New method for measuring intimal component of pulmonary arteries

JUNE M FERNIE,* D LAMB†

From the *Institute of Occupational Medicine, Edinburgh, and the †Department of Pathology, University of Edinburgh Medical School, Edinburgh

SUMMARY Current methods for assessing the intima of pulmonary arteries produce measurements of intima and artery size that are affected by the constriction or collapse of the arteries and the generally patchy distribution of intimal abnormality. Our new method measures intimal area and defines artery size as the total length of the internal elastic lamina; these measurements are unaffected by the constriction or collapse of the arteries and are easily and directly obtained from histological sections, using a light microscope with a camera lucida attachment in conjunction with a microcomputer linked to a digitising board. The measurements produced are consistently repeatable.

We considered that the extent of intimal change in a pulmonary artery was most readily understood when expressed not as an area measurement but in the form of an “intima index”, in which intimal area is calculated as a proportion of the area enclosed by the internal elastic lamina in its theoretically unwrinkled state. Values for intima index range from $>0$ to $\leq 1$, indicating minimal through to total occlusion of the artery lumen. Although values for the intima index increased as artery size decreased in the subjects studied, there was no consistent overall correlation between intima index and artery size for different subjects. We therefore concluded that subjects should be compared by calculating mean intima indexes for arteries subdivided into groups according to size.

Although there have been many quantitative studies of pulmonary vasculature, especially the muscular pulmonary arteries, the emphasis has always been on measurements of the media; few studies have included any measurement of the intimal component. There are two likely explanations for this. Firstly, there has been a tendency to underestimate the potential importance of intimal changes as a cause of increased pulmonary vascular resistance and hence pulmonary hypertension. Secondly, estimating the nature and distribution of the intimal changes that occur in pulmonary arteries has been difficult. In most disease states these changes are patchy, rarely affecting the entire circumference of an artery to a uniform extent.12 Furthermore, variable numbers and different size groups of arteries may be affected.12 These factors make the measurement of the intima difficult, and few methods of measurement have been reported. The simplest method involves subjectively scoring each artery on a scale $0$, $+$, $++$ to $+++$. according to the degree of intimal change considered to be present. Other methods include measurement of the thickness of the intima in each artery at the site of least,5 or least and greatest6 intimal thickening.

Intimal thickness has also been expressed as a percentage of some indicator of vessel size, either the internal diameter7–9 (distance between diametrically opposed points on the internal elastic lamina) or the external diameter.10 These methods of measurement may be criticised for the same reasons as the “wall thickness” methods for assessing medial hypertrophy; the measurements produced do not accurately reflect either the extent of intimal change or the size of artery affected, as they vary with the degree of constriction or collapse present.

We have recently developed a new technique using a semiautomatic digitising system for measuring the medial component of pulmonary arteries,11 in which the size of an artery is defined in terms of the length of internal elastic lamina, a variable that is unaffected by constriction or collapse. We showed that this variable was a more reliable indicator of vessel size than others previously used and that the measurements obtained by the new technique were consistently repeatable.11

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This paper describes how the technique may be applied to the measurement of patchy intimal changes and discusses the repeatability of the measurements obtained.

Material and methods

Table 1 gives details of the six subjects included in this study. Whole lungs were obtained at necropsy from five subjects; one lung was a resection specimen. Only one lung from each subject was studied.

Preparation of specimens

The lungs were inflated by running formalin from a height of 45 cm into the main bronchus; the formalin was allowed to run in until the pleura was firm with rounded edges. Fixation was then allowed to continue for at least one week by placing each lung in a basin of formalin, which was covered to prevent drying.

After fixation the lungs were sliced at intervals of 1 cm in the sagittal plane, and representative tissue blocks selected. In general, a total of 12 blocks was taken, covering the upper, lower, and, in the case of a right lung, middle lobes. The selected blocks, measuring about 2.5 cm × 2.5 cm were taken from throughout the lung (cases 1–5). These blocks were embedded in paraffin and sectioned at 5 μm. Weigert’s elastic stain with a Van Gieson counterstain or Miller’s elastic stain were used. Twelve blocks were randomly selected (using a random number table and template) from the two most lateral slices of the lower lobe (the upper lobe was excluded because it contained a carcinoma) (case 6); these were a standard size 1.91 cm × 1.91 cm. The 12 blocks were embedded in glycol methacrylate and sectioned at 3 μm according to the method of Sims.12 Staining of elastic tissue was carried out using a modification of Verhoeff’s elastic stain.13

Method of measurement

All measurements were recorded using the Graphic digitising systems 1 equipment supplied by Graphic Information Systems Ltd. This equipment comprises a digitising board with an electronic cursor, together with a microcomputer and printer.

Measurements of pulmonary arteries were obtained directly from histological sections by means of a camera lucida attachment to the light microscope; the attachment was orientated so that it overhung the digitising board. This enabled the pinpoint of light emitted from the cursor on the digitising board to be superimposed on the artery in the field of view. Before starting measurement the digitising board was calibrated to the light microscope; this was effected by assigning each of the four buttons on the cursor to a specific lens objective on the microscope.

Table 1 Details of six subjects studied

<table>
<thead>
<tr>
<th>Case No</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Smoking history</th>
<th>Cardiopulmonary pathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>56</td>
<td>M</td>
<td>smoker</td>
<td>COLD*, severe RVH**</td>
</tr>
<tr>
<td>2</td>
<td>64</td>
<td>M</td>
<td>unknown</td>
<td>Rheumatic heart disease</td>
</tr>
<tr>
<td>3</td>
<td>59</td>
<td>M</td>
<td>smoker</td>
<td>Normal</td>
</tr>
<tr>
<td>4</td>
<td>67</td>
<td>M</td>
<td>unknown</td>
<td>Atrial septal defect</td>
</tr>
<tr>
<td>5</td>
<td>24</td>
<td>M</td>
<td>unknown</td>
<td>Pulmonary sequestration</td>
</tr>
<tr>
<td>6</td>
<td>59</td>
<td>M</td>
<td>smoker</td>
<td>Small peripheral carcinoma in right upper lobe</td>
</tr>
</tbody>
</table>

* COLD = chronic obstructive lung disease,
** RVH = right ventricular hypertrophy
Several variables of each artery, determined by the program used, were measured by moving the pinpoint of light across or along the specified parts of the artery while depressing the appropriate button on the cursor. All coordinates "activated" on the digitising board during the measurement of each variable were recorded and passed to the microcomputer, which translated the information into distances between points, lengths, or areas, depending on the variable. (All measurements may be printed directly or stored on tape for analysis at the operator's convenience.)

Programs

Three programs were used in this study; these were written in BASIC. Program 1 and the statistical program "simple regressions" were provided by Graphic Information Systems. The intima index program was obtained from the Institute of Occupational Medicine.

Program 1 was designed to measure the variables of muscular pulmonary arteries that satisfied the criteria of being cut in good cross section and having a well defined internal elastic lamina round the major part (at least 7/8ths) of their wall. The variables measured by the observer were marked with an *; measurements for the remaining variables were produced by the computer: *circumference of lumen (LC); *total length of internal elastic lamina (IEL); *total length of external elastic lamina (EEL); areas of lumen (LA), intima (IA), and media (MA) (Fig. 1).

Intimal area was calculated by the microcomputer as the area enclosed by the internal elastic lamina less the area enclosed by the lumen circumference. Similarly, medial area was calculated as the area enclosed by the external elastic lamina less the area enclosed by the internal elastic lamina. Arteries satisfying the above criteria, and therefore measured using Program 1 were termed "digitisable".

The statistical program simple regressions was used to determine the relation between measurements of the intima and size of artery (defined in terms of total length of internal elastic lamina). This program enabled several functions, linear and non-linear, to be fitted to the data and selected that giving the best fit.

The intima index program was designed to enable the intimal area of vessels measured using Program 1 to be expressed as a ratio of the area enclosed by the internal elastic lamina in its theoretically unwrinkled state (Fig. 2). This was required to accommodate differences in the degree of collapse or constriction between arteries.

\[
\text{Intima index} = \frac{\text{area of intima}}{\text{area enclosed by theoretically unwrinkled internal elastic lamina}}
\]

Fig. 2 Diagrammatic representation of part of muscular pulmonary artery showing internal elastic lamina and intima area measured (a) and theoretically unwrinkled (b) state.

\[
\text{intimal area} = \frac{\text{length internal elastic lamina}^2}{4\pi}
\]

Values for the intima index ranged from $>0$ to $\leq 1$. Thus a value of 1 would indicate total occlusion (actual and theoretical) of the lumen by intimal change.

Repeatability of Intimal Area Measurements

Twenty muscular pulmonary arteries were selected from the histological sections of the six subjects. These arteries were specifically chosen because they covered the full range of size of muscular pulmonary arteries and, more importantly, they showed a wide variation in the degree of intimal change and degree of constriction or collapse present. Each artery was digitised three times in succession using Program 1 to provide a baseline (mean) value for each variable measured and once on a further three occasions, each separated by a minimum period of six weeks. A subgroup of 18 arteries was additionally digitised at different magnifications.

Results

Repeatability of Measurements

This study was concerned solely with the repeatability of measurements of intimal area in muscular pulmonary arteries; the repeatability of measurements of all other variables of these arteries has been described in detail elsewhere.\[11\]

For each of the 20 selected muscular pulmonary arteries the mean values of intimal area (measured using Program 1) were calculated from those obtained at three consecutive digitisations. The maximum percentage deviation from the mean value was also calculated. Of the 20 arteries, 11 showed deviations of less than 1% and 16 showed deviations of...
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![Graph showing area against baseline area](image)

Fig. 3 Long term reproducibility of measurements of intimal area expressed in relation to value for intimal area.

less than 2% on consecutive digitisations; the maximum percentage deviation observed was 5.2%.

Using the mean values obtained from the initial consecutive digitisations as a baseline, the long term repeatability of measurements of intimal area was investigated. On each of three further digitisations the percentage deviation from the baseline value of intimal area was calculated for each artery. Fig. 3 shows the maximum percentage deviation observed over the three independent digitising sessions in relation to the baseline value of intimal area. In general, the long term repeatability of the measurements was excellent, with only three of the arteries showing deviations of more than 5% from the baseline value; these were small arteries with a thin intima.

The effect of magnification on the measurement of intimal area was investigated in a subgroup of eighteen arteries which fulfilled the criterion of being digitisable at a minimum of three out of four lens objective magnifications (×4, ×10, ×20, ×40). The data were expressed as ratios:

Measurement at specified magnification

Measurement at ×4 magnification

Table 2  Effect of magnification on measurements of intimal area in 18 arteries, expressed as measurement at specified magnifications : measurement at ×4 magnification.

<table>
<thead>
<tr>
<th>Artery number</th>
<th>Specified magnifications</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>×10</td>
</tr>
<tr>
<td>2</td>
<td>0.65</td>
</tr>
<tr>
<td>3</td>
<td>0.87</td>
</tr>
<tr>
<td>4</td>
<td>0.92</td>
</tr>
<tr>
<td>5</td>
<td>0.93</td>
</tr>
<tr>
<td>6</td>
<td>0.88</td>
</tr>
<tr>
<td>7</td>
<td>1.13</td>
</tr>
<tr>
<td>9</td>
<td>0.93</td>
</tr>
<tr>
<td>10</td>
<td>0.86</td>
</tr>
<tr>
<td>11</td>
<td>0.96</td>
</tr>
<tr>
<td>12</td>
<td>1.04</td>
</tr>
<tr>
<td>13</td>
<td>1.02</td>
</tr>
<tr>
<td>14</td>
<td>1.00</td>
</tr>
<tr>
<td>15</td>
<td>0.97</td>
</tr>
<tr>
<td>16</td>
<td>1.02</td>
</tr>
<tr>
<td>17</td>
<td>0.97</td>
</tr>
<tr>
<td>18</td>
<td>0.90</td>
</tr>
<tr>
<td>19</td>
<td>1.00</td>
</tr>
<tr>
<td>20</td>
<td>1.08</td>
</tr>
</tbody>
</table>

These data are ratios.

![Graph showing intima index against length of internal elastic lamina](image)

Fig. 4 Relation between area of intima and size of artery (case 6). \( y = A + B \times \log x \), \( A = -3898.95 \), \( B = 7155.3 \). \( r = 0.8 \), \( n = 96 \).

A ratio of 1 throughout for any artery would indicate that the measurement of intimal area was unaffected by magnification. Measurement at a magnification of ×4 generally produced underestimation or overestimation of intimal area, which could be quite

![Graph showing correlation](image)

Fig. 5 Correlation between intima index and size of artery (case 6). \( y = A + Bx \), \( A = 0.28 \), \( B = -0.0001 \). \( r = 0.51 \), \( n = 96 \).
severe—that is, arteries 7 and 2, respectively (Table 2). Intimal area measurements did not vary much with magnification from $\times 10$ upwards (Table 2). To summarise, the described method of measurement produced values for intimal area that were generally reproducible to within 5%. To obtain accurate measurements of area, however, it is advisable to digitise at a magnification of $\times 10$ or greater.

**Correlation between Intimal Area and Size of Artery**

Histological sections from the six subjects were scanned, and each muscular pulmonary artery considered to be digitisable was measured using Program 1, the data being recorded on tape. The correlation between intimal area and size of artery (defined in terms of length of internal elastic lamina) was then investigated in each subject using the statistical program simple regressions: for any size of artery the values for intimal area within all the subjects varied enormously, emphasising the irregular distribution of intimal abnormality.

The functions giving the best fit between intimal area and artery size also varied. For the six subjects studied three different best fit functions were observed; these took the form $y = A + bx$, $y = Ae^{bx}$, and $y = A + B\log x$, the last of which is shown in Fig. 4. The observed increase in intimal area with a corresponding increase in size of artery was as expected and was evident in all six subjects.

**Correlation between Intima Index and Size of Artery**

An intima index was calculated for each artery, a value of 1 indicating complete occlusion (actual and theoretical) of the lumen. These indexes were then plotted against size of artery (length of internal elastic lamina) for three subjects (cases 1, 4, and 6) who showed different correlations between area of intima and size of artery. It was evident from these plots that the smallest arteries were those most affected by intimal change (case 6) (Fig. 5). There was no consistent correlation, however, between the intima index and the size of artery in different subjects.

**Discussion**

Measuring the intimal component of pulmonary arteries comprises two separate stages: firstly, the measurement of the intima in the individual artery and secondly, the analysis of that data.

With regard to methods of measuring the intima, we consider our described technique to be an improvement on those previously reported for three main reasons. Perhaps the most important of these is that our method produces a measurement for the area of the intima, and as such is entirely unaffected by the distribution of the intimal layer round the arterial wall. In contrast, those methods, requiring measurement of intimal thickness, lead to an inaccurate estimation of the intimal layer if it is irregular, eccentric, or crescent shaped, as is often the case.

The second reason is the variable used to describe vessel size and to which measurements of the intima are related. We chose the total length of the internal elastic lamina as it is a variable that is unaffected by constriction or collapse; the use of this indicator of vessel size was validated in an earlier study. Other workers have chosen to relate intimal thickness to vessel diameter, either internal or external, a measurement that varies considerably with the degree of constriction or collapse present. Expression of intimal thickness in relation to it will lead, therefore, to an overestimation of the intimal component.

Thirdly, there is the factor of the reproducibility of our measurements of intimal area, which varied less than 5% on subsequent digitisations. Poorer reproducibility (up to 12%) was evident only in arteries in which the intimal area was very small. Magnification did not seem to have much effect on the measurements of intimal area with the exception that digitisation at a lens objective magnification of $\times 4$ tended to produce either too great or too small an estimation of the intimal area. The obvious solution to this problem is to digitise at magnifications of $\times 10$ upwards.

Although we are confident that our method of measurement accurately assesses the intimal component of individual arteries, there are problems in handling the data and deciding how best to compare subjects. Regression of intimal area against vessel size for our six subjects showed that no single function could describe the correlation for all the subjects. This was in strong contrast to the correlation between medial area and vessel size that generally takes the form $y = Ax^n$. As there was no consistent correlation between intimal area and vessel size subjects could not simply be compared by assessing whether the slopes of the correlation were the same or not, a comparison which was possible with the media.

One disadvantage of relating area of intima to size of artery is that it is not immediately obvious which arteries are most affected by intimal change in relative terms. For this reason we decided to express the data in another form, the intima index, in which the intimal area is expressed as a ratio of the area enclosed by the internal elastic lamina in its theoretically unwrinkled state. We considered that this index gave a readily understandable impression of the extent of intimal change in an artery as it ranged from $>0$ to $\leq 1$, indicating minimal through to total.
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collection of the artery lumen. It must be remembered, however, that while the index accurately reflected the extent of intimal abnormality in an artery, the value produced for lumen occlusion was a minimum possible value, as the index was independent of the degree of collapse or constriction present.

Regression of intima index against size of artery showed which arteries were most affected by intimal change and to what extent. Invariably the smaller vessels were most affected, although there was considerable variation in the intima indexes of arteries in this group, emphasising the patchiness of intimal change. No single function described the correlation between intima index and size of artery for all the subjects.

These problems with the relation between the two measures (area and intima index) of the intimal component and size of artery led us to conclude that the most sensible method of comparing subjects would be to calculate a mean intima index for arteries subdivided by size (length of internal elastic lamina). Some workers simply calculated a mean value for their measurements of the intima based on all arteries measured. It is, however, essential to subdivide by size as the smaller arteries are more affected by intimal change.

Finally, there is the problem of selecting arteries for the measurement of the intima. Using our method, the number of arteries measured was less than 35 per subject with paraffin embedded tissue. Taking into account the patchiness of intimal change, the question is whether these 35 or fewer arteries were a truly representative sample of the total population.

Although it is possible to compare the measured arteries with the total population, such a comparison has to be based on the variables of intimal thickness and vessel diameter, and for the reasons already discussed, it is of questionable value. A simple solution to the problem would be to increase the number of arteries measured. This can be accomplished by embedding tissue in glycol methacrylate in preference to paraffin—for example, 96 arteries were considered to be digitisable using Program 1. Adaptations to Program 1 which might permit this variation are currently being investigated.

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References


Requests for reprints to: Dr June M Fernie, Institute of Occupational Medicine, 8 Roxburgh Place, Edinburgh EH8 9SU, Scotland.
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J M Fernie and D Lamb

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