Technical Considerations of the Microscopic Evaluation of Intrapulmonary Vessels

ARTHUR S. GRANSTON, M.D.

Department of Pathology, West Suburban Hospital, Oak Park, IL 60302

ABSTRACT

The methods of microscopic evaluation and measurement of the intrapulmonary blood vessels as reported in the literature are reviewed. Difficulties in these procedures and their practical application are considered.

The intrapulmonary vasculature has been studied in the past two decades and now forms an important part of our body of knowledge of cardiopulmonary pathophysiology. This review will cover some aspects of the methods of microscopic evaluation of the intrapulmonary vessels as used by different workers and will consider some of the difficulties and practicalities of procedures.

Staining Technique

The staining of the microscopic sections for examination of the blood vessels must demonstrate elastic tissue and include a nuclear stain and a counterstain to distinguish collagen and muscle tissue. One of the more popular elastic stains is the Verhoeff method using an iodine-ferric chloride-hematoxylin mixture, which also stains the nuclei; it is commonly counterstained with the Van Gieson picrofuchsin stain to distinguish collagen and muscle. This method provides a sharp contrast with black elastic fibers, but the stain seems to lack sensitivity for the fine elastic fibers in the smallest blood vessels. O'Neal et al14 found that overdifferentiation of the Verhoeff stain was a hazard, especially in the lungs of infants. The histotechnologist commonly relies on the large vessels to judge the degree of differentiation of the stain and may use a section of aorta as a staining control. It is difficult for the technologist to examine the small vessels in an uncovered slide taken from a water solution, which would be necessary for microscopic control of the staining procedure.

Civin and Edwards, using the Verhoeff elastic stain, recorded that the age was variable for the development of the elastic lamina of the pulmonary arteriole, but that the elastic lamina had appeared by the 20th postnatal year.2 On the other hand, O'Neal et al used the aldehyde fuchsin stain for elastic tissue and could easily identify the elastic laminae of arterioles in older fetuses. The differences in these observations may be related to the elastic staining technique.

The Weigert resorcin-fuchsin elastic stain is a sensitive agent for elastic tissue and stains deeply to give a sharp contrast. This stain is an iron resorcin lake with basic
fuchsin or crystal violet, the color of the elastic staining varying from black to dark green depending upon whether or not basic fuchsin, crystal violet or a combination of the two is used. A separate nuclear stain is required, such as iron hematoxylin, and the Van Gieson stain is commonly used as a counterstain. The staining technique is not difficult, and it has not been a problem when newly introduced into the histology laboratory. The Weigert elastic stain is recommended and its details may be found in standard references.

Quantitative Measurement

The quantitative measurement of the small vessels has been frequently employed in microscopic studies. This is performed with a calibrated ocular scale in the microscope. An outline of a thickened muscular pulmonary artery and the various measurements that may be taken are illustrated in figure 1. Various parameters and ratios that have been measured and calculated by different authors are listed in table I. This is not intended to be an exhaustive compilation. It is evident that there is no unanimity among different workers and that almost every possible ratio has been used for evaluation. These values are interrelated and their choice may largely depend upon whether or not one can better visualize the numerical values as increasing or decreasing with progressive pathologic change. The important difference is whether or not the intima or media or both are included in the ratio. Evaluation of medial thickness in hypertensive pulmonary vascular disease is meaningful only in early stages before severe intimal changes have occurred with associated thinning of the media.

The description in the literature of quantitative microscopic assessment often sounds deceptively simple. It has been found, rather, that it is tedious and frustrating. First, measurements are limited to vessels which are cut in an approximately transverse section. The orientation of the muscle nuclei in the media parallel to the circumference is a helpful criterion of the plane of sectioning. In addition, the artery should maintain an approximately round

| FIGURE 1. Outline of a thickened muscular pulmonary artery and various measurements that may be taken. L, diameter of lumen; I, thickness of intima; M, thickness of media; IE, internal diameter measured between internal elastic lamina on opposite sides of vessel; EE, external diameter measured between external elastic lamina on opposite sides of vessel. |

| TABLE I |
| DIFFERENT RATIOS AND INDICES USED BY VARIOUS AUTHORS IN THE QUANTITATIVE MICROSCOPIC EVALUATION OF SMALL PULMONARY ARTERIES |
| Linear measurements |
| Lumen/Wall | L/2W<sup>1.4</sup> |
| Lumen/Media | L/2M<sup>3</sup> |
| Wall/Lumen | W/L<sup>1.9</sup> |
| Wall/External. Diam. | W/EE<sup>19</sup> |
| Media/External. Diam. | M/EE<sup>0.6-0.5,17-20,24</sup> |
| External. Diam./Media | EE/M<sup>9</sup> |
| Intern. Diam./Media | IE/M<sup>1.3</sup> |
| Intern. Diam./External. Diam. | IE/EE<sup>0.18</sup> |
| Area measurements |
| Area of Media<sup>13,15</sup> |
| Area of Media/Area of Intima<sup>2</sup> |
| Index of Medial Area/Surface |
| Area of Lung<sup>20</sup> |
contour, which it more often does not. The apparent thickness of the media at different sites in the vessel wall may vary considerably, owing to artifact, such as angulation of the contour of the wall and shrinkage and other distortion occurring in the processing of the tissue (figure 2). The selection of the points on the circumference at which to take measurements will be a subjective decision, unless exact directions are set forth, such as specified by Wagenvoort and Wagenvoort.24

Those arteries with abnormally thickened media maintain their configuration better than do normally thin vessels. Consequently, there will be a tendency for a bias in the measurements in favor of thickened vessels,8 especially in lungs in which the arteries present a wide range of wall thickness. One may be able to make a more valid assessment of the vessels in some cases by subjective grading.

The elastic membranes and their convolutions represent a significant proportion of the thickness of the vessel wall (figure 3). Only occasionally have authors specified exactly where they take their measurements with respect to the elastic laminae.15,20 The ratios relating vessel wall and diameter will differ in small arteries as compared to large arteries,8 and many authors have selected arteries of specified range of diameter for evaluation. This division of vessels on the basis of diameter is not an exact comparison of contracted and dilated vessels at corresponding levels of the arterial tree, though the discrepancy may not be significant.

Considering the variable and subjective factors in the microscopic measurement of vessels, each group of investigators can compare its observations only with its own control values. Some authors have used a grading system for evaluating medial thickness and other pathologic changes.14,18 Another approach has been the estimation of the proportion of pulmonary arteries classified as elastic, transitional, muscular, and endothelial in specified ranges of external vessel diameter to demonstrate changes in congenital heart disease.10

The morphologic distinction between arterial contraction and actual hypertrophy of medial muscle mass has taken the attention of several workers. The problem has been to find a constant reference standard for comparison of the medial muscle. O'Neal, Thomas, and Hartroft calculated the area of the media of arteries lying adjacent to specified levels of the respiratory bronchiole which they identified by the histologic characteristics.15 The validity of this technique has been criticized.25 Wagenvoort compared the total calculated index of cross-sectional area of the media...
of arteries in relation to the cross-sectional area of the paraffin sections of the lung, in this way determining an index of the total quantity of arterial muscular tissue per unit of lung.\textsuperscript{20}

Naeye used the cross-sectional area of the intima plus the internal elastic membrane as his constant point of reference with which to compare the area of the arterial media.\textsuperscript{12} It was found that the intimal area for arteries and arterioles in two specified size ranges of luminal diameter, remained constant up to approximately 16 weeks of age. This was similar for both systemic and pulmonary vessels. Naeye tabulated the mean ratio of the area of the media to that of the intima and internal elastic membrane for the vessels in the separate size ranges.\textsuperscript{12} This technique can be applied only in young individuals before intimal thickening occurs either as an aging or pathologic change in studies of cases of pulmonary hypertension, as in the perinatal period studied by Naeye.\textsuperscript{13}

Naeye used a camera lucida and planimeter for measuring the areas microscopically.\textsuperscript{12} This offered a method of circumventing the irregularity of the blood vessels in microscopic sections. Projection of the microscopic image may be used as an alternative to a camera lucida, if a darkened room is available. A microscope\textsuperscript{*} with a quartz halogen lamp may be adapted with a drawing tube by using a dual viewing adapter and a vertical monocular tube to which is attached a drawing prism with a 10× wide field ocular. A light shield should be inserted above the substage light source. The outline of the vessel is sketched on a piece of paper, and the measurements with a planimeter can be carried out at a later time.

Discussion

Although differences of technique and possible sources of discrepancy have been mentioned, the quantitative observations on

\textsuperscript{*} American Optical Company, series 20.
the peripheral pulmonary vasculature by many workers has been of considerable value in cardiovascular research. The results have been frequently corroborated by different reports. However, as a general procedure, quantitative microscopic observations are too time consuming to serve a useful purpose on a routine pathology service.

By applying the grading classification of Heath and Edwards, the pathologic changes of the small pulmonary vessels can be well categorized by distinctive qualitative characteristics. With adequate tissue sections, a good evaluation of the pulmonary vessels can be made in a short time by qualitative description. Quantitative measurements may be needed for adequate interpretation in cases with mild pathologic changes of medial hypertrophy, in some situations with medial thinning or in the first postnatal year when there is a period of change in the medial thickness of the peripheral pulmonary arteries and a normally greater variation of the vessels between individuals and within the same individual than in other age periods. The classification of Heath and Edwards into six grades of pathologic change of the small pulmonary vessels has been found in congenital heart disease to correlate with hemodynamic data and post-operative reversibility of pulmonary hypertension.

Special preparation of the lung for study is applicable only to autopsy or experimental studies. Methods of fixation and sampling of the lung are not uniform. There are differing views on the significance of postmortem contraction of the peripheral pulmonary arteries, in altering their appearance and leading to an inaccurate evaluation in contrast to vessels distended by an arterial injection mass. This may depend on the parameters that are used in the microscopic examination of the vessels. Other techniques which have been fruitful in studying the pulmonary vasculature are not discussed in this article and include such tools as serial sections for microscopy, angiography, corrosion casts and other injection techniques including the use of radioactive media. Other quantitative microscopic methods and the theoretical principles of morphometric studies of the lung have been discussed by Weibel.

References