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# What are the components that contribute to variability in echocardiographic measurements in aortic stenosis?

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## Abstract

### Background

Echocardiography is the standard clinical approach for quantification of the severity of aortic stenosis. A comprehensive examination of its overall reproducibility and the simultaneous estimation of its variance components by multiple operators, readers, probe applications, and beats have not been undertaken. Such knowledge is key for three groups of people. Guideline authors need to be able to both state what level of precision can be expected with a technique, and provide evidence-based steps to improve the precision. Clinicians need such data so that they can differentiate true biological changes from random noise. Researchers need such data for both designing and powering trials, and developing targeted techniques to improve their precision.

### Method + Results

As part of a quality improvement program, 27 subjects with aortic stenosis were scanned over 7 months in the echo-department by myself and a median of 2 other operators (range 1 to 3). From each patient and each operator multiple runs of beats from multiple probe positions were stored for later analysis by multiple readers. A mixed effects model was constructed to extract the variance components.

The coefficient of variation was 13.3%, 15.9%, 17.6%, and 20.2% for the aortic peak velocity ($V_{max}$), and velocity time integral (VTI), and left ventricular outflow tract (LVOT) $V_{max}$ and VTI respectively. The largest individual contributors to the overall variability were the beat-to-beat variability (9.0%, 9.3%, 9.5%, 9.4% respectively) and that of inability of an individual operator to precisely apply the probe to the same position twice (8.3%, 9.4%, 12.9%, 10.7% respectively). The tracing (inter-reader) and reader (inter-reader), and operator (inter-operator) contribution were less important.

**Conclusions**

This thesis found that the reproducibility of measurements in aortic stenosis is poorer than often reported in the literature. However, it is comparable with a retrospective study I have previously participated in. Furthermore, the large variability of these simple parameters makes composite measurements for the assessment of the severity of aortic stenosis even more unreliable. The source of this variability does not appear, as traditionally believed, to result from a lack of training or operator and reader specific factors. Rather the unavoidable beat-to-beat biological variability, and the inherent impossibility of applying the ultrasound probe in exactly the same position each time are the largest contributors.

Consequently, guidelines suggesting greater standardisation of procedures and further training for sonographers are unlikely to result in an improvement in precision. Clinicians themselves should be wary of relying on even three-beat averages as their expected coefficient of variance is 10.3% for the peak velocity at the aortic valve.

# Index

# Introduction

Echocardiography is the standard clinical approach for the quantification of aortic stenosis, both at baseline and at follow-up. While echocardiographic follow-up of aortic stenosis severity is recommended by every national and international guideline, the test-retest variability of this is actually rather poor[1], although discussion of this seems to be a "*taboo"*.

The science of aortic stenosis measurement suffers from two weaknesses. First measurement technologies that have not undergone detailed, dispassionate analysis of their measurement properties are taken straight through to end-point studies, and become a guidelines recommended standard. Secondly, test-retest variability is not directly taken into consideration by guidelines and mention of such variability is answered by advice to undergo further training.

In this thesis I set out to address under formal, blinded, scientific conditions how large the variability between one measurement of aortic stenosis and another actually is. I designed the experiment to permit the sources of variability to be decomposed so that we would be able to give sensible advice to those seeking more reliable measurements – trying to do something more specific than to "*undergo more training*".

**Test-retest variability**

The true test-retest variability of the various measures of aortic stenosis is much higher than previously thought. I participated in a retrospective review of repeated echocardiograms in 70 patients with aortic stenosis[1] which demonstrated that the coefficient of variation for the simplest estimate of the gradient, the peak instantaneous pressure drop, was 19.1%. The dimensionless index using VTI, traditionally thought to be more accurate as it corrects for ventricular function, was

higher still at 25%. The variabilities of mean trans-aortic pressure drop and left ventricular outflow tract VTI were no better; 26.9% and 22.1% respectively[1].

These values contrast to those typically quoted in the literature of 3-4 %[2]

The following questions were left open:

1) Were these findings a consequence of its retrospective design and the scans not having being originally acquired for research purposes?

2) Could this have been explained by some element of disease progression over time since there was up to several months between successive scans?

3) Is the variability really caused by inadequate training or are there some fundamental components which can be separated and quantified, as an essential step to reducing their impact?

**Guidelines**

The European Society of Cardiology guidelines for the assessment of aortic stenosis[3], in addition to the simple maximum aortic velocity which requires one measurement to be made, details eleven other composite measures that can be used for assessing the severity of aortic stenosis; one of which requires 5 different parameters to be measured.

The test-retest variability of composite measures will be greater than those simple ones as errors are propagated and compounded through an equation (unless the errors are significantly correlated). Therefore, the addition of further parameters to a measure to “correct” for other sources of error such as left ventricular dysfunction may actually worsen the accuracy of the measure. This may explain why the clinical utility of these more complex measures is less than initially believed[2].

**Identifying a measure**

It is inadequate to advise echocardiographers to make numerous measurements without a clear algorithm about what to do with the different results, since there will almost always be discrepancy between the grading categorisations between different variables[4]. Typically echocardiographers are advised to use their clinical judgement, which is understandable advice in the absence of evidence that could suggest something more meaningful. In a research environment where echocardiography is used as a measurement, for entrance criteria, or as an endpoint, it is unacceptable for the echocardiographer to incorporate other information into a variable that is then presented as if it were a pure echocardiographic measurement.

When individual measurements are too variable they will never be a reliable basis for the categorisation of individual patients or as an end-point measurement for individual patients[5].

**Reliable measurements**

Instead of recommending piling one unreliable measurement upon another, it is more rational to carefully assess why the measurements are unreliable in order to determine whether reliability can be improved.

To select the most appropriate measure, it is essential for clinicians to be provided with the appropriate test-retest variability. In addition, researchers in echocardiography who hope to improve the reliability of aortic stenosis severity assessment also need information on its constituent components. Within echocardiography these components of variation that can be attributed to either analytical or temporal (biological) variability. The analytical component will comprise of differences in interpreting the Doppler tracings (intra-reader and inter-reader), and the small changes in the probe position that the same and different operators will obtain (intra-operator and inter-operator). The temporal component will operate on

different time-scales: beat-to-beat reflecting fluctuations in autonomic activation; day-by-day reflecting filling; and over longer time periods [Figure 1]

Unfortunately, credible prospective data assessing the true test-retest variability and its constituent components is lacking in echocardiography. Whilst some studies report presenting reproducibility data, on close examination it is very rarely formal, blinded, test-retest variability. The most common type of reproducibility data is remeasuring the same stored traced, either by the same reader or a different reader, and these characteristically are reported as a showing an extremely strong agreement. However, such reports omit most of the scientifically relevant variability, as shown in the figure above.

**Language of quantification**

For some reason, reproducibility reports often emphasise the correlation coefficient between the two repeated measurements. This statistic is a composite of two pieces of information:

1) The variability of the measurement.

2) The range of the variable in the patients tested.

It is an unsatisfactory summary of reproducibility for two reasons. First, the breadth of the spectrum of patients has an overwhelming effect on the correlation coefficient observed. If the spectrum is wide, including very mildly diseased and very severely disease, the correlation coefficient is forced to be high even if the measurement technique is poorly reproducible. Second, it does not present variability in units that allow a clinician to determine for an individual patient what the uncertainty is[6].

This is in contrast to other physical sciences which use well established experimental designs and modern statistical methods to estimate the reproducibility of measurement device[7]; with national laboratories, such as NIST, which provide blinded and unbiased assessment culminating in an estimate of both the overall precision of a measurement device and its constituent components.

We therefore designed and conducted a reproducibility experiment aiming to use such techniques to simultaneously estimate both the total test-retest variability and its constituent components [Figure 1]:

***Tracing***

Working from the same frozen acquired Doppler image, the same reader is asked to trace around the same beat on three occasions with enough time elapsing and intervening beats measured for there to be no realistic possibility for the reader remembering exactly how he or she decided to trace around the beat on the previous occasion. This is sometimes called intra-reader variability.

***Beat-to-beat***

If the same reader is asked to analyse successive beats acquired in a single Doppler run, the two measurements will of course be different. It is important to note that there are two contributors to this difference. The more obvious component is that due to the change of beat. However, the second component will always be present, namely, the variability due to the act of tracing.

The only way to extract the component specifically due to the beat-to-beat difference is to start overall observed variability between beats and then subtract the variability when the same beat is measured twice. In statistical terms this subtraction is valid if the variability is measured as a variance, i.e. the square of the standard deviation.

In figure 1, the overall variance observed in the beat-to-beat experiment is composed of the tracing component (purple) and the true beat-to-beat component (red). The true beat-to-beat component can only be calculated by subtracting the tracing component (purple) from the overall beat-to-beat variability (red + purple).

***Reader***

A second reader carrying out a tracing will obtain different values from the first. However, contrary to common belief amongst Authors, this should not be assumed to be the result of the *junior* of the two operators being inadequately trained to match the *senior* operator. It could simply be a manifestation of the within reader variability of each. In general it is not reasonable to expect two readers to agree with each other, better than each agrees with themselves. If they do agree better with each other, better than with themselves, this is likely due to inadvertent collusion. If they agree just as well with each other as they do with themselves, there is no difference attributable to the difference in reader, only the variability introduced by having the tracing performed by anyone.

As long as the same beat is being addressed, it is only the tracing variability that is must be subtracted.

If on the other hand the new reader is only able to address a new beat, then the total observed variability observed in that experiment is a combination of three components: tracing (purple), beat-to-beat (red), reader (blue). It will be therefore necessary to subtract the tracing and beat variabilities to obtain the pure reader variability.

***Position***

Working backwards to the time of acquisition, the exact position of the probe is unique for each acquisition and can never be perfectly reproduced even with a high level of training. To determine how much variability this causes step of probe positioning causes it is not sufficient to repeatedly do measurements placed afresh onto the chest each time. This is because any observed difference between probe placements will always include variability arising from tracing (purple) and from beat-to-beat (red). These latter two components must always be subtracted from the experimental variability to obtain the true variability arising from probe position (orange).

***Operator***

If a second operator acquires data in the same patient, the measurements will be different, but of course, the probe will be in a different position, the heart beats will be different, and it will be a separate process of tracing. Therefore to determine the true, incremental effect of changing operator, one must subtract the variability due to probe position (orange), beat-to-beat variability (red), and tracing (purple). Conveniently this can be done by subtracting when one operator removes the probe from the chest, puts it back, and makes a fresh measurement, i.e. the experiment described above as “position”.

***Clinical status***

There can be some genuine change over time in the status of patients. For example, over a period of weeks or months, the disease can progress, or, theoretically improve Moreover, over short periods of time changes in volume status can increase or decrease velocities. Treatment effects can also occur, over short, medium, or longer-terms.

In my study I decided to specifically set these potential sources of variability aside and focus exclusively on an accurate assessment of the five components shown above.

**Experimental design required**

In my pilot studies[(1)] with my colleagues Dr. Finegold and Dr. Manisty we observed the large variability in a retrospectively analysed dataset. I decided to carry out a quality improvement program within our echocardiography department. I arranged that whenever I was in the echo laboratory and a patient with aortic stenosis in sinus rhythm was scanned, I ensured that the LVOT and aortic valve velocities were measured from more than one probe position, and by more than one operator, typically three. The clinical report was then issued by the primary operator after viewing all the acquired data using a standard approach. The acquired data were

then anonymised with respect to patient identity, beat-sequence, probe position iteration, and operator and subjected to further analysis to extract the components shown in Figure 1.

I decided to address the variability of the key measurements - peak velocity ($V_{max}$) and velocity time integral (VTI) at the aortic valve (AV) and left ventricular outflow tract (LVOT).

# METHODS

Patients attending an echocardiography department at a tertiary-referral centre in London for routine surveillance of known aortic stenosis underwent a standard echo according BSE guidelines[8] by myself, a consultant in cardiology with particular interest in echocardiography. After performing a standard BSE echo[8], continuous-wave tracings across the aortic valve and pulse-wave tracings of the LVOT were taken in the five-chamber view. Once a satisfactory position had been obtained, rather than selecting a single beat, and performing on-line measurement, long runs (up to 15) of beats were captured and stored for off-line, blinded, analysis. The probe was then removed from and then reapplied to the patient, before capturing another run of beats. For each patient two to three other accredited sonographers captured continuous and pulse wave traces in a similar manner.

All images were exported as TIFFs and anonymised with respect to patient demographics. For each beat an image was generated that removed all other beats, leaving only it and the calibration markings. The operator, position, beat number, along with calibration data were entered into a database and then identifiers were removed from the image. Images were then screened for quality. No whole patient, operator, or position were excluded from the database, but 16/1880 (<1%) were considered to be of poor quality to prevent further analysis. Images were then analysed using an imaging software[9] to select the maximum velocity, and VTI on a 15 inch MacBook Pro (Apple Inc., California, USA) in a darkened room. Sonographers were given the choice of using a mouse or trackball. An independent statistician and programmer designed software and protocols to present appropriate images to readers randomised and blinded to the patient, operator, position, and beat number. I traced every image selecting the $V_{max}$ and tracing the VTI independently, whilst multiple sonographers reviewed a selection (123) beats evenly distributed across patients and operators measuring both the $V_{max}$ and VTI.

The measurement data was then combined with the database describing the origin of the images within R (Version 2.15.1). A mixed-effects model was generated, with each patients as a fixed effect, and the arbitrary beat-number nested under position-position, which itself was nested under operator. Estimates of the variance components with associated confidence (or credible) intervals of these components were generated using MCMC techniques by the MCMCglmm package in R[10] (using default priors, number of iterations = 100,000 burn-in 15,000 thin 50). The measurements were log-transformed before processing to allow the variance components to be easily converted to coefficients of variation[6].

These estimates were plotted individually as bar charts, but also as a series of nested squares (as it is the variances that are additive), along with a population coefficient of variation to provide context.

# RESULTS

### Baseline characteristics

Twenty-seven patients with aortic stenosis ($V_{max}$ mean 4.00, range 2.31 to 6.06m/s) were scanned between (November 2011 to June 2012) in the department where I worked when I was present. I worked as part of a pool of 17 operators, although many only worked for a short window of time during that period, or were only part time during the week. Each patient was scanned by a median of 3 operators (range 2 to 4). On average 3.6 (range 1-7) runs of beats were recorded by each operator for each patient, consisting of a mean of 14.8 (range 1 to 51) for a total of 1880 beats. The baseline characteristics are in Table 1.

### Components contributing to the variability

The overall total coefficient of variation across readers, operators, positions, and beats at for the aortic valve $V_{max}$ and VTI, and LVOT $V_{max}$ and VTI were 13.3% 15.9%, 17.6%, 20.2% respectively. Figure 2 and Table 1 show its constituent parts.

### Order of priority of sources of variability

Position was the component that on average contributed most to variability, namely a average coefficient of variation of 10.5%. A close second was true beat-to-beat difference 9.3%. Further behind came variability arising from the act of tracing, at 7.3%. Coefficient of variation from the reader was only 4.2% on average.

### Behaviour of different parameters

The four parameters behaved broadly similarly in the pattern above. However, LVOT VTI showed a particularly large variability component attributable to tracing 11.3% coefficient of variation whilst the other parameters the other coefficient of variation were between 3.4% for the $V_{max}$ at the aortic valve and 7.1% for the VTI at the aortic valve.

**Display of variability components in an understandable manner**

To help clinical echocardiographers understand the meaning of the results I am presenting them graphically in a way that lets the subtraction of components become intuitive, namely showing the variances as areas. This is shown for peak aortic velocity in Figure 3.

The interpretation is as follows:

For peak aortic velocity, the tracing variability has a coefficient of variation 3.4%. This is shown as a purple square whose side is 3.4%. In the experiment of changing beats a total variability of 9.7% is seen, of course 3.4% must be allocated tracing variability, and therefore only 9.0% is attributable it being a different beat. It is the squares of the variance that summate rather than the standard deviations. This is graphically shown by a larger red square with sides of length 9.7% surrounding the purple square. The overall area of the larger square encompasses both sources of variability and therefore only the outer strip of red, which is not tracing variability, is genuinely due to the different beats.

This process can be repeated with progressively more sources of variability added until the full variability is shown for a new operator acquiring new pictures interpreted by a new reader that is represented by the outermost coloured square. The areas of colour represent the size of variance introduced by each component of variability. Note how once the squares get large, the addition of an additional variance component has less and less effect on the magnitude of the overall coefficient of variation.

The corresponding “peel-away variance maps” for LVOT peak velocity, aortic VTI and LVOT VTI are shown in figures 3B, 3C, and 3D.

# DISCUSSION

The coefficient of variation was 13.3%, 15.9%, 17.6%, and 20.2% for the aortic peak velocity ($V_{max}$), and velocity time integral (VTI), and left ventricular outflow tract (LVOT) $V_{max}$ and VTI respectively. This variability is clinically large, and larger than reported in the literature. It should be remembered that, this variability does not contain the biological components of variability that develop over a period of days. It only includes variability in the very short term arising from rapid biological variations or by the many steps of the measurement process.

This study suggests that the usual advice given to clinicians and researchers to help obtain reliable measurements not accurate enough. They are typically advised to follow a strict protocol to try to use a consistent member of staff on each occasion for image acquisition, and a consistent member of staff to carry out each reading of the acquired images. In reality the contribution to variance made by having a different operator acquiring images or a different reader analysing images, is small by comparison to the variance introduced by the act of tracing, by spontaneous beat-to-beat variability, and probe position during acquisition.

**How clinicians are advised by guidelines to reduce variability.**

Multiple readings can be combined to reduce the apparent variability of a measurement, and indeed guidelines bodies such as the ESC[11] and ASE[12] recommend averaging across three to five beats. However, they neither provide an estimate of what level of reproducibility before, nor after averaging. Furthermore, as I have shown in this thesis there are multiple sources of variability; beat-to-beat and biological variability at longer time-scales, intra- and inter-reader, and intra and inter-operator. Averaging only across a few sequential beats will only reduce a few of the components, and the effect on the total variability will therefore less than expected.

For example, if three consecutive beats are averaged only the tracing and beat-to-beat variance will be reduced by a third, rather that the total variance. In this dataset, this is equivalent to reducing the coefficient of variation from 13.3 to 10.3 for the assessment of the aortic $V_{max}$. If however, the three beats are taken from different probe positions, the expected coefficient of variation will be 7.9%. Table 2 lists such calculations for the other parameters.

## Implications for clinical practice

### *The maximum achievable precision*

Should the clinician wish to reduce the coefficient of variation to, for example <5%, he would need to average 9 beats from 9 different probe positions for the aortic $V_{max}$. If however they were to follow current guidelines and only average consecutive beats, as the position coefficient of variation is in excess of 5% for the aortic $V_{max}$, such precision could not be achieved as the position variability is 8.3% and without varying that it cannot be averaged out. Indeed, the theoretical best precision that could be achieved for the aortic $V_{max}$ with a large number of beats would be 8.6%.

Knowledge of the true test-retest variability of a measurement and its constituent parts is therefore essential for both the clinician and the researcher.

### *Advice for sonographers*

Whilst the typical advice to a sonographer who fails to match the reading produced by a more experienced colleague it to undergo more training, this thesis find that the most significant contributor to variability is not related to the reader or the operator, which have the smallest contribution to the overall coefficient of variation, but that of beat-to-beat and probe position. Consequently, as a science, efforts should be directed to utilising this information in developing more robust measurement techniques and strategies (as described below), rather than producing ever

expanding guidelines with recommendations that appear to be based on the belief that a lack of precision results from a lack of standardisation[13].

***Effect on management***

An accurate assessment of aortic stenosis severity is crucial in terms of patient's management, especially when the AS is in the "grey area" between moderate and severe grade and a decision to refer the patient to surgery or postpone must be taken[14]. The variability of even a simple measure like aortic $V_{max}$ will lead to occasional extreme values being seen that exceed a certain threshold. If guidelines are followed and the operator selected the highest values obtained, they would occasionally capture a beat with a $V_{max}$ higher than the threshold of 4m/s even though the vast majority of the beats are lower. Such an error could lead to a significant change in management (coloured in red). By requiring selection of the maximum value, the value selected will become dependent upon the number of beats examined as the operator waits for more and more extreme beats. Furthermore the variability of the maximum value of a distribution is greater than that of the mean[15].

Both these effects conspire to further diminish the parameters reproducibility.

***Effect on ability to measure change***

Guidelines suggest that a combination of a markedly calcified valve with a rapid increase in velocity of 0.3 m/s within one year has been shown to identify a high-risk group of patients (about 80% death or requirement of surgery within two years). In light of the variability identified in this thesis, this guideline may be unwise. The 95% confidence interval for detecting a change is from -33% to +42% for $V_{max}$ (the confidence interval is symmetric on a log scale), which is larger than the approximate 10% progression represented by 0.3 m/s in a patient with moderate aortic stenosis. If three consecutive beats are averaged the coefficient of variation is reduced to 10.3%, therefore the 95% confidence interval for a change is from -24.7% to +32.9%. However, if three beats from three different probe applications are used the

coefficient of variation becomes 7.9%, and the associated the 95% confidence interval for a change is from -19% to +24%. None of these confidence intervals is sufficiently small to allow for confident detection of a true change of 10% in maximum aortic velocity.

***Allows for the introduction of bias***

It has been previously demonstrated that the variability of measurements from serial echo data is reduced when performed unblinded to the study order and patient[16]. Readers, when confronted with serial measurements of a parameter that they expect to at least stay the same and may often progress, such as with aortic stenosis will be reluctant to report a value that the lower that the preceding one. Errors in the opposite direction, however, will not be automatically detected by this process, and may instead be accepted as evidence of genuine deterioration. As a result, the will be a consistent bias towards exaggeration to the rate of progression of disease.

***Effect on composite measures***

If the errors are not correlated, which is unlikely as they are not acquired simultaneously (e.g. pulse wave and continuous wave Doppler tracings cannot be recorded at the same time) the reproducibility of composite measures will be worse than those of its constituent measurements. E.g. Whilst the coefficient of variation of $V_{max}$ at the aortic valve and LVOT is 13.3% and 17.6% respectively, for the dimensionless index the ratio of the two it would be 31%. It is even worse if VTIs are used - the VTI at the AV and LVOT is 15.9% and 20.2% giving a predicted coefficient of variation of 36% for the dimensionless index by DI. That reproducibility of the aortic valve area, which incorporates a third measure, that of the diameter of the LVOT squared, will be even worse. These calculations assume that the errors in measurement are completely uncorrelated. In fact it is likely that the errors LVOT and AV velocity are partly correlated - if an operator was particularly fastidious or lax about ensuring correct alignment both the AV and LVOT would likely be high or low, therefore, these estimates may represent the upper limit on the estimated coefficient

of variation. Indeed, in my retrospective analysis for real world data[1] these figures were lower at 13% and 25% for the dimensionless index by $V_{max}$ and VTI respectively.

## Implications for research

***Effect on studies using aortic as end-points***

Accurate assessment of the progression of aortic stenosis is crucial for determining the effects of potential clinical interventions on the disease as a whole. The power of potential future clinical trials to detect the effect of interventions on the progression of aortic stenosis[17] is reduced by the lack of reliability in the measurements.

***Appreciating that the agreement between two different measurement techniques is limited by their individual reproducibility***

The high concordance of simultaneous in vivo Doppler and dual catheter measurements of aortic gradients established echocardiography as the primary technique for the quantitative assessment of aortic stenosis[18]

Nevertheless, even these original authors' enthusiasm was tempered by the significantly worse concordance with non-simultaneous echocardiographic measurements[19] (such as those values obtained from the referring clinic).

This finding is not unexpected. The analytical and biological variability of a measurand will naturally limit the agreement between two non-simultaneous techniques[20]. A lack of test-retest reproducibility limits how much a variable can correlate with itself, let alone another noisy variable.

Consequently, when comparing one technique with another for assessing aortic stenosis clinical researchers should first assess its true test-retest variability before pitting it against another noisy measure.

***Designing mechanisms to improve the precision of aortic stenosis assessment***

The comprehensive analysis provided by this thesis of the components that contribute to the variability of aortic stenosis assessment allows for a targeted approach for future improvements. Removal of the variability associated with tracing

and readers (intra- and inter-reader variability) should be straightforward with computer algorithms. Once such algorithms are in place, the unbiased analysis of multiple captured beats and positions becomes easier, and their associated variability can be reduced in proportion to the square root of the number of positions and beats captured. Such systems have already been developed for the mitral[21][22] and other valves[23].

**Limitations**

This thesis did not examine the variability associated over time periods longer than the minimum to allow for at most four operators to capture continuous and pulse wave Doppler tracings of the aortic valve and LVOT as we had investigated this previously, although in a retrospective manner[1]. Due to operational constraints I did not investigate the impact of the equipment manufacturer or training of the operator.

This was not a multicentre study. However, whilst the coefficients of variation may vary from between centres due to operator experience my study did utilise 17 different operators, some sonographers and some doctors with a range of years of experience since accreditation. Consequently, I expect these results to be generalizable to similar large echocardiography units.

I did encounter some statistical issues. Whilst between two and four operators scanned each patient, within a quality improvement programme it would have been difficult to ensure that it were the same operators for every patient. The computational method and statistical package (MCMCglmm) used for solving the mixed model provided unstable estimates with large confidence intervals for the operator coefficient of variation as it was a partially crossed factor. Other authors have run into such issues[24], we are unaware of a straightforward, direct solution to this problem. Therefore, the current estimate of the true operator contribution must be considered as unreliable.

**Comparison with existing literature**

Within cardiology, and much of echocardiography, such analyses are rare, though are not without precedent[24][25]. Often results are presented as not a standard deviations or coefficients of variations (or corresponding Bland-Altman limits of agreement), but as a regression line and correlation coefficient. Interpreting these can be fraught with hazards[6]. Furthermore, whilst the Bland-Altman limits of agreement technique is a marked improvement upon reporting correlation coefficients and p-values it tends to lead to an experimental set-up limited to two specific observers, readers, or operators. This provides a good estimate for the agreement between the two specified operators, but may be poorly generalisable to multiple operators.

This thesis therefore uniquely provides estimates for the individual contribution of many of the components that contribute to the variability of measurements of aortic stenosis. These estimates are comparable with our previous retrospective analysis[1], but larger than previous estimates. Other studies[26][27] similar levels of accuracy, reporting a coefficient of variation for $V_{max}$ ranging from 5% to 7%. The difference for this is unclear. In one early study which reported a test-retest coefficient of 5%[27] inspection of the provided raw data shows them to be incredibly accurate. In five out of the twenty patients examined exactly the same $V_{max}$ to two decimal places was found on three independent, scans taken at baseline, one week, and one month. Review of the methods provides for no special techniques to improve precision; perhaps inadvertent unblinding could have led to their apparent accuracy.

# CONCLUSION

This thesis provides both an accurate assessment of the coefficient of variation that a typical large echocardiography department would be able to ascertain for four of the key parameters that are used to assess the severity of aortic stenosis. It finds that there reproducibility is poorer often reported in the literature, but comparable with a retrospective study I had previously conducted.

This large variability for these simple parameters of $V_{max}$ and VTI at the aortic valve and LVOT makes composite measures for the assessment of the severity of aortic stenosis even more unreliable. Within clinical practice, such variability can lead to classification errors near the severity thresholds, or can results in inaccurate assessments of a progression in severity. The source of this variability does not appear, as traditionally believed, to result from a lack of training or inter-operator and inter-reader factors, but rather the beat-to-beat biological variability, and the inherent impossibility of applying the ultrasound probe in exactly the same position each time are the largest contributors. Consequently, guidelines suggesting greater standardisation of procedures and further training for sonographers are unlikely to result in an improvement in precision.

# Footnotes

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## Table 1.

| Coefficient of variation attributable to each component | Aortic | | LVOT | |
|---|---|---|---|---|
| | **$V_{max}$** | **VTI** | **$V_{max}$** | **VTI** |
| **Tracing** | 3.4% (3.3 to 3.6%) | 7.1% (6.8 to 7.5%) | 4.5% (4.3 to 4.7%) | 11.3% (10.7 to 11.9%) |
| **Beat-to-beat** | 9.0% (8.4 to 9.6%) | 9.3% (8.5 to 10.2%) | 9.5% (8.9 to 10.2%) | 9.4% (8.1 to 10.7%) |
| **Position** | 8.3% (7.2 to 9.8%) | 9.4% (8.0 to 10.9%) | 12.9% (11.2 to 14.9%) | 10.7% (9 to 12.8%) |
| **Operator** | 0.6% (0.0% to 5.8%) | 0.3% (0.0 to 2.4%) | 0.4% (0.0 to 5.0%) | 0.9% (0.0% to 7.5%) |
| **Reader** | 2.3% (1.5 to 3.6%) | 3.3% (2.0 to 5.6%) | 3.4% (2.2 to 5.3%) | 6.6% (4. 0 to 10.7%) |

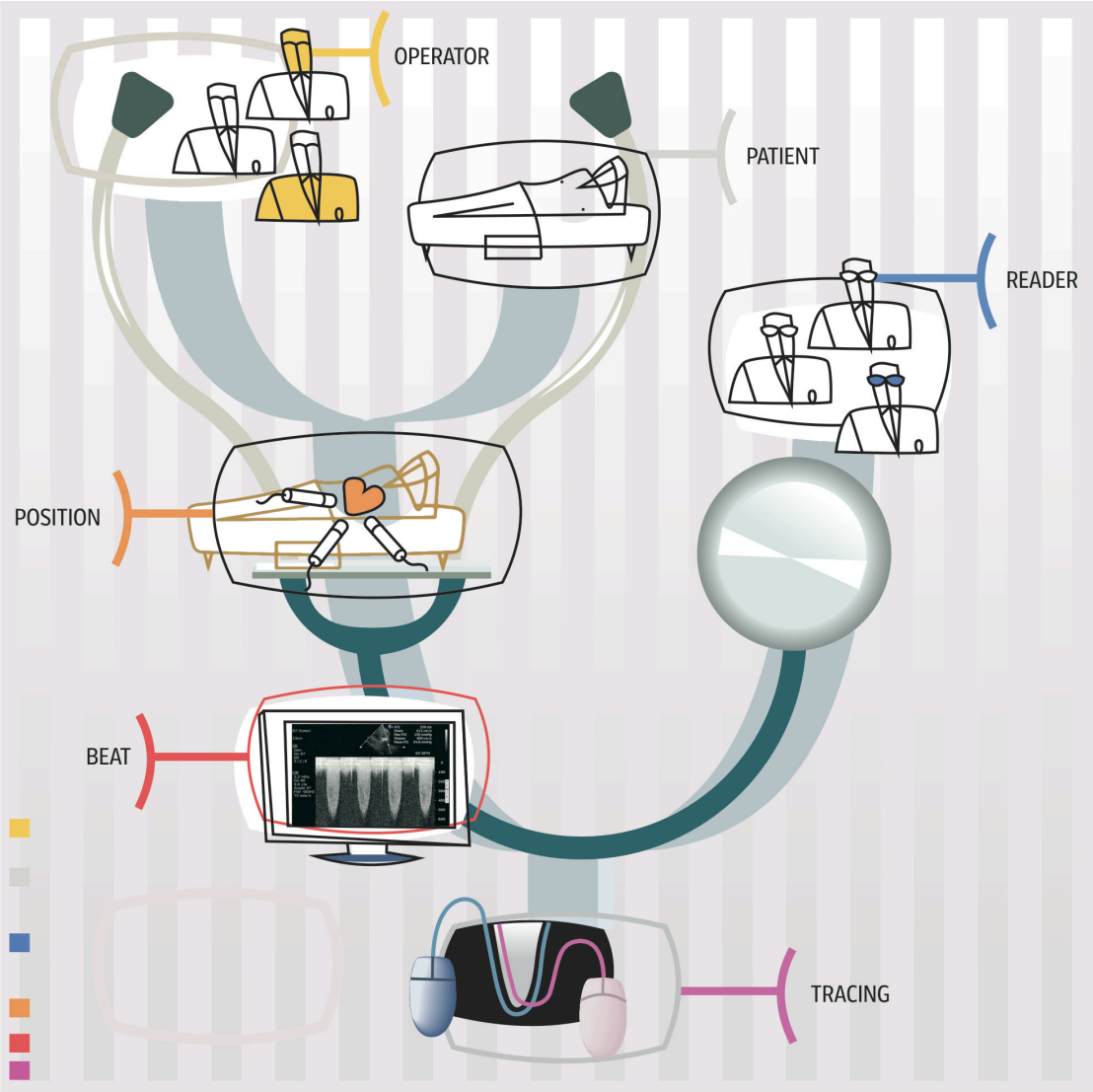
The values presented are the coefficients of variation attributable to each component individually.
Number in brackets - 95% confidence interval.

## Table 2.

| Overall variability at each level | Aortic | | LVOT | |
|---|---|---|---|---|
| | **$V_{max}$** | **VTI** | **$V_{max}$** | **VTI** |
| **Reader** | 13.3% | 15.9% | 17.6% | 20.2% |
| **Operator** | 13.0% | 15.5% | 17.1% | 18.9% |
| **Position** | 13.0% | 15.5% | 17.1% | 18.9% |
| **Beat** | 9.7% | 11.9% | 10.6% | 15.0% |
| **Tracing** | 3.4% | 7.1% | 4.5% | 11.3% |

The overall variability at each level, which includes all the underlying levels.
For example, the variability associated tracing around the same beet is 3.4%. If a different operator is used, the variability must also include that of a different position, beat, and tracing.

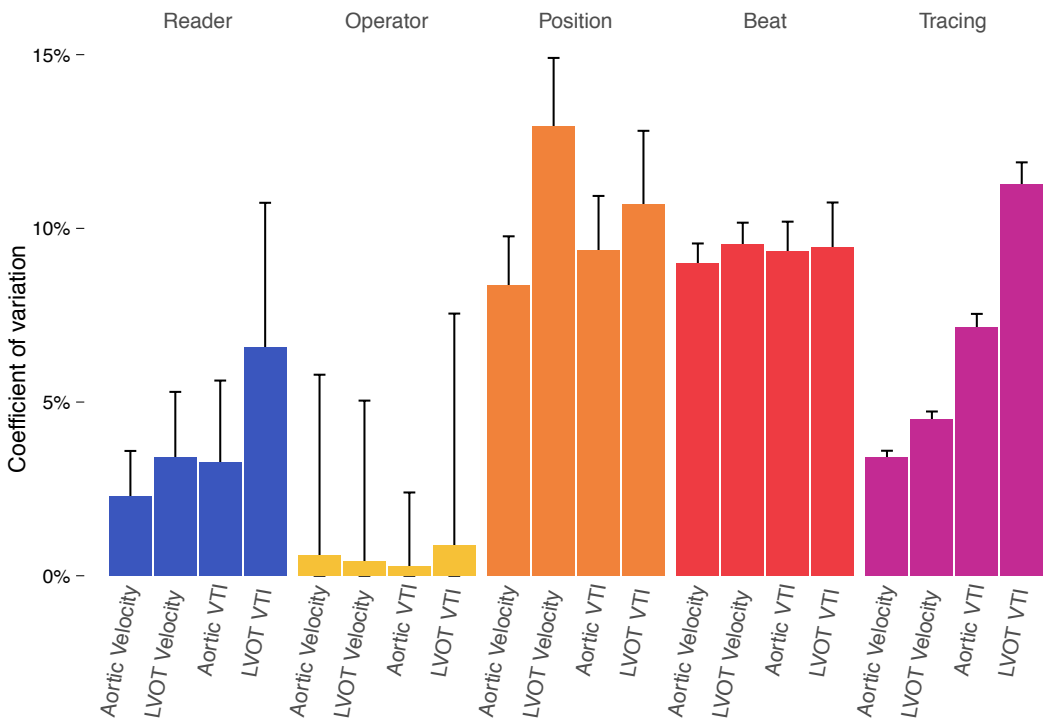
# Figure 1.



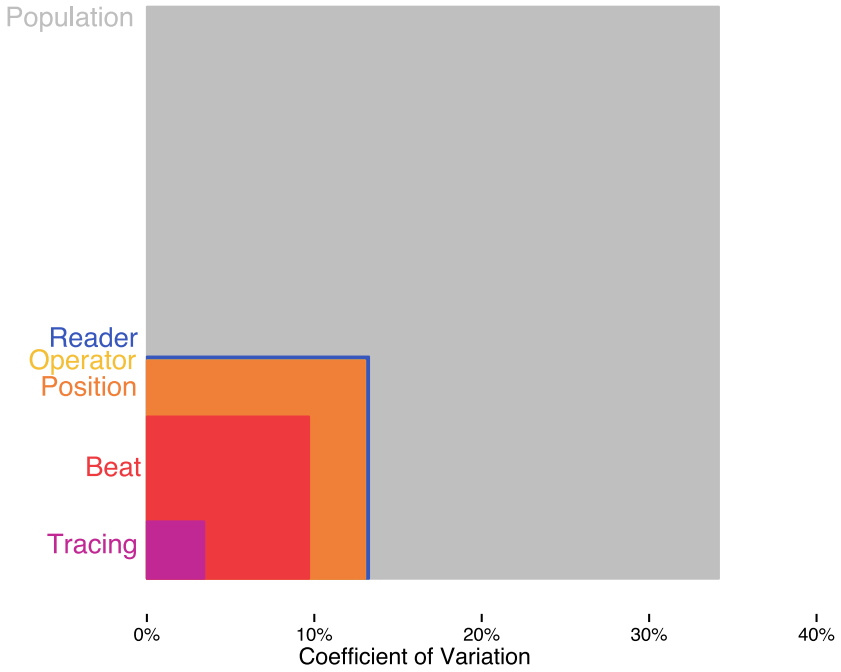

This diagram above shows the potential sources of variability associated with making a measurement, such as the aortic valve $V_{max}$ or LVOT VTI. It shows the hierarchy of the components that contribute to variability. Change in one of the components will naturally lead to a change in lower down components. For example, if the operator is changed, then so will the probe position, beat, and tracing. If a different beat is selected for measurement, then a separate tracing must also occur.

# Figure 2

Legend:

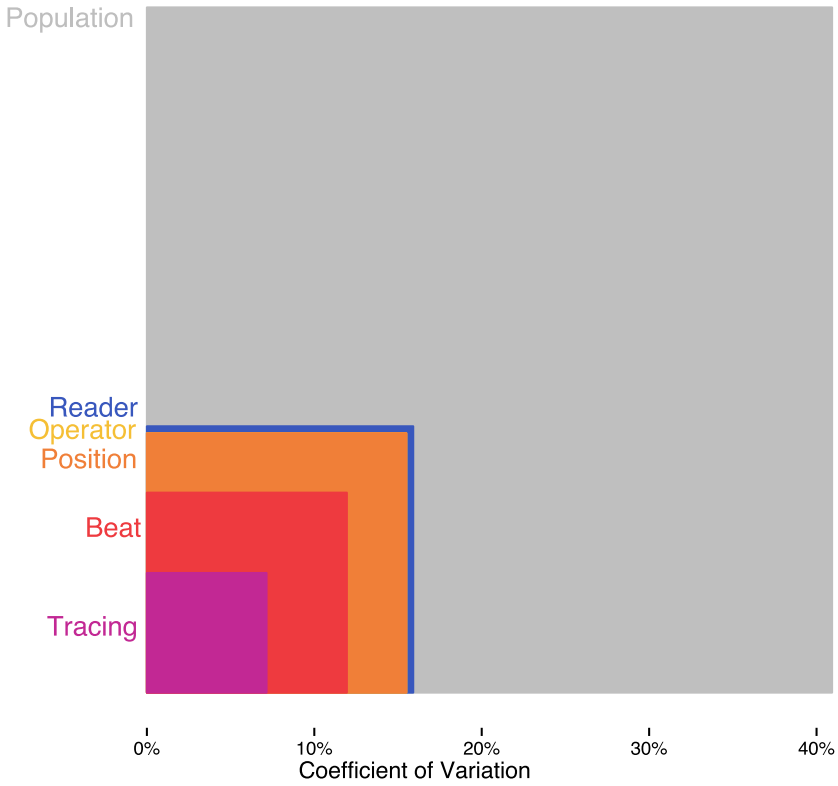


## Figure 3 A.



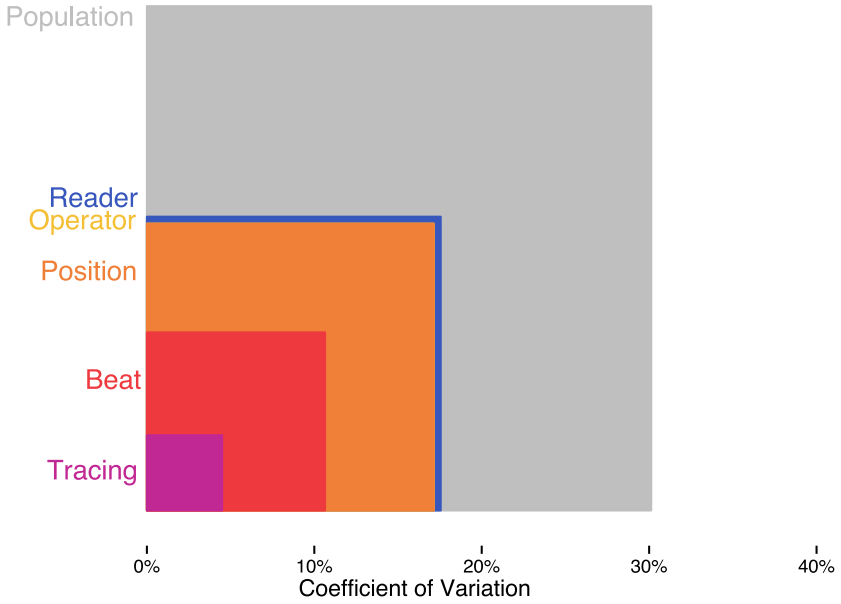

“Peel-away plot” of the coefficient of variation for the aortic valve maximum velocity.

## Figure 3 B.



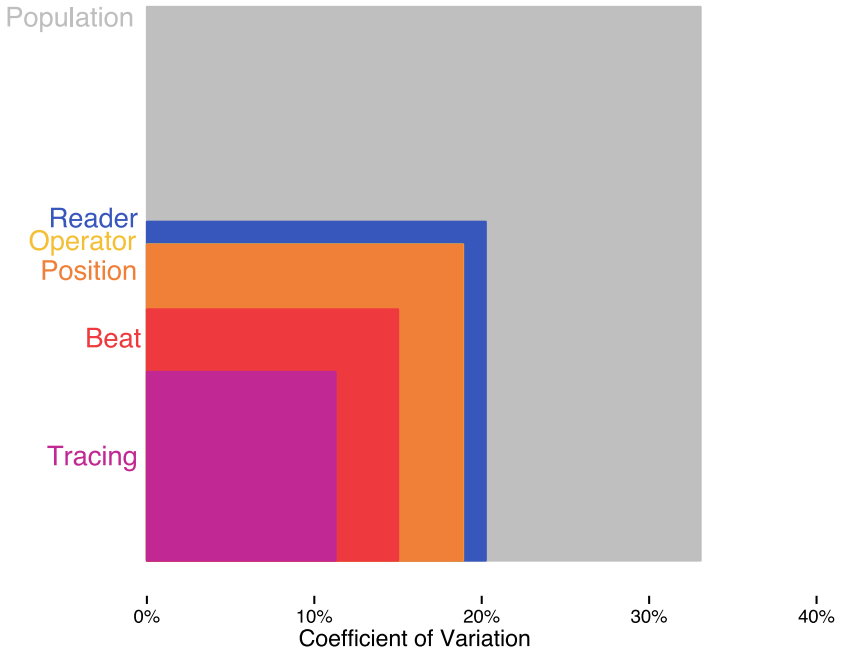

"Peel-away plot" of the coefficient of variation for the aortic valve velocity time integral.

## Figure 3 C.




"Peel-away plot" of the coefficient of variation for the LVOT maximum velocity.

## Figure 3 D.




“Peel-away plot” of the coefficient of variation for the LVOT velocity time integral.