MORPHOMETRIC ANALYSIS OF CORONARY ARTERY STENOSIS: AN ACCURACY AND RELIABILITY STUDY

LYNETTE MANWARRING*, DIANNE L. O'CONNELL†, BRUMDUTT S. BHAGWANDEEN*, IBRAHIM M. ZARDAWI* AND ANNETTE J. DOBSON‡

**Discipline of Histopathology, Faculty of Medicine, University of Newcastle; †Centre for Clinical Epidemiology and Biostatistics, Faculty of Medicine, University of Newcastle; and ‡Department of Statistics and Centre for Clinical Epidemiology and Biostatistics, University of Newcastle, New South Wales 2308, Australia*

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SUMMARY

Luminal narrowing was assessed in 238 transverse segments obtained from coronary arteries removed at post-mortem. In each segment, narrowing was assessed by gross visual estimation before and after fixation, and on histological sections by stereological point counting and computer-assisted planimetry.

Computer-assisted planimetry was found to be accurate and reliable but the equipment needed is expensive, and requires specialized software and an experienced user.

Morphometric measurement by stereologic point counting was accurate, rapid, simple, and inexpensive.

In comparison with computer-assisted planimetry visual estimation was found to be neither accurate nor reliable.

Our results indicate point counting as the method of choice for assessment of coronary artery luminal narrowing by atherosclerosis.

KEY WORDS—Visual estimation, stereologic point counting, computer-assisted planimetry, coronary artery stenosis, morphometry.

INTRODUCTION

Reliable and accurate measurements of coronary artery stenosis are essential for the quantitative evaluation of coronary atherosclerosis and are important prerequisites for epidemiological studies.[1–3] There is as yet no standard procedure used for evaluating the degree of stenosis in cross-sectional areas of coronary arteries. The measurement techniques currently in use vary in complexity, accuracy, and reliability.[4–8] The diversity of the methods and the widespread use of non-quantitative techniques make epidemiological studies of the prevalence, extent, and severity of atherosclerosis across and within populations difficult.[9–12]

Several studies have been based on the appearance of the intimal surface of coronary arteries after they have been opened longitudinally.[9–10] The extent of atherosclerosis is assessed as the percentage of intimal surface affected by the different grades of lesions.[13] Assessment of longitudinally opened arteries has been made by visual estimate, point counting, and planimetry.[1] However, such studies provide no indication of the severity of luminal narrowing.

Injection with radio-opaque contrast media and subsequent angiography of coronary arteries allow quantification of arterial stenosis by comparison of the luminal diameter at the point of narrowing with the diameter of a more proximal segment assumed to be normal.[10] However, coronary angiography often underestimates the severity of disease, as subsequent post-mortem findings have confirmed.[14]

By examining transversely-cut coronary arteries it is possible to examine vessel wall thickness and to assess stenosis as a measure of the effect of atherosclerosis.[9] Direct assessment of the extent of luminal narrowing is also possible. In addition, areas of calcification, the presence of major thrombi, and complete occlusions can also be assessed.

Addressee for Correspondence: B. S. Bhagwandeen, Histopathology, Faculty of Medicine, University of Newcastle, New South Wales 2308, Australia.

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This study was undertaken to determine a reliable and accurate technique for assessment of coronary artery stenosis in transverse sections. The methods used for the study were gross visual estimation, stereological point counting, and computer-assisted planimetry.

MATERIALS AND METHODS

Coronary arteries

The three major branches of coronary arteries were dissected during hospital post-mortem examinations from 11 subjects.

Visual estimation

At the time of autopsy, the degree of luminal narrowing was assessed by examining transverse sections at 5 mm intervals along the entire length of each major vessel (Fig. 1).

The degree of cross-sectional luminal narrowing in each 5 mm segment irrespective of the nature of the pathology was categorized as <50 per cent, 50–75 per cent, or >75 per cent narrowing. A total of 238 5 mm artery segments from the 33 vessels was examined. Three independent observers visually estimated luminal narrowing in 199 fresh 5 mm segments. The remaining 39 segments were assessed by two observers only.

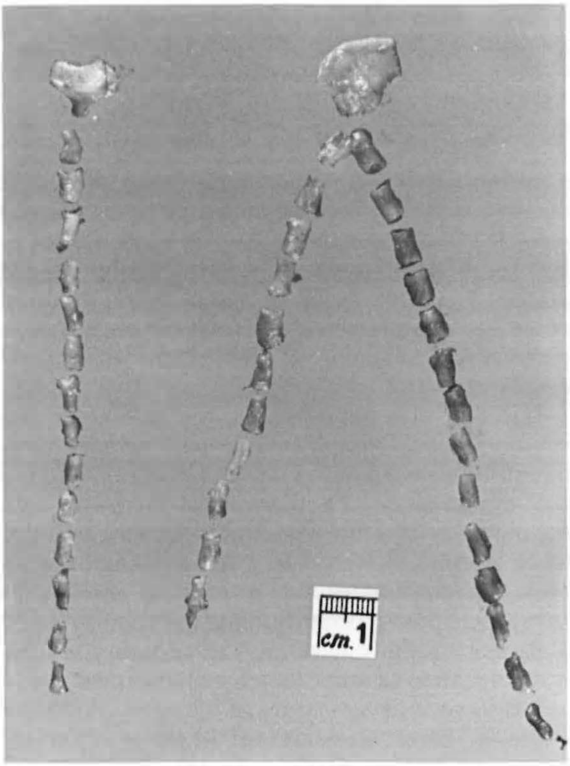

Fig. 1—The right, left anterior descending, and left circumflex coronary arteries cut at 5 mm intervals for visual estimation of cross-sectional luminal narrowing

Each segment was then labelled and transferred to 10 per cent buffered formalin for fixation and preparation for subsequent histological examination. Prior to embedding, each post-fixed segment of vessel was again independently examined and assessed for luminal narrowing by the three observers.

Histology

After formalin fixation all segments were decalcified in 10 percent formic acid. Routine tissue processing, including dehydration, clearing, and filtration, was carried out before embedding in paraffin wax and 5 μm sections were stained using Verhoeff's elastic stain with van Gieson counterstain.

Point counting

To determine the percentage of affected lumen, stained sections were examined using a Zeiss stereomicroscope with an integrating eyepiece graticule which had 121 points (intersections of horizontal and vertical lines). The optimal number of points was obtained from a pilot study using the following formula:

$$\mathrm{RSE} = \sqrt{1-A_A}/\sqrt{n}$$

where RSE is the relative standard error, A_A is the area fraction, and n is the total number of points. The RSE in the pilot study was approximately 5 per cent.[15] This was thought to be acceptable for the type of tissue used in the study. Like Cheung *et al.*,[7] we took the internal elastic lamina (IEL) as the original luminal border. The number of points within the lumen and the number of points lying on and within the area bounded by the internal elastic lamina were counted (Fig. 2). The percentage of the lumen narrowed was calculated from the ratio of these two numbers. A subset of sections ($n=42$) was also counted by the second observer (I.Z.) for determination of inter-observer relibiality. A further subset ($n=25$) was recounted by the first observer (L.M.) to assess intra-observer reliability.

Computer-assisted planimetry

The extent of luminal narrowing of the same coronary arteries was also measured by computer-

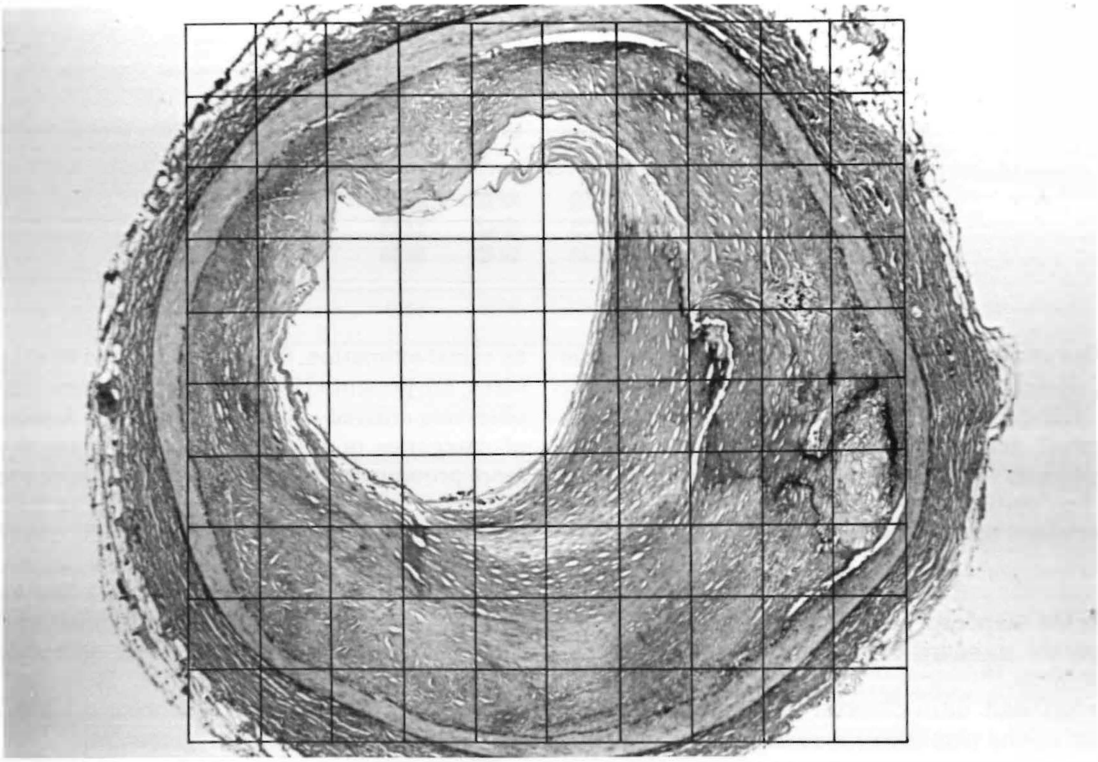

Fig. 2—Integrating eyepiece graticule with 121 intersecting points superimposed on a section of coronary artery stained with Verhoeff's/van Gieson. In this section, 74 points lie within the area bounded by the IEL and 13 points within the still patent lumen. Eighty-two per cent of the lumen is obliterated

assisted planimetry. A microscopic image of the cross-section of the coronary artery was projected onto the surface of a digitizing tablet linked to an Apple IIe computer. The perimeters of the internal elastic lamina and the effective functional lumen border were traced using an electronic pen and the area measurements calculated automatically by the computer.

The extent of luminal narrowing was determined according to the formula:

$$100-\left(\frac{A_i}{A}\times 100\%\right)$$

where A_i is the area of functional lumen and A is the area within the IEL. A subset of sections ($n=45$) was examined independently by the second observer (I.Z.) to determine inter-observer reliability. The first observer (L.M.) re-examined a subset ($n=44$) for assessment of intra-observer reliability.

Enlarged photomicrographs of the same subset of sections were initially examined by a similar planimetric method to determine any human error in tracing the luminal borders from projected images. Comparison of the results obtained from photomicrographs with their direct tracings from projected images showed no errors.

Assessment of reliability

To assess the degree of inter-observer reliability of visual estimation a generliazed kappa statistic was calculated. This statistic is adjusted for the amount of agreement that can occur by chance and assesses the agreement across the three observers simultaneously.[16] The agreement beyond chance is calculated as $P_o - P_e$ where P_o is the proportion of agreement across all subjects and P_e is the agreement which would be expected by chance alone. The ratio of the actual agreement beyond chance to the maximum possible agreement beyond chance is the index of observer agreement, kappa:

$$K = (P_o - P_e)/1 - P_e)$$

A kappa value of 1 represents perfect agreement, while a kappa value of zero indicates that the agreement was no better than chance.

Table I—Relative frequency of use of the three categories of severity by each observer for fresh and fixed specimens

Category	Observer 1 Fresh	Observer 1 Fixed	Observer 2 Fresh	Observer 2 Fixed	Observer 3 Fresh	Observer 3 Fixed
<50%	0·68	0·52	0·73	0·54	0·45	0·30
50–75%	0·22	0·25	0·15	0·22	0·29	0·36
>75%	0·10	0·23	0·12	0·24	0·26	0·34

The unweighted form of kappa was used since in this context only assignments to the same category are relevant, the clinical outcomes being quite different across the three categories of luminal narrowing.

The statistical significance of kappa was determined by comparing

$$z = K/\mathrm{SE}(K)$$

with the standard normal distribution, where SE (K) is the standard error of kappa under the null hypothesis.[16]

Inter- and intra-observer reliabilities of point counting and planimetry were determined using the intra-class correlation coefficient (rho).[17] The intra-class correlation coefficient is a measure of agreement for continuous data, with values close to 1 representing good agreement. The statistical significance of the intra-class correlation coefficient is determined by comparing the ratio of the mean square error within subjects, obtained from a one-way analysis of variance, with the appropriate F distribution.

Measurement of accuracy

Image-projected planimetry was taken as the standard against which point counting and visual estimation were compared. The accuracy of point counting was measured by determining the agreement, using the intra-class correlation coefficient, with the results obtained by planimetry. The accuracy of visual estimation was investigated by comparing the distributions of planimetry measurements within the categories of narrowing obtained by visual estimation.

RESULTS

Visual estimation: inter-observer reliability

The relative frequencies with which the three observers allocated the three categories of severity by visual estimation, for the unfixed and fixed specimens, are presented in Table I. It is evident that the observers differed substantially in their assessment of categories of severity. These differences were more pronounced when assessing fresh specimens.

The overall inter-observer agreement on the fresh specimens was $K = 0{\cdot}46$ ($\mathrm{SE}(\kappa) = 0{\cdot}03$, $z = 16{\cdot}40$, $P < 0{\cdot}001$). Similarly for fixed specimens, kappa was 0·43 ($\mathrm{SE}(\kappa) = 0{\cdot}02$, $z = 17{\cdot}92$, $P < 0{\cdot}001$). The kappa values are statistically significantly different from zero, indicating that inter-observer agreement is significantly, better than chance. However, the observed agreement beyond chance is still less than half the maximum possible aggreement.

Kappa was also calculated for each category of severity (Table II). Inter-observer agreement was best within the categories < 50 per cent and > 75 per cent, for both fresh and fixed specimens, and although the z scores showed agreement to be statistically significantly better than chance for all categories, the kappa values for the category 50–75 per cent were small (0·22 and 0·15 for fresh and fixed specimens, respectively).

Point counting: inter- and intra-observer reliability

The intra-class correlation coefficient for the assessment of inter-observer reliability of point counting measurements was 0·97. This value indicates almost perfect agreement between the two sets of point counting measurements ($F = 67{\cdot}76$ with 40 and 41 degrees of freedom; $P \ll 0{\cdot}01$). The intra-class correlation coefficient for estimating intra-observer reliability of point counting was also 0·97 ($F = 73{\cdot}73$ with 24 and 25 degrees of freedom; $P \ll 0{\cdot}01$).

Planimetry: inter- and intra-observer reliability

Inter-observer reliability for image-projected planimetry was also very high, the intra-class correlation coefficient being rho $= 0{\cdot}91$ ($F = 21{\cdot}06$ with 44 and 45 degrees of freedom; $P \ll 0{\cdot}01$). For intra-

Table II—Kappa, standard error of kappa, and *z* scores for agreement on use of each category of severity by three observers on fresh and fixed specimens

	Fresh			Fixed		
Category	κ	SE (κ)	z	κ	SE (κ)	z
<50%	0·58	0·04	15·73*	0·51	0·03	14·70*
50–75%	0·22	0·04	5·60*	0·15	0·04	4·19*
>75%	0·54	0·03	14·56*	0·61	0·03	16·82*
Overall	0·46	0·03	16·40*	0·43	0·02	17·92*

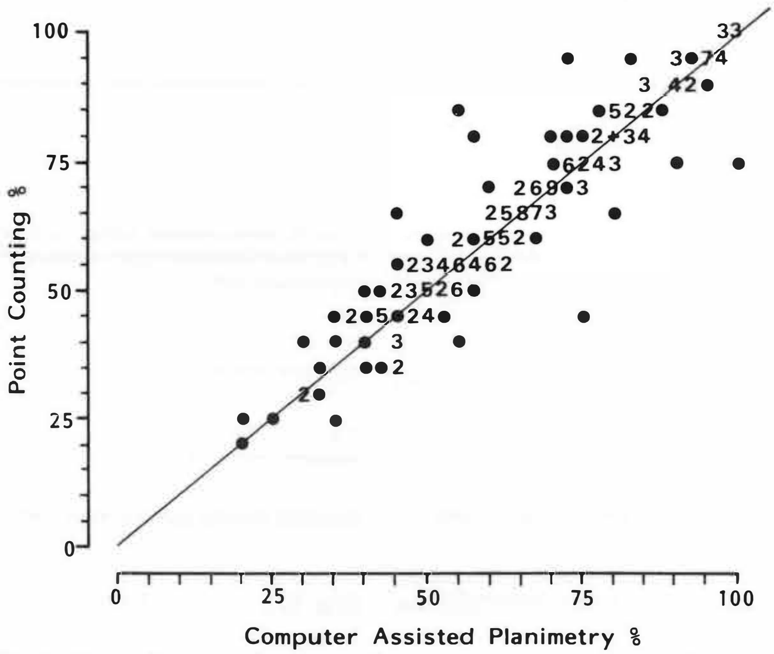

Fig. 3—Scatter diagram to demonstrate the agreement between point counting and computer-assisted planimetry in assessing luminal narrowing. The points on the diagonal line indicate perfect agreement. (Numerals represent numbers of arterial sections with similar degree of narrowing; ● = 1; 2 = 2 sections, etc.)

observer reliability rho = 0·97, again indicating almost perfect agreement (F = 66·41 with 24 and 25 degrees of freedom; $P \ll 0{\cdot}01$).

Comparison of point counting and planimetry

Point counting and image-projected planimetry were compared using the intra-class correlation coefficient. Again agreement was found to be almost perfect, the intra-class correlation coefficient being significantly different from zero (rho = 0·94, F = 37·91 with 237 and 238 degrees of freedom; $P \ll 0{\cdot}01$). These results are further demonstrated in Fig. 3, which is a graphical representation of measurements from point counting compared with image-projected planimetry. Points on the line passing through the origin indicate perfect agreement between the two methods. If image-projected planimetry is taken as the gold standard, these results show that point counting was extremely accurate.

Comparison of visual estimation and planimetry

Figure 4 shows the actual distribution of the continuous scale measurements obtained by image-projected planimetry within each of the visually estimated categories of narrowing assessed by

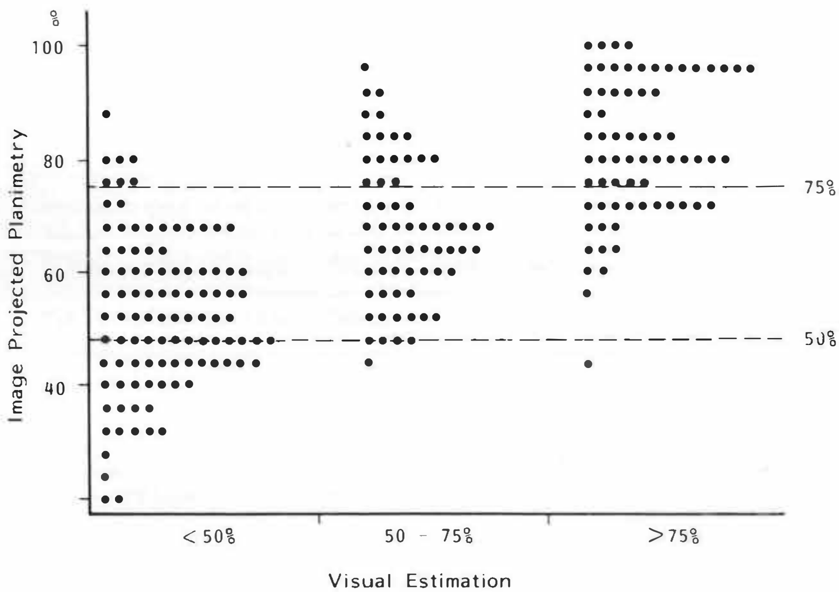

Fig. 4—Distribution of computer-assisted planimetric measurements within each of the categories as estimated by visual observation. It illustrates the unreliability of simple visual estimation

one observer (other observers had similar results). The graph shows considerable overlap of values among the categories, indicating substantial misclassification by visual estimation.

DISCUSSION

The results of our study indicate that gross visual estimation of luminal narrowing on cross-sections of coronary arteries is not reliable. Observers were often not able to agree satisfactorily when using a three-point scale of severity.

Agreement between observers differed for the categories of severity. Agreement was best within the categories < 50 per cent and > 75 per cent. These two categories represent the least amount (< 50 per cent) and the most severe narrowing (> 75 per cent), respectively. Agreement was worst in the category 50–75 per cent.

The results of visual assessment did not accord with those obtained by point counting or by image-projected planimetry. The poor accuracy of visual estimation was demonstrated by the wide and overlapping distributions of measurements determined by image-projected planimetry within each of the categories estimated by visual observation alone.

Point counting also proved to be more reliable than visual estimation. The measures of agreement for inter- and intra-observer comparisons indicate that the method is highly reproducible.

There were few technical difficulties in employing the point counting method or image-projected planimetry. On histological cross-sections, the arterial structures were well preserved and the internal elastic lamina was easily identified. Even in vessels where the internal elastic lamina was interrupted its former position could easily be identified (Fig. 1).

In a small proportion of healthy arteries, the effective functional lumen was artefactually narrowed by segmental collapse of the vessel wall. Assessment of luminal narrowing by point counting in these cases obviously gave erroneous results (Fig. 3). Planimetric measurement, however, was not affected as the determination of luminal narrowing depended on tracing of the actual and potential (as outlined by the internal elastic lamina) lumens. Planimetric measurement in this situation is therefore superior to the point counting method. However, the number of distorted arteries in all preparations was surprisingly small and did not adversely affect our overall results (Fig. 3). This is apparent from the comparative study, where point counting proved as accurate as planimetry.

Although point counting is slower and more tedious than visual estimation there are inherent advantages in the use of this method.[18–20] Point counting is a non-automatic procedure requiring simple and inexpensive instrumentation. Accuracy is reported to improve if the density of points is increased, but counting large numbers of points beyond an optimal number can be tedious, time-consuming, and confusing, and does not significantly improve accuracy.[18,21]

Computer-assisted planimetry is a semi-automated procedure and is regarded as the gold standard for morphometry.[19] The method is accurate and reliable and can be used to measure a variety of characteristics. However, the equipment is expensive and requires specialized software. Until the user becomes experienced with the use of an electronic pen for accurate tracing of the structures being measured, the procedure can be time-consuming.

Despite the advantages of being able to make an immediate and rapid assessment of the extent of coronary occlusion by visual estimation, the results obtained on fresh and fixed specimens proved unreliable. Its use for epidemiological studies of prevalence, extent, and severity of atherosclerosis would therefore be unsuitable. Compared with planimetry, the point counting method proved to be as reliable and the two methods gave nearly identical results. Our results for inter- and intra-observer reliability and accuracy and the advantages of simple and inexpensive instrumentation indicate point counting to be the method of choice for measuring coronary artery stenosis for epidemiological studies.

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