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Method for the morphometric analysis of arterial structure

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Changes in arterial structure are seen in many diseases and their morphometric quantitation has been used as an objective measure of such alterations. A critical review of techniques for arterial mensuration has been presented by Cook and Yates.¹ They described a planimetric method for the assessment of arterial structure using the perimeter of the internal elastic lamina as a reference value to indicate the true size of vessels, which were fixed without perfusion. They noted that linear measurements made on partly collapsed vessels were of dubious value as the degree of contraction and collapse is an inconsistent phenomenon. Their method did not, however, take account of the intimal component of the arterial wall. The fact that intimal thickening is often a patchy process affecting the circumference of a vessel in an irregular manner makes isolated linear measurements of little value for accurate and reproducible assessment of this lesion. The method described here allows reproducible measurements of arterial structure to be made on histological sections. It uses an adaptation of the method of Cook and Yates to transform area measurements of intima and media to fit the mathematically "circularised" measured perimeter of the internal elastic lamina.

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Theoretical considerations

A muscular artery is shown diagrammatically in Fig. 1 and an ideal circular vessel in Fig. 2. The perimeter of the internal elastic lamina, L, is assumed to be constant after collapsing down from its expanded (perfused) state. The area of the media, A1, and intima, A2, are also assumed to be constant after collapsing down from a stretched (perfused) state. Direct linear measurements of lumen radius, intimal thickness, and medial thickness are unreliable if made in vessels of this type.

The radius of the ideally circular contour of the

Fig. 1 Contracted muscular artery

Fig. 2 Mathematically ideal muscular artery

internal elastic lamina RE, may be derived from its measured perimeter, L, and is given by:

$$RE = \frac{L}{2\pi}$$

The area of the intima, A2, and the area of the media, A1, may be obtained by direct planimetric measurement from the section. In a theoretical circular expanded state the intima is assumed to lie in a uniform layer within the internal elastic lamina. The area inside the expanded elastic lamina, Aiel, is given by:

$$Aiel = \pi RE^2$$

The area of the ideal lumen, Alum, is calculated as the Aiel minus the measured area of the intima, A2.

$$Alum = Aiel - A2$$

and hence the radius of the ideal lumen, RL, is given by:

$$RL = \sqrt{\frac{Alum}{\pi}}$$

In a similar fashion the media is assumed to be arranged in a uniform layer outside the internal elastic lamina. The total area of the ideal vessel, Atot, is given by:

$$Atot = A1 + Aiel$$

The radius to the outside of the media, RA, is hence given by:

$$RL = \sqrt{\frac{Atot}{\pi}}$$

From the derived linear measurements of lumen radius (RL), vessel radius (RA), and radius of internal elastic lamina (RE), the ideal intimal thickness (TI) is given by:

$$TI = RE - RL$$

and the medial thickness, Tm, is given by:

$$Tm = RA - RE$$

Other values derived from the measurements may be made such as the degree of occlusion of the area inside the internal elastic lamina by intima, (A0%), given by:

$$A0\% = \frac{A2}{Aiel} \times 100$$

This value A0% is a measure of the degree of severity of occlusion of the arterial lumen.

Practical considerations

Paraffin wax embedded sections of tissue stained with elastic Van Gieson are used. Sections are cut at 5 µm on a rotary microtome.

Planimetric measurements are made using a Kontron Videoplan microcomputer linked to a digitising tablet. A microscope linked to a television camera provides a video image of the slide, which is displayed on a high resolution monitor. This image is overlaid by a video cursor image generated by the computer. The video cursor is moved around the contour of the vessel as it is linked to movements made by a magnetic cursor on the digitising tablet. This system is scaled for magnification factors and generates measured values in microns. Vessels are analysed at a final magnification sufficient to ensure a trace diameter on the tablet of between 5 and 15 cm for the outer contour of the vessel. This reduces intraobserver errors due to slight inaccuracies in tracing contours.

The software used for the planimetric evaluations is the YX system (Kontron). This allows user defined mathematical transformations of the planimetric data to be made as measurements are performed, enabling rapid access to derived results.

In practice three traces are made; around the lumen, internal elastic lamina, and media of each vessel. All derived values as illustrated in Fig. 2 are instantly calculated and may be stored for statistical analysis.

Technical methods

Discussion

There are many practical aspects to be considered in the application of this method.

Only vessels which are in true cross section are measured. This is judged by looking for widening of the media at the major axis of elliptical vessels which would suggest an oblique section. Contracted vessels which are non-circular in profile may be measured provided that they are in transverse section. The contour of the elastic lamina must be traced precisely. The degree of resolution of the folding of the elastica depends on magnification and for this reason a high magnification is used for the assessment of small vessels, typically $\times 200$ for vessels of $500\text{ }\mu\text{m}$ external diameter and $\times 1000$ for vessels of $100\text{ }\mu\text{m}$ external diameter (magnifications are final image magnifications). Small muscular vessels with a high area of media in relation to their cross sectional area show the most severe degree of contraction artefact and it is therefore important to resolve the elastica as precisely as possible in order to avoid these errors. Another factor in dealing with small vessels at high magnification is that the width of the elastic lamina becomes significant in comparison to the width of the measuring cursor. Tracing along the outer contour of the elastic lamina gives the most reproducible results on repeated measurements of the same series of vessels. For vessels with no significant intimal thickening the inner contour of the internal elastic lamina is traced as being the intimal layer.

In dealing with vessels showing a diseased intima there is often reduplication of the internal elastic lamina with fragmented elastic lamellae. This is a problem in the application of this technique. Often, however, the original elastic lamina remains as an obviously denser structure with thin lamellae incor-

porated into the intimal thickening. In these cases the observed dense outer lamella is measured as the original internal elastic lamina. With severe disease of the vessel wall and particularly with the onset of collagenous replacement of intima and media, the dense original elastic lamina may be lost. In this case it is not possible to use this method as there is no reliable index to assess contraction.

In practice the above method has been used to assess the patterns of small vessel disease in limbs amputated for critical ischaemia.² It allows the assessment of between 60 and 90 vessels an hour.

This technique is most suitable for the assessment of small muscular arteries and relies on the adequate demonstration of an internal elastic lamina. For large vessels, a sufficiently low magnification which will allow the tracing of the entire vessel will entail poor resolution of the contour of the elastic lamina. The error consequently generated by this poor resolution is offset by the fact that larger vessels have a lower media to lumen ratio and hence do not tend to show large degrees of elastica contraction in relation to the size of their lumen.

Further work is underway to compare results derived by this method with those obtained from direct measurements on vessels fixed in a perfused state at physiological pressures.

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Letters to the Editor

Further evaluation of a new filter for leucocyte depletion of blood

Since the evaluation of the leucocyte depleting filter Sepacell R-500 (Asahi Medical Co Ltd, Tokyo, Japan) was reported,¹ the manufacturer has modified the design of the filter by incorporating into the filtration chamber a prefilter for the removal of microaggregates. The purpose of this modification was to reduce the prolonged filtration time seen in some of the original filters. We report the results obtained using the modified Sepacell R-500 filter.

Units of blood were collected into

CPDA-1 anticoagulant in single or triple packs (Fenwal FKR 0844 or FKR 1369, Travenol Laboratories Ltd, Thetford, England). Whole blood and plasma reduced blood (11 units of each) were stored at $+4^{\circ}\text{C}$ for two or three days before filtration. The priming of the filter with 0.9% sodium chloride and filtration of the blood was done in accordance with the manufacturer's instructions. The mean ($\pm$ SD) filtration time was 8.5 ± 1.8 min and the range from 5 to 12 min. Red cell, leucocyte, and platelet counts were determined on samples taken before and after filtration using a Technicon H-6000 automated flow cytochemistry cell counter. The efficiency

of the filter in removing leucocytes and platelets and the associated red cell loss were expressed as a percentage of absolute numbers of leucocytes, platelets and red cells in the blood before filtration: the mean values ($\pm$ SD) were $99.1 \pm 1.3\%$, $83.1 \pm 7.6\%$, and $8.9 \pm 7.2\%$, respectively. Non-haemolytic febrile transfusion reactions have not been reported in any of the seven recipients of filtered blood, all of whom had a history of such reactions in the past.

Our tests have shown that the modified Sepacell R-500 filter is highly efficient in removing leucocytes and that it is possible to filter a unit of whole blood or plasma