ± sem): 7.39 ± 0.01 (pH), 45 ± 3 mmHg (pCO_2), 154 ± 4 mmHg (pO_2) and 93 ± 2% (O_2 saturation, measured with an ABL-3, Radiometer, Copenhagen, Denmark).

Left ventricular and central aortic pressure were monitored with microtipped catheters (Honeywell-Philips, Best, the Netherlands). After the heart was exposed using a midsternal split, a hydraulic occluder (RE Jones, Silver Spring, MD, USA) was placed around the proximal left anterior descending coronary artery (LADCA) just distal to its first diagonal branch and connected to a 1 ml syringe (Hamilton Bonaduz, Bonaduz, Switzerland) which was driven by a micrometer. Regional myocardial function was estimated from myocardial wall thickness recordings obtained with the aid of a 4.6 MHz ultrasonic transducer (Krautkramer-Branson, Lewistown, PA, USA) sutured onto a the epicardial surface in the middle of the region perfused by the distal part of the LADCA that was to be occluded. The transducer remained at this site during the entire experiment. The transducer was connected to an echocardiographic ultrasound system (Organon Teknika, Oss, the Netherlands).

Experimental protocol

After systemic hemodynamics had been stable for at least 30 minutes following completion of the surgical procedures, baseline values of systemic hemodynamics and regional myocardial function were obtained. LADCA flow was then gradually reduced by slowly inflating the balloon until regional wall thickening was abolished. Wall thickening remained absent during the episode of ischemia. If necessary, minor adjustments in the degree of flow reduction were performed during the first 5 minutes of ischemia, but, thereafter, no further manipulations were allowed [10].

Data acquisition

The measurements were made at two episodes during the experiments: the baseline measurements were obtained just before the occlusion was applied and after 10 minutes of subsequent acute ischemia. The ultrasound transducer (Krautkramer-Branson, Lewistown, PA, USA) was connected to an in-house developed transmitter/wide band amplifier (input impedance 100 Ohm, fixed gain 29 dB, -6 dB cut-off frequencies 1.2 and 10 MHz). Amplified ultrasound signals were filtered by a 5th order 10 MHz low-pass Chebyshev filter and connected to one channel of a dual channel digital oscilloscope (LeCroy 9400), digitizing the time interval of interest (4.5 μs to 24.5 μs following the transmitter pulse) with an

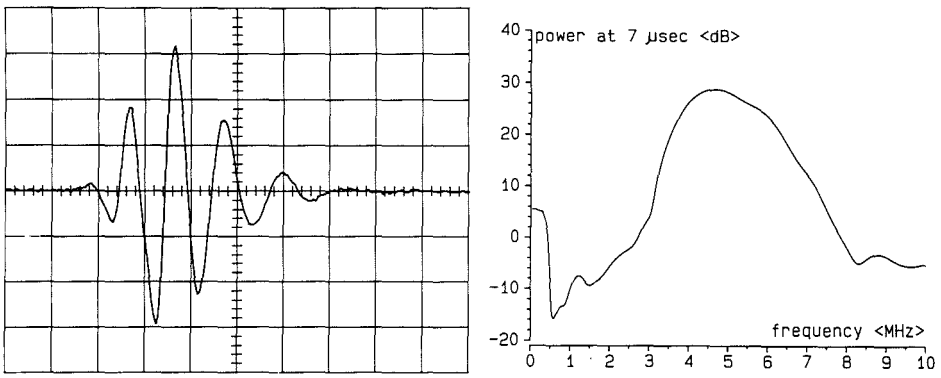

Fig. VIII-1. The time domain response (left-hand side, horizontal time scale: 0.2 µs per division) and the power spectrum (right-hand side) of the ultrasound reflection from a stainless steel plate at a distance of 5.4 mm (7 µs). The reference spectrum is the Fourier transform of the time domain signal and was used to correct the acquired myocardial ultrasound spectra.

8 bit resolution at a sample frequency of 25 MHz. Simultaneously a left ventricular pressure signal was digitized by the oscilloscope. The oscilloscope was interfaced with an IBM computer system (AT-3).

Measurement sequences were controlled by the computer system. At the onset of the pressure curve (begin systole), 62 broad band transmitting pulses (duration approximately 1 µs; see Fig. VIII-1) were generated with a repetition rate of 50 times the pig's heart rate. Following the last transmitter pulse the accumulated high frequency ultrasound data were stored on disc. Data acquisition of ten cardiac cycles could be accomplished within 30 to 40 seconds. This procedure was repeated for every episode of the study.

Data processing

Data were processed off-line. Ultrasound power spectra were calculated by a Fourier transformation using an array processor (Data Translation 7020). A split cosine bell window (p = 0.1) [11] of the time interval from 5 to 8 µs following the transmitter pulse was chosen to select the region of interest and to exclude the reflections of the endocardial wall from the spectra. Spectra were corrected for the frequency response of the transducer and the wide band amplifier by a reference spectrum measured from a flat stainless steel reflector (Fig. VIII-1). This was achieved by division of the measured power spectrum by the reference power spectrum for every frequency bin. Integrated backscatter was calculated by

averaging the corrected power spectra over the useful frequency band width from 3.2 to 7.2 MHz and expressed in decibel units.

To measure the myocardial wall thickness the acquired ultrasound signals of complete cardiac cycles were displayed in brightness mode on a video monitor using a 30 dB logarithmic signal compression. The endocardial wall was traced manually using the 'mouse' of the computer system. Only the measurements of cardiac cycles having clearly visualized borders were selected for further analysis.

The time dependent measurements of backscatter, percentage wall thickness and left ventricular pressure of the 10 consecutive cardiac cycles of every sequence were matched in time on the basis of the pressure curve. The time scales of the cardiac cycles were normalized to a linear scale expressed as a percentage of the cardiac cycle ranging from 0% (begin systole) to 100% (end diastole) with 2% increments. Mean values and standard deviations of the measurements were calculated for every 2% time increment of the cardiac cycle in every pig.

The measurements of integrated backscatter and wall thickness were related to their respective end-diastolic value taken during the baseline measurement interval in order to remove the inter subject differences of backscatter levels from the data set [5]. In every pig the end-diastolic integrated backscatter level at baseline was defined as 0 dB. Measurements of myocardial wall thickness were normalized with respect to the end-diastolic value measured during normal myocardial contractile performance and expressed as a percentage.

Statistical analysis

The relationship between relative backscatter and percentage wall thickening in normal myocardium was estimated by simple linear regression of the relative backscatter measurements and percentage wall thickening obtained during normal myocardial contractile performance for each pig.

The zero hypothesis was made that the relationship between backscatter and myocardial wall thickness as observed in normal myocardium is maintained during the state of acute ischemia. If a statistically significant additional contribution of the state of acute ischemia was found the zero hypothesis was rejected.

On the basis of the estimated relationship estimators of ischemic relative backscatter levels were calculated using the percentage wall thickening measured during acute ischemia in the same pig. The estimated relative integrated backscatter and the actual relative integrated backscatter measurements obtained in acutely ischemic myocardium were compared in the separate pigs using a paired t-test. A significance level of $p < 0.01$ rejected the zero hypothesis. In every pig the mean values relative integrated backscatter measurements and their estimators

were calculated and a paired t-test was performed on the data as a group.

For visual inspection, combined scatterplots were made of the relative integrated backscatter versus percentage wall thickening of the measurements obtained during normal myocardial contractile performance and during acute ischemia.

RESULTS

A typical example of the measurements of relative integrated backscatter, percentage myocardial wall thickening and left ventricular pressure taken at baseline and during acute ischemia plotted versus percent cardiac cycle is in given in Fig. VIII-2.

Combined scatterplots of the relative integrated backscatter measurements versus percentage wall thickening taken in the same pigs during baseline and during acute myocardial ischemia are depicted in Fig. VIII-3.

The results of the simple linear regression between relative integrated backscatter and percentage myocardial wall thickening
during normal contractile performance are given in Table VIII-1. The observed slopes in the individual pigs ranged from -0.163 dB/% to 0.034 dB/% (mean -0.036 dB/%, standard deviation 0.060 dB/%).

The mean values and standard deviations of the paired differences between the estimated relative integrated backscatter and the actually measured relative integrated backscatter including the results of the paired t-tests are given in Table VIII-1. In 15 out of 16 pigs a statistically significant contribution of ischemia was found and the zero hypothesis was rejected. The paired differences ranged from -3.84 dB to 5.76 dB (group mean value 0.31 dB, standard deviation 2.72 dB). In eight pigs a positive contribution in dB was found whereas the other eight pigs showed a negative contribution. The additional contribution of ischemia to the backscatter measurements, although statistically significant, proved to be strongly dependent on the individual pig.

A paired t-test of the group mean differences resulted in a p-value of 0.65 (n.s.). Therefore no predictable contribution of ischemia could be shown in the group of pigs. A scatterplot of this comparison is depicted in Fig. VIII-4.

DISCUSSION

The cyclic variation of myocardial ultrasonic integrated backscatter has been shown to be a parameter of myocardial contractile performance [3]. An empirical relationship between integrated backscatter and myocardial wall thickness would explain this.

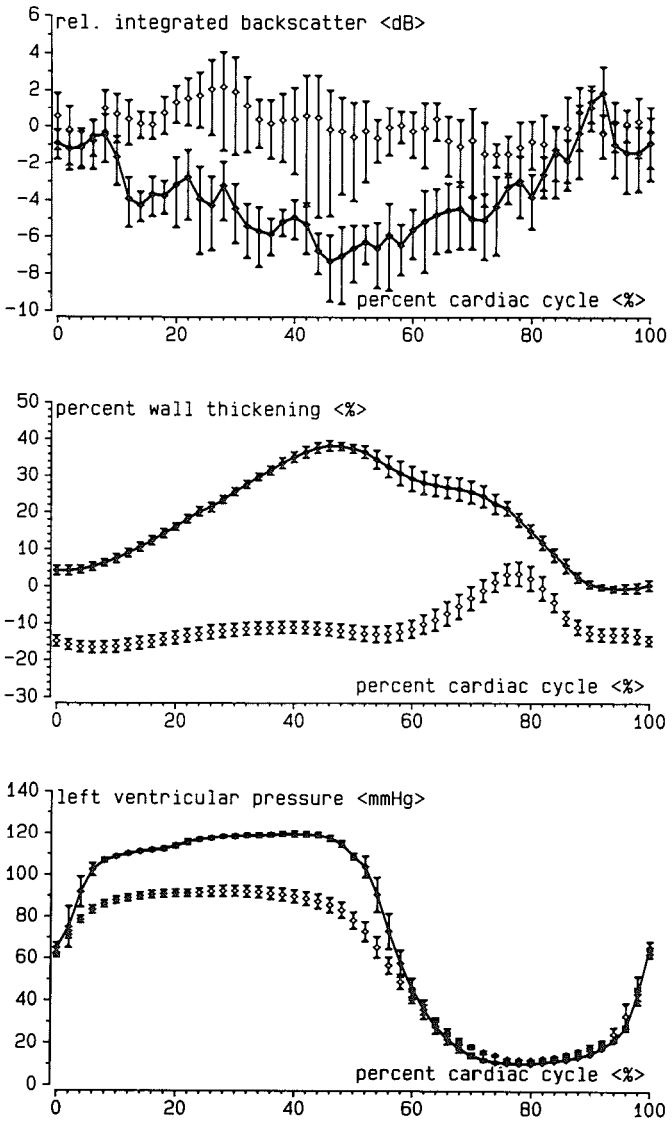

Fig. VIII-2. The time dependent measurements of relative integrated backscatter (top), percentage myocardial wall thickness (middle) and left ventricular pressure (bottom). Shown are the mean values (diamonds) and standard deviations (bars) of ten cardiac cycles of pig no. 6 during baseline (connected with a solid line) and after 10 minutes subsequent acute ischemia.

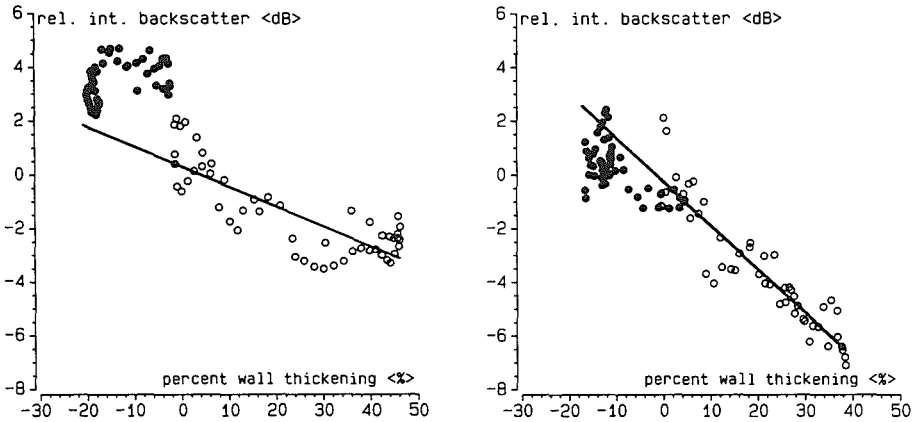

Fig. VIII-3. Combined scatterplots of the relative integrated backscatter measurements versus percentage myocardial wall thickness taken in pig no. 1 (left-hand side) and in pig no. 6 (right-hand side). Shown are the mean values of ten cardiac cycles. The line of regression was obtained by simple linear regression of the measurements taken at normal myocardial performance (open circles). The closed circles depict the measurements obtained during acute ischemia in the same pig.

The purpose of this studies was to investigate the relationship between integratedbackscatter and wall thickening in an experimental animal model under normal and ischemic conditions. It should be realized that in acute ischemia the wall thickness of a pig's myocardium is decreased during most of the cardiac cycle as compared to its non-ischemic end-diastolic value as can be appreciated from Fig. VIII-2. The experiments were also designed to distinguish whether the increased backscatter levels during acute ischemia were related to a decreased wall thickness or to the ischemia itself.

The relationship between backscatter and wall thickness

Myocardial ultrasonic backscatter is caused by the interaction between ultrasound and small structures within the myocardium. It is assumed that the size of these structures (scatterers) is small as compared to the wave length of the applied ultrasound frequency. Part of the acoustic energy is returned to the ultrasound transducer by these scatterers. According to the backscatter theory [12] the energy level of ultrasonic backscatter is determined by the density of the scatterers within the myocardium, the size of the scatterers and the frequency of the applied ultrasound. The change of myocardial integrated backscatter level at a given frequency during the cardiac cycle may therefore be explained by a change in

	normal tissue rel. IB versus % wall thickness		acute ischemic tissue paired differences actual rel. IB minus estimated rel. IB		
pig no.	slope (dB/%)	offset (dB)	mean (dB)	sd (dB)	t-test
1	-0.075	0.32	2.06	1.02	$p < 0.01$
2	-0.067	-0.08	-1.79	1.16	$p < 0.01$
3	0.034	0.04	5.76	1.58	$p < 0.01$
4	0.011	0.97	1.82	1.31	$p < 0.01$
5	-0.018	-1.64	4.17	1.41	$p < 0.01$
6	-0.163	-0.26	-1.15	0.84	$p < 0.01$
7	0.012	-0.46	2.56	1.62	$p < 0.01$
8	0.022	-0.37	0.42	1.41	$p = 0.04$
9	-0.023	0.67	-1.63	1.34	$p < 0.01$
10	0.033	-0.95	-2.62	1.21	$p < 0.01$
11	0.012	0.73	-0.95	1.33	$p < 0.01$
12	-0.114	1.13	-1.98	1.54	$p < 0.01$
13	-0.006	-1.28	3.22	1.12	$p < 0.01$
14	-0.071	0.29	0.72	0.82	$p < 0.01$
15	-0.122	0.53	-3.84	0.97	$p < 0.01$
16	-0.041	0.02	-1.82	1.82	$p < 0.01$
mean	-0.036	-0.02	0.31		
sd	0.060	0.79	2.72		

Table VIII-1. The slope and offset of the relationship between relative integrated backscatter (rel. IB) measurements and percentage myocardial wall thickness as determined by simple linear regression (column 2 and 3). On the right-hand side the results are given of the paired t-tests between the estimated relative integrated backscatter based on this relationship and the actual measurements.

scatter density or a change in scatter size or both. But the actual cause of the cyclic variation of myocardial integrated backscatter has not yet been assessed. Some information about this may be obtained by analyzing the corrected power spectrum (backscatter transfer function). Since integrated backscatter is the frequency average of the backscatter transfer function, the information on whether the cyclic variation is caused by change in scatter density or by a change in scatter size is lost. This is currently under investigation.

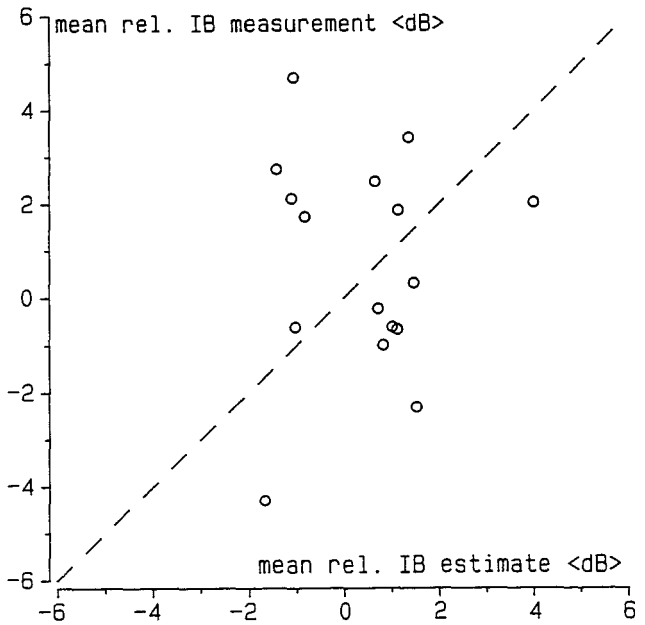

Fig. VIII-4. Scatterplot of the mean values of the estimated relative integrated backscatter (rel. IB) measurements versus the actually measured relative integrated backscatter obtained in 16 pigs. A measurement above the line of identity (dashed line) signifies a positive contribution of the state of acute ischemia to the relative integrated backscatter measurement.

The empirical relationship between myocardial integrated backscatter and myocardial wall thickness has been studied in animal experiments before [4,5] but not to a large extent. The result of the present study showed an average slope of -0.036 dB/%. This would result in a mean cyclic variation of 1.8 dB for a 50% change in wall thickness. An interesting observation of this study is that the ranges of the estimated slopes proved to be quite large (-0.163 dB/% to 0.034 dB/%). This indicates that there are consistent individual differences between the pigs, notwithstanding the controlled conditions under which this in vivo study was performed. Since the ultrasound transducer was sutured directly upon the pig's myocardium the integrated backscatter measurements were marginally influenced by a change of the angle of insonification during the cardiac cycle. Secondly, these measurements are not corrupted by an unknown frequency dependent attenuation of the ultrasound path in between the transducer and the region of interest opposite to closed chested in vivo experiments.

The additional contribution of ischemia

Fifteen out of sixteen pigs showed statistically significant contributions to the integrated backscatter levels by ischemia. But the contribution does not appear to be consistent in the individual pig: 50% of the pigs examined showed a positive contribution, the other 50% a negative contribution. Therefore other parameters than myocardial wall thickness and ischemia must be involved. However since no overall contribution of ischemia could be proven in the analysis of the data as a group the unknown factor also appeared to cancel out. The hypothesis that the relationship between integrated backscatter and wall thickness is maintained during acute ischemia of the myocardium was rejected in all but one of the individual pigs but not in the group as a whole.

The variability of myocardial integrated backscatter measurements

In a previous study [5] we reported a large inter subject variation of absolute integrated backscatter levels in the myocardium of different pigs. In the current study we observed a large inter subject variation of the relationship between integrated backscatter and myocardial wall thickness during normal contractile performance. Three different explanations may be given here. Applying the backscatter theory to these observations one has to conclude that there are individual differences in the structure of a pig's myocardium.

A second explanation may be individual differences in layout of the coronary arteries of a pig's heart. In the experiments the position of the ultrasound transducer was referenced to the actual layout of the LADCA. Therefore small differences in position of the transducer with regard to the outer geometry of the heart may be introduced.

A third explanation may be that in practice the ultrasound signals which are returned by the pig's myocardium consist of a combination of backscatter signals and weak specular reflections. In the time domain one cannot distinguish genuine backscatter signals, originating from scatterers smaller than the wavelength in size, and weak specular reflections, caused by structures larger than the wavelength. Analysis of the backscatter transfer function of myocardial tissue may solve this problem. This should be a subject for further investigation.

CONCLUSION

The increased myocardial integrated backscatter level during acute ischemia are more related to the wall thickness than to the state of acute ischemia itself. On the average there was no consistent additional contribution to the level of integrated backscatter measurements if the dependency of wall thickness is removed from the

data. In addition it may be concluded that integrated backscatter measurements in pigs are subjected to a large inter subject variation. In normal myocardium the slope relationship between myocardial integrated backscatter level and wall thickness showed a large variation.

ACKNOWLEDGEMENT

These investigations are supported by the Netherlands Technology Foundation (STW).

REFERENCES

1. Miller JG, Pérez JE, Mottley JG, Madaras EI, Johnston PH, Blodgett ED, Thomas LJ and Sobel BE. Myocardial tissue characterization: an approach based on quantitative backscatter and attenuation. Proc IEEE Ultrasonics Symp 83 CH1947-1, 1983: 782-793.
2. Mottley JG, Glueck RM, Pérez JE, Sobel BE and Miller JG. Regional differences that parallel regional differences in contractile performance. J Acoust Soc Am 76, 1984: 1617-1623.
3. Wickline SA, Thomas III LJ, Miller JG, Sobel BE and Pérez JE. The dependence of myocardial ultrasonic integrated backscatter on contractile performance. Circulation 72, 1985: 183-192.
4. Wickline SA, Thomas III LJ, Miller JG, Sobel BE and Pérez JE. Sensitive detection of the effects of reperfusion on myocardium by ultrasonic tissue characterization with integrated backscatter. Circulation 74, 1986: 389-400.
5. Rijsterborgh H, Mastik F, Lancée CT, van der Steen AFW, Sassen LMA, Verdouw PD, Roelandt J and Bom N. Ultrasonic myocardial integrated backscatter and myocardial wall thickness in animal experiments. Ultrasound in Med & Biol 16, 1990: 29-36.
6. O'Donnell M, Bauwens D, Mimbs JW and Miller JG. Broadband integrated backscatter: an approach to spatially localized tissue characterization in vivo. Proc IEEE Ultrasonics Symp 79 CH1482-9, 1979: 175-178.
7. Glueck RM, Mottley JG, Miller JG, Sobel BE and Pérez JE. Effects of coronary artery occlusion and reperfusion on cardiac cycle-dependent variation of myocardial ultrasonic backscatter. Circ Res 56, 1985: 683-689.
8. Lancée CT, Mastik F, Rijsterborgh H and Bom N. Myocardial backscatter analysis in animal experiments. Ultrasonics 26, 1988: 155-163.
9. Office of Science and Health Reports. Guide for the care and use of laboratory animals. DHEW Publication No. (NIH)80-23, DRR/NIH Bethesda.
10. Sassen LMA, den Boer MO, Rensen RJ, Saxena PR and Verdouw PD. B-isoprolol improves perfusion of ischaemic myocardium in anaesthetized pigs. Br J Pharmacol 95, 1988: 361-370.
11. Bloomfield P. Fourier analysis of time series: an introduction. New York: John Wiley, 1976: 80-85.
12. Morse PM and Ingard KU. Theoretical acoustics. New York: Mcgraw-Hill company, 1968: 400-441.

SUMMARY

This thesis on the various aspects of quantitative cardiac ultrasound is divided into three parts. The subject of the first four chapters deals with problems of the assessment of the reproducibility of echocardiographic measurements. These chapters are focussed on quantitative Doppler echocardiography and cross-sectional echocardiography.

The second part of this thesis deals with reference ranges of echocardiographic measurements. Reference ranges, established in healthy subjects, are mandatory to make the distinction between health and disease. In Chapter V reference ranges of M-mode echocardiographic measurements of the normal population within the age range between 4 and 64 years are provided. Chapter VI is devoted to the reference ranges of cross-sectional left ventricular measurements established in healthy male adult subjects.

The third part constitutes a new aspect of quantitative cardiac ultrasound. Chapter VII and Chapter VIII describe the initial experience with myocardial tissue characterisation.

In **Chapter I** a general survey is given on the various methods which are at our disposal to assess the reproducibility of the echocardiographic measurements in terms of bias and random error. The concepts of bias and random error are discussed as well as the methods to estimate their magnitude. A method is described separating the various components of the total random variation of echocardiographic measurements: observer variability and beat-to-beat variation. The problems of comparing two or more methods of measurements are discussed. It is shown that, even without having a true 'gold standard', the clinically relevant aspects of the reproducibility of echocardiographical measurements can be assessed.

Continuous wave Doppler echocardiography is the obvious non-invasive means to evaluate the valvular function in adult patients with mitral stenosis or mitral prosthesis. The reproducibility of Doppler blood flow velocity measurements in this group of patients represent the subject of **Chapter II.** The routinely made Doppler measurements were being evaluated: the early peak velocity, the mean diastolic velocity, the mean temporal velocity and the pressure half-time.

The differences in bias between observers due to interpretational differences of the same velocity tracings were investigated. The overall random variation of the Doppler parameters was estimated separately in patients in sinus rhythm and in patients in atrial fibrillation. In addition the various components of the overall random variation could be assessed: the observer random variation, the beat-to-beat variation due to changes in heart rate and the beat-to-beat variation caused by respiratory effects.

With the knowledge of the specific components of the overall random variation of these measurements one can evaluate the properties of the measurement itself. For instance, the magnitude of the overall random variation of the pressure half-time measurement was found to be relatively large and this could not be explained by contributions of changes in heart rate or by respiratory effects. In that case the Doppler method of measuring the pressure half-time must be in doubt. The overall variation of the other Doppler parameters studied could be explained, in part, by the components of the beat-to-beat variation.

Another clinically relevant application of the results of this study can be made in the field of follow-up studies. If measurements taken in the same patient are to be compared, as in a follow-up study, the random measurement variation will introduce a random component in the differences of the measurements. However with the information on the magnitude of the overall intra-patient variation one can calculate 'threshold' differences needed to designate a genuine, or statistically significant, change of the measurements of an individual patient. In Chapter II a table of the threshold differences was presented of the Doppler parameters included in this study.

The subject of **Chapter III** is a simple hydrodynamic model which describes the relationship between the pressure drop across an obstruction in a flow channel and the velocity of the fluid at the site of the obstruction. The model was developed more than two centuries ago by Bernoulli and it is applied nowadays in cardiology as a non-invasive estimation of the pressure drop across a stenotic valve on the basis of a Doppler echocardiographic blood flow velocity measurement.

In this chapter it is shown that, if the Bernoulli equation is verified in an *in vitro* experiment, both the measured velocities and the measured pressure drops are too large as compared to their respective values predicted by the Bernoulli theory. This observation is the more remarkable if one realises that the Bernoulli equation is based on fundamental physical laws such as the law of conservation of mass and the law of conservation of energy. Therefore one must conclude that the implicit assumptions to be made before applying the Bernoulli equation are not valid. A detailed analysis is given about the phenomena causing the discrepancies: energy losses at the inlet of the obstruction, the magnitude of the blood flow velocity proximal to the obstruction, the consequences of a non-uniform spatial velocity profile and the pressure recovery in the region distal to the obstruction.

A review is presented on the results of the *in vivo* verifications, published in the literature, comparing the non-invasive estimation of transvalvular pressure drops in aortic stenosis, using the simplified Bernoulli equation, on one hand and the

catheterisation data representing the 'gold standard' on the other. The observed differences measured to be up to 25 mmHg.

The conclusion must be that, although the Bernoulli theory does not describe the procedures in the vicinity of a flow channel obstruction in full, in practice its effectiveness can be ascribed to the fortunate circumstances that the overestimation and the underestimation of the actual transvalvular pressure drop seem to cancel out to some extent.

Chapter IV is about a subject which is seldom reported upon in the medical literature: the estimation of the overall measurement variability within patients of the echocardiographically determined left ventricular volumes and ejection fraction. As in Chapter II, the overall variability is composed of the random beat-to-beat variation and the random measurement error introduced at the time of the interpretation of the cross-sectional echocardiograms. This is a practical approach, since all routinely made measurements will be influenced by the sum of these components.

The various standardised examination techniques by which cross-sectional images can be obtained as well as the corresponding methods of the determination of the volumes and the ejection fraction were explored: the area-length method in images obtained in four-chamber views and apical long-axis views as well as the modified Simpson's rule applied on parasternal short-axis images.

The magnitude of the overall variation of the volume measurements obtained in both apical views turned out to be within the same range. The overall random variation of the volume measurements from short-axis images was significantly smaller as compared to the random variation of the apical images. An explanation may be found in the fact that the Simpson's rule volume determination includes an extra independent cross-sectional area measurement as compared to the area-length method.

Detailed analysis, based on a large number of measurements, showed that the overall random variation of the left ventricular volume measurements increased significantly with the magnitude of the volume itself. A small, non-significant, decrease of the random variation of the ejection fraction measurements with increasing ejection fraction was found.

The clinical relevance of the results of this study is twofold: one can estimate the number of measurements to be made in an individual patient in order to reduce the random variation of the obtained mean value to a desired level. Secondly one can calculate a minimum difference in order to detect a real, or statistically significant, change in the left ventricular function of an individual patient. Plots are presented of these threshold differences of volume and ejection fraction

measurements given the number of measurements and the level of statistical significance.

Chapter V and **Chapter VI** deal with reference ranges of echocardiographic measurements established in healthy subjects. In Chapter V the reference ranges are reported of the standard M-mode echocardiographic measurements, whereas Chapter VI is on the subject of 'normal values' of the left ventricular volumes, stroke volume and ejection fraction as determined by cross-sectional echocardiography. The topic of these chapters is the same: to find the best predictor of a specific echocardiographic measurement of a healthy subject given the person's attributes age, weight, height, heart rate and sex.

To solve this problem a multivariate regression model was explored in which the echocardiographic measurements represent the dependent variables and the attributes of the normal subjects constitute the independent variables. The applied strategy was to remove the independent variables, having a non-significant contribution to the estimation, from the regression equation until a regression equation was obtained consisting of significant contributors only.

The conclusion of both studies was the same: the person's weight proved to be the best predictor of the echocardiographic measurements. In the study of the M-mode echocardiographic measurements the person's sex proved to be an additional contributor. This could not be investigated for the cross-sectional echocardiographic measurements (Chapter VI), since this study included only adult male persons.

An interesting point here is that the body surface area of a person, a traditional estimator of a number of clinical measurements, did not prove to be the optimal predictor of the echocardiographic determinations. In addition it is explained, in Chapter V, why body surface area could even be an incorrect predictor of left ventricular wall thickness for theoretical reasons.

With the results of these studies nomograms were constructed of the ninety percent confidence limits of the respective measurements versus the weight of a normal subject to be used as a valuable reference in the daily practice of the routine echocardiographic laboratory.

A new issue in quantitative cardiac ultrasound is the application of ultrasound to distinguish normal cardiac muscle tissue from diseased tissue. The basic concept of ultrasound myocardial tissue characterisation is that the amount of acoustic energy (integrated backscatter), returned by a cardiac muscle, will change if the structure of the tissue changes. **Chapter VII** and **Chapter VIII** describe our initial experience with myocardial tissue characterisation during experimental ischemia. In Chapter VII absolute levels of integrated backscatter measured during ischemia

and during normal contractile function are compared. During end diastole no significant differences were found of integrated backscatter levels measured in normal cardiac tissue and ischemic tissue. End-systolic integrated backscatter from ischemic tissue was 5.3 dB higher than that of normal tissue. An inverse relationship between integrated backscatter and myocardial wall thickness was observed. In Chapter VII this relationship was investigated in more detail.

SAMENVATTING

Dit proefschrift over quantitatieve toepassingen van ultrageluid voor gebruik in het hart is bestaat uit drie gedeelten. Het onderwerp van de eerste vier hoofdstukken is het schatten van de reproduceerbaarheid van echocardiografische metingen. Deze hoofdstukken zijn specifiek gericht op de quantitative Doppler echocardiografie en de quantitatieve tweedimensionale echocardiografie.

Het tweede gedeelte van dit proefschrift heeft als onderwerp de normale waarden van echocardiografische metingen. Normale waarden, zoals die gemeten worden in gezonde proefpersonen, zijn een essentieele voorwaarde teneinde onderscheid te kunnen maken tussen een normaal en een abnormaal functionerend hart. Het betreft hier de normale waarden van eendimensionale echocardiografische M-mode metingen (Hoofdstuk V) en die van tweedimensionale metingen in gezonde volwassen mannelijke personen (Hoofdstuk VI).

Het derde gedeelte behandelt een geheel nieuw aspect van quantitatieve echocardiografie. Hoofdstuk VII en Hoofdstuk VIII beschrijven de eerste ervaringen met weefsel-identificatie van de hartspier met behulp van ultrageluid.

In **Hoofdstuk I** wordt een overzicht gegeven van de verschillende methoden voor het schatten van de reproduceerbaarheid van echocardiografische metingen in termen van consistente meetfouten ('bias') en toevallige meetfouten ('random error'). Het onderscheid tussen consistente en toevallige meetfouten wordt bediscusieerd evenals de methoden voor het schatten van de grootte van deze meetfouten. Een methode wordt beschreven om de verchillende componenten van de totale variatie van echocardiografische metingen tengevolge van toevallige fouten te scheiden: variabiliteit veroorzaakt door de interpretatie en toevallige variatie tengevolge van spontane varanderingen van het hartritme. De problemen van het vergelijken van twee of meer meetmethoden worden behandeld. Hier wordt duidelijk gemaakt dat, ook zonder 'gouden standaard', de klinisch relevante aspecten van de reproduceerbaarheid van echocardiografische metingen in kaart kunnen worden gebracht.

Doppler echocardiografie met behulp van continu ultrageluid is de aangewezen niet-invasieve weg voor de evaluatie van de klepfunctie van volwassen patienten met mitraalklepstenose of mitraalklepprothese. De reproduceerbaarheid van Doppler snelheidsmetingen in deze groep patienten vormt het onderwerp van **Hoofdstuk II**. De Doppler snelheidsmetingen van het bloed in het hart welke routinematig worden uitgevoerd werden geevalueerd: de diastolische pieksnelheid, de gemiddelde diastolische snelheid, de gemiddelde snelheid over de hartcyclus en de halfwaardetijd van het diastolische drukverloop.

De verschillen in consistente fout tengevolge van de interpretatie door de waarnemers werden onderzocht. De totale toevallige variatie van de Doppler metingen werd geschat bij patienten in sinus ritme en bij patienten met atrium vibrillatie. Ook werd een schatting gemaakt van de verschillende componenten van de toevallige variatie van de metingen: de toevallige fout tengevolge van de interpretatie door de waarnemer, de slag-tot-slag variatie tengevolge van de veranderingen van het hartritme en de variatie van de metingen als gevolg van de ademhaling.

Met behulp van deze kennis van de verschillende componenten van de totale toevallige meetfout werden de eigenschappen van de metingen geevalueerd voor wat betreft de reproduceerbaarheid. Een van de conclusies was dat de totale toevallige variatie van de meting van de halfwaardetijd van het drukverloop over de mitraalklep relatief groot was en niet veroorzaakt werd door variatie van het hartritme of door de ademhaling. In dit geval moet de reproduceerbaarheid van de meting van de halfwaardetijd in twijfel worden getrokken. De totale variatie van de andere Doppler parameters kon, gedeeltelijk, worden verklaard met de variatie van het hartritme.

Een andere klinisch relevante toepassing van de resultaten van deze studie is bij vervolgstudies. Bij het vergelijken van metingen van dezelfde patient zal de toevallige variatie van de metingen een toevallige fout introduceren in het verschil van de metingen. Met de kennis van de grootte van de variatie van de metingen binnen een patient kan het minimale verschil van de metingen worden berekend wat nodig is om een statistisch significant verschil te detecteren. In Hoofdstuk II worden deze minimale verschillen voor de bestudeerde Doppler parameters in de vorm van een tabel gepresenteerd.

Een eenvoudig hydrodynamisch model is het onderwerp van **Hoofdstuk III.** Dit model beschijft de relatie tussen het drukverval over een vernauwing in een vloeistofleiding en de snelheid van de vloeistof op de plaats van de vernauwing. Het basale idee werd meer dan twee eeuwen geleden bedacht door Bernoulli en wordt tegenwoordig in de cardiologie toegepast om op niet-invasieve manier het drukverschil over een stenotische hartklep te schatten op basis van een Doppler snelheidsmeting.

Aan de hand van een *in vitro* experiment wordt in dit hoofdstuk duidelijk gemaakt dat in de praktijk zowel de gemeten vloeistof snelheden als de gemeten drukverschillen groter zijn dan de berekende waarden gegeven door de theorie van Bernoulli. Aangezien de wet van Bernoulli gebaseerd is op fundamentele natuurkundige wetten zoals de wet van behoud van massa en de wet van behoud van energie moeten we concluderen dat de impliciete aannamen, welke gemaakt

dienen te worden alvorens de theorie van Bernoulli toe te passen, in de praktijk niet geldig zijn. Er wordt een gedetaileerde analyse gemaakt van de verschillen tussen de theorie en de prakijk: de energieverliezen van het instroomgebied van de obstructie, het verwaarlozen van de bloedstroomsnelheid vóór de obstructie, de gevolgen van een niet-uniforme ruimtelijke snelheidsverdeling en het herstel van de druk na de obstructie.

Er wordt een overzicht gegeven van de in de litteratuur gepubliceerde *in vivo* vergelijkingen van drukmetingen verkregen tijdens hartcatheterizatie en drukmetingen geschat met de vereenvoudigde formule van Bernoulli op basis van een niet-invasieve Dopplersnelheidsmeting. De praktijk leert dat deze verschillen kunnen oplopen tot 25 mmHg.

Alhoewel het eenvoudige model van Bernoulli geen complete beschrijving geeft van de verschijnselen rond een vernauwing in een transportleiding met een stromende vloeistof, moet worden geconcludeerd dat de effecten welke aanleiding geven tot over- en onderschatting van het drukverschil over een obstructie in de praktijk elkaar gedeeltelijk opheffen.

Hoofdstuk IV heeft als onderwerp de totale variabiliteit, binnen een patient, van tweedimensionale echocardiografische metingen van de linker hartkamer (volumes en ejectie fractie). Evenals in Hoofdstuk II wordt de totale variabiliteit verondersteld samengesteld te zijn uit de slag-tot-slag variatie en de toevallige fout gemaakt bij de interpretatie. Aangezien elke routine meting beinvloed wordt door deze twee toevallige factoren is dit een practische benadering.

De verschillende gestandaardiseerde onderzoekstechnieken om tweedimensionale cardiale beelden te registreren werden onderzocht evenals de verschillende methoden om het volume van het hart en de ejectie fractie te bepalen: de 'area-length' methode toegepast op beelden verkregen vanuit de vier kamer doorsnede en vanuit de apicale lange as doorsnede; de gemodificeerde regel van Simpson toegepast op korte as beelden.

De totale variatie van de volume metingen verkregen uit de beide apicale doorsneden bleek ongeveer even groot te zijn. De totale variatie van de volume metingen van de korte as opnamen bleek kleiner te zijn vergeleken met die van de apicale doorsneden. Dit laaste kan verklaard worden uit het feit dat de volume meting gebaseerd op de gemodificeerde regel van Simpson een extra onafhankelijke oppervlakte meting impliceerd.

Uit een gedetaileerde analyse, gebaseerd op een groot aantal metingen, bleek dat de totale toevallige variabiliteit van de volume metingen significant toeneemt naarmate het gemeten volume groter wordt. De variabliteit van de ejectie fractie metingen nam, niet significant, af met grotere waarden van de ejectie fractie.

De klinische toepassing van de resultaten van deze studie worden geillustreerd op twee manieren. Op grond van de grootte van de variabiliteit kan bepaald worden hoeveel metingen nodig zijn om de toevallige fout van de gemiddelde waarde op het gewenste nivo te krijgen. Ten tweede is het mogelijk om het minimale verschil te berekenen wat nodig is om een significant verschil te kunnen detecteren in een vervolgstudie van dezelfde patient, gegeven het aantal waarnemingen en het gewenste significantienivo. Deze minimum verschillen worden als functie van de gemeten waarde in grafiek weergegeven.

Hoofdstuk V en **Hoofdstuk VI** zijn gewijd aan een ander aspect van de quantitatieve echocardiografie: de normale waarden van echocardiografische routine metingen. In Hoofdstuk V worden de normale waarden gepresenteerd van echocardiografische M-mode metingen, terwijl Hoofdstuk VI de normale waarden van het linker kamervolume, de ejectie fractie en het slagvolume, bepaald met behulp van tweedimensionale echografische afbeeldingstechnieken, beschrijft. De vraagstelling in beide hoofdstukken is dezelfde: wat is de beste voorspeller van de echocardiografische parameter van een persoon, gegeven de leeftijd, lengte, gewicht, geslacht en hartritme ?

De oplossing van dit probleem werd gezocht in een multivariaat regressie model waarbij de echocardiografische parameters de afhankelijke variabelen vormden en de attributen van de normale personen de onafhankelijke variabelen representeerde. De strategie was om achtereenvolgens de onafhankelijke variabele, welke geen significante bijdrage leverde aan de regressie vergelijking, te verwijderen uit het model zodat een gereduceerde regressie vergelijking ontstond met alleen maar significante afhankelijke variabelen.

De conclusie van beide studies was dezelfde: het gewicht van de persoon is de beste schatter van de bestudeerde echocardiografische parameters. Bij de studie van de M-mode parameters bleek dat het geslacht van de persoon ook van bepalend te zijn voor de afmetingen van cardiale structuren.

Een interessant punt was dat het lichaamsoppervlak, een traditionele schatter van verschillende klinische parameters, niet de beste voorspeller bleek te zijn van de echocardiografische metingen. In Hoofdstuk V wordt toegelicht dat het lichaamsoppervlak als voorspeller van de dikte van de hartspier van de linker kamer op theoretische gronden zelfs incorrect is.

Gebaseerd op de resultaten van deze studies werden nomogrammen gemaakt van de negentig procent betrouwbaarheids intervallen van de echocardiografische parameters tegen het gewicht voor praktisch gebruik in de kliniek.

Een geheel nieuw aspect van de quantitatieve echocardiografie is het toepassen van ultrageluid voor het onderscheiden van normaal en ziek hartspierweefsel. Het

basale idee achter deze vorm van weefselidentificatie is dat de hoeveelheid akoestische energie ('integrated backscatter'), welke door het hartspierweefsel wordt geretourneerd, veranderd indien de structuur van het weefsel een verandering heeft ondergaan. **Hoofdstuk VII** en **Hoofdstuk VIII** beschrijft de eerste studie van weefselidentificatie met behulp van ultrageluid tijdens experimentele ischemie. In Hoofdstuk VII worden de absolute energienivo's van het ultrageluid afkomstig uit normaal spierweefsel en uit ischemisch weefsel met elkaar vergeleken. Eind diastolisch bleek er geen onderscheid te kunnen worden gemaakt tussen normaal en ischemisch hartspierweefsel. Eind systolisch was het energienivo gemeten in ischemisch hartspierweefsel 5.3 dB hoger dan dat in normaal weefsel. Ook werd een omgekeerde relatie geconstateerd tussen de dikte van de hartspier en het nivo van de backscatter. In Hoofdstuk VII werd dit verband nader bestudeerd.

CURRICULUM VITAE

De schrijver van dit proefschrift werd geboren op 1 december 1946 te Amsterdam. Hij volgde het lager onderwijs in Amsterdam en bezocht het Keizer Karel College te Amstelveen. Na het behalen van het HBS-B diploma ging hij in 1964 naar Enschede voor een studie aan de Technische Hogeschool Twente. Hier werd in 1965 de algemene propaedeuse afgerond en werd in 1970 het baccalaureaatsexamen in de elektrotechniek afgelegd.

Er werd gekozen voor een afstudeeropdracht bij de vakgroep Lage Temperaturen van de afdeling Technische Natuurkunde: het meten van spin-rooster relaxatietijden met behulp van elektronspinresonantie in het temperatuurgebied van 1,2 tot 4,2 graden Kelvin.

Na het behalen van het ingenieursdiploma, in 1972, was hij nog gedurende twee jaar als research assistent en wetenschappelijk medewerker verbonden aan de vakgroep Lage Temperaturen van de afdeling Technische Natuurkunde van de Technische Hogeschool Twente. In deze periode werd het onderzoek aan het afstudeeronderwerp voortgezet.

In 1974 kwam hij bij de afdeling Neurologie van het Academisch Ziekenhuis Dijkzigt te Rotterdam. Onder leiding van dr. de Vlieger werkte hij aan de neurologische toepassing van het lineaire ultrageluidssysteem, wat toen juist ontwikkeld was door de afdeling Experimentele Echocardiografie van het Thoraxcentrum teneinde tweedimensionale afbeeldingen te maken van het hart.

In 1976 volgde een aanstelling bij het Interuniversitair Cardiologisch Instituut waar hij achtereenvolgens werkte aan project V (prof. Bom) en project VIII (prof. Roelandt). Beide projecten betroffen de evaluatie van de verschillende technische en klinische aspecten van de echocardiografie in de centra van het Interuniversitair Cardiologisch Instituut in Nederland. Dit proefschrift is gebaseerd op studies welke in die periode werden uitgevoerd.

Sinds 1988 werkt hij aan een project van de Stichting Technische Wetenschappen voor het toepassen van weefselindentificatie in de hartspier met behulp van ultrageluid.

PUBLICATIONS OF THE AUTHOR

De Vlieger M, Rijsterborgh H, Van Eijndhoven JHM, Megens PHA and Bom N. Two-dimensional echo-encephalography with mechanical B-scan and Multiscan. In: White D, Brown RE (eds) Ultrasound in Medicine 3A. Plenum Publishing Corporation, New York NY, 1977: 813-820.

Van Zwieten G, Vogel JA, Bom AHA and Rijsterborgh H. Computer assisted analysis of M-mode echocardiograms. In: Computers in Cardiology, IEEE Cat No. 77CH1254-2C, 1977: 285-287.

Ligtvoet CM, Rijsterborgh H, Kappen L and Bom N. Real time ultrasonic imaging with a hand-held scanner (Part I: Technical description). Ultrasound Med & Biol 4, 1978: 91-92.

Voogd PJ, Rijsterborgh H, Van Zwieten G and Lubsen J. Percentiles of echocardiographic dimensions in healthy children and young adolescents. In: Lancée CT (ed) Echocardiology. Martinus Nijhoff Publishers, The Hague, Boston, London, 1979: 299-307.

Rijsterborgh H (ed) Echocardiology - Proceedings of the 4th Symposium on Echocardiology Erasmus University Rotterdam June 1981 - Martinus Nijhoff Publishers, The Hague, Boston, London, 1981.

Bom N and Rijsterborgh H. New echocardiographic techniques. In: Kurjak A (ed) Progress in medical ultrasound, vol 3. Excerpta Medica, Amsterdam-Oxford-Princeton, 1982: 173-181.

Bom N, Rijsterborgh H and Roelandt J. The present and future state of M-mode and real-time echocardiography. In: Hunter S, Hall R (eds) Echocardiography 1. Churchill Livingstone, Edinburgh, London, Melbourne, New York, 1982: 1-11.

Rijsterborgh H, Van Zwieten G and Bom N. Derivation of cardiac parameters in M-mode echocardiography. Comp Cardiol, IEEE Cat No. 81CH1750-9, 1981: 3-5.

Bom N and Rijsterborgh H. Real-time cardiac imaging. In: Short MD, Pay DA, Leeman S and Harrison (eds) Physical techniques in cardiological imaging. Adam Hilger Ltd, Bristol, 1983: 31-39.

Lubsen J, Roelandt J, Rijsterborgh H and Van Domburg RT. Quantitative aspects of measurement error in echocardiography. In: Roelandt J (ed) The practice of M-mode and two-dimensional echocardiography. Developments in cardiovascular medicine 23. Martinus Nijhoff Publishers. The Hague, Boston, London, 1983: 74-89.

Bom K, De Boo J and Rijsterborgh H. On the aliasing problem in pulsed Doppler cardiac studies. J Clin Ultrasound 12, 1984: 559-567.

Rijsterborgh H and Bom N. The next decade in echocardiology. In: Hunter S, Hall R (eds) Echocardiography 2. Churchill Livingstone, Edinburgh, 1984: 139-147.

Voogd PJ, Rijsterborgh H, Lubsen J, Arntzenius AC, Monsjou LK and Godijn EH. Reference ranges of echocardiographic measurements in the Dutch population. Eur Heart J 5, 1984: 762-770.

Bom N, Rijsterborgh H, Meijboom EJ, De Boo JAJ and Roelandt J. Problems in measuring cardiac Doppler values, In: Gill RW, Dadd MJ (eds) Proceedings WFUMB 1985. Pergamon Press, Sydney, Oxford, New York, Toronto, Frankfurt, 1985: 374-375.

Bom N and Rijsterborgh H. Principles of cardiac Doppler ultrasound. In: Hunter S and Hall R (eds) Clinical Echocardiography. Castlehouse Publications Ltd, Tunbridge Wells, Kent, 1986: 33-38.

Roelandt J, McGhie J, Vletter WB and Rijsterborgh H. Assessment of pulmonary flow disorders by Doppler echocardiography. In: Hunter S, Hall R (eds) Clinical Echocardiography. Castlehouse Publications Ltd, Tunbridge Wells, Kent, 1986: 54-59.

Roelandt J, Verdouw PD, Rijsterborgh H and Hartog J. Aerobic exercise and cardiac size: an echocardiographic study of Rotterdam marathon runners. In: Fagard RH, Bekaert IE (eds) Sports Cardiology. Martinus Nijhoff Publishers, Dordrecht, Boston, Lancaster, 1986: 85-91.

Roelandt J, Vletter WB, McGhie J and Rijsterborgh H. Doppler echocardiography: een nieuwe sprong voorwaarts in de onbloedige diagnostiek van hartaandoeningen. Hart Bull 17, 1986: 37-48.

Rijsterborgh H and Roelandt J. Estimation of transvalvular pressure drops by Doppler echocardiography: the Bernoulli equation revisited. In: Roelandt J (ed) Color Doppler flow imaging and other advances in Doppler echocardiography. Martinus Nijhoff Publishers, Dordrecht, Boston, Lancaster, 1986: 19-28.

Van der Borden SG, Roelandt J and Rijsterborgh H. Computer aided analysis of Doppler echocardiograms. In: Roelandt J (ed) Color Doppler flow imaging and other advances in Doppler echocardiography. Martinus Nijhoff Publishers, Dordrecht, Boston, Lancaster, 1986: 39-49.

Roelandt J, Vletter WB, Rijsterborgh H and Gussenhoven WJ. Color coded Doppler flow imaging: a major advance for non-invasive cardiology? In: Roelandt J (ed) Color Doppler flow imaging and other advances in Doppler echocardiography. Martinus Nijhoff Publishers, Dordrecht, Boston, Lancaster, 1986: 123-140.

Meijboom EJ, Rijsterborgh H, Bot H, De Boo JAJ, Roelandt JRTC and Bom N. Limits of reproducibility of blood flow measurements by Doppler echocardiography. Am J Cardiol 59, 1987: 133-137.

Meijboom EJ, Horowitz S, Valdes-Cruz LM, Larson DF, Bom N, Rijsterborgh H, Olivera Lima C and Sahn DJ. A simplified mitral valve method for two-dimensional echo Doppler blood flow calculation: validation in an open-chest canine model and initial clinical studies. Am Heart J 113, 1987: 335-340.

Rijsterborgh H and Roelandt J. Doppler assessment of aortic stenosis: Bernoulli revisited. Ultrasound Med & Biol 13, 1987: 241-248.

Roelandt J, Vletter WB and Rijsterborgh H. Colour coded Doppler flow imaging. In: Visser CA (ed) Proceedings Cardiac Imaging Amsterdam 1987: 65-78.

Lancée CT, Rijsterborgh H and Bom N. Monitoring aspects of an ultrasonic esophageal transducer. Initial experience. Med Progr Technol 13, 1988: 131-138.

Lancée CT, Mastik F, Rijsterborgh H and Bom N. Myocardial backscatter analysis in animal experiments. Ultrasonics 26, 1988: 155-163.

Van der Giessen WJ, Verdouw P, Ten Cate F, Essed CE, Rijsterborgh H and Lamers JMJ. In vitro cyclic amp induced phosphorylation of phospholamban: an early marker of long-term recovery of function following reperfusion of ischaemic myocardium? Cardiovascular Research 22, 1988: 714-718.

Zeelenberg C, Bom N, Bos E, Oomen JAF, Rijsterborgh H, Slager CJ. Proposal for a standard storage, coding and manipulation of cardiac contour data. In: Computers in Cardiology Los Angeles: IEEE Computer Society Press, 1988: 635-637.

Huang H, Scheffers M, Rijsterborgh H and Roelandt J. Does echocardiography allow the monitoring of the cardiac effect of nitrates? Eur Heart J 9, 1988: 51-55.

Forster T, McGhie J, Rijsterborgh H, Van der Borden S, Laird-Meeter K, Balk A, Essed C and Roelandt J. Can we assess the changes of ventricular filling resulting from acute allograft rejection with Doppler echocardiography? J Heart Transplant 7, 1988: 430-434.

Roelandt J, Tirtaman C, Vletter W, Ten Cate F, Romdoni D, Gussenhoven W and Rijsterborgh H. Contrast echocardiography. In: Cikes (ed) Interventional Echocardiography. Kluwer Academic Publishers, Dordrecht, 1989: 149-177.

Rijsterborgh H, Romdoni R, Vletter W, Bom N and Roelandt J. Reference ranges of left ventricular cross-sectional echocardiographic measurements in adult men. J Am Soc Echo 2, 1989: 415-418.

Rijsterborgh H, Mastik F, Lancée CT, Van der Steen AFW, Sassen LMA, Verdouw PD, Roelandt J and Bom N. Ultrasonic myocardial integrated backscatter and myocardial wall thickness in animal experiments. Ultrasound in Med & Biol 16, 1990: 29-36.

Rijsterborgh H, Mayala A, Forster T, Vletter W, Van der Borden B, Sutherland GR and Roelandt J. The reproducibility of continuous wave Doppler measurements in the assessment of mitral stenosis or mitral prosthetic function: the relative contributions of heart rate, respiration, observer variability and their clinical relevance. Eur Heart J 11, 1990: 592-600.

Rijsterborgh H. Echografie van het hart. Techniek in de gezondheidszorg, beheer en toepassing, 1990: 18-19.

Van Doorn BA, Van der Does E, Lubsen J en Rijsterborgh H. Betrouwbaarheid van bloeddrukmeting; vergelijking van een elektronische meter en een kwikmanometer in de huisartspraktijk. Ned Tijdschr Geneeskd 134, 1990: 1646-1650.

Rijsterborgh H, Mastik F, Lancée CT, Sassen LMA, Verdouw PD, Roelandt J and Bom N. The relative contributions of myocardial wall thickness and ischemia to ultrasonic myocardial integrated backscatter during experimental ischemia. Ultrasound Med & Biol, 1990 (accepted for publication).

Bom N, Rijsterborgh H, Lancée CT and Roelandt J. Possibilities of tissue identification in the heart. In: Iliceto S, Rizzon P and Roelandt JRTC (eds) Ultrasound in coronary artery disease. Kluwer Publishers Dordrecht, 1990 (accepted for publication).

Rijsterborgh H, Tirtaman C, Domenicucci S and Roelandt J. Reproducibility of left ventricular volume and ejection fraction measurements from cross-sectional echocardiograms (submitted for publication).

Rijsterborgh H, Mastik F, Lancée CT, Verdouw P, Roelandt J and Bom N. Ultrasound myocardial integrated backscatter signal processing: frequency domain versus time domain (submitted for publication).

Rijsterborgh H, Mastik F, Lancée C, Verdouw P, Roelandt J and Bom N. The principles of myocardial tissue characterisation. In: Roelandt JRTC and Sutherland GR (eds) Textbook of cardiac ultrasound. Gower Medical Publishing, London (submitted for publication).

Van der Steen AFW, Rijsterborgh H, Mastik F, Lancée CT, van Hoorn W and Bom N. The influence of attenuation on measurements of ultrasonic myocardial integrated backscatter during the cardiac cycle (submitted for publication).