MORPHOLOGIC STUDIES

Limitations of Postmortem Assessment of Human Coronary Artery Size and Luminal Narrowing: Differential Effects of Tissue Fixation and Processing on Vessels With Different Degrees of Atherosclerosis

ROBERT J. SIEGEL, MD, FACC,* KATHERINE SWAN, BA,* GRETCHEN EDWALDS, BA,† MICHAEL C. FISHEIN, MD, FACC‡

Los Angeles, California

Numerous studies have utilized histologic sections of coronary arteries as the standard for testing the validity of the angiographic determination of coronary artery dimensions. However, little attention has been given to artifactual dimensional changes that occur during fixation and histologic processing of tissues (dehydration, clearing, embedding, sectioning and staining). Using planimetric techniques, the dimensional changes that occurred with fixation and processing were quantitated in 61 coronary artery segments with minimal or moderate to severe atherosclerosis obtained from 12 patients studied at autopsy.

In vessels with minimal atherosclerotic narrowing, fixation and processing resulted in a decrease in total vessel cross-sectional area and luminal cross-sectional area (p ≤ 0.05), whereas absolute wall area (total vessel cross-sectional area minus luminal cross-sectional area) did not change (p = NS). These disproportionate changes resulted in an alteration in the relation between lumen and wall areas so that luminal cross-sectional area decreased from 47.6 ± 8.5% of the total vessel cross-sectional area observed before fixation to 36.2 ± 7% after processing (p ≤ 0.05). In vessels with moderate to severe atherosclerosis, both the total cross-sectional area and wall area decreased after fixation and processing (p ≤ 0.05), but luminal area did not change (p = NS).

As a result, the percent luminal cross-sectional area in these vessels increased from 21.1 ± 10.1% before fixation to 28.7 ± 9.7% after processing (p < 0.05).

This study demonstrates that: 1) fixation and processing are associated with disproportionate dimensional changes in human coronary arteries, and 2) different dimensional changes occur in coronary artery segments with different degrees of atherosclerosis. Therefore, this study emphasizes the limitations of measuring coronary artery dimensions and estimating the degree of luminal narrowing in routinely prepared histologic sections.

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Technical limitations, anatomic variations and other factors that may lead to inaccurate cineangiographic assessment of coronary anatomy, have been a lack of critical attention to the pathologic techniques used in the validation studies of angiography. Some investigators (20–22) have addressed technical factors involved in postmortem analysis of coronary artery dimensions, but the potential effects of tissue fixation and processing on postmortem histologic measurements have not been considered in many studies.

By the time the fixation and processing procedures of histologic sections of coronary arteries are completed, a number of changes have occurred that could affect coronary artery dimensions: 1) the tissue has died; 2) it has gone from a state of relatively high intraluminal pressure to zero pressure; 3) it has undergone fixation; and 4) it has been processed for histologic study (dehydrated, cleared, embedded in paraffin, cut, stained and mounted on glass slides). Since changes in volume of other tissues are known to occur with fixation and processing (23), this study was designed to...
determine if such changes were introduced during fixation and processing of coronary arteries for pathologic study. If so, this could explain some of the discrepancies between arteriographic and necropsy evaluation.

**Methods**

**Tissues studied.** The major epicardial coronary arteries in 12 human autopsy cases were studied. At autopsy, before fixation, the left main, left anterior descending, left circumflex and right coronary arteries were excised and trimmed of excess tissue. X-ray films of all arteries were obtained to identify calcified areas (Hewlett-Packard Faxitron unit, Kodak XO Mat TL film). Each vessel was cut perpendicular to the lumen into serial 2 mm segments in areas with and without X-ray evidence of calcification. Then gross photographs of 2 mm cross sections were taken at 7.5 x magnification. A millimeter ruler was included in each photograph as an internal reference for determining magnification.

Vessel segments were classified as having either minimal disease or moderate to severe coronary atherosclerotic narrowing. Twenty-nine vessel segments were free of X-ray evidence of calcification, and by planimetry had less than 50% cross-sectional luminal area narrowing; these were classified as having "minimal" disease. Thirty-two segments with X-ray evidence of calcification and greater than 50% cross-sectional luminal area narrowing were classified as having "moderate to severe" disease.

The 2 mm cross sections were then fixed overnight by immersion in 10% neutral-buffered formalin. They were then rephotographed (same side up). Sixteen segments with severe calcification underwent decalcification in 5% nitric acid before further processing. These coronary artery segments were again rephotographed. All vessels were then routinely processed by dehydration in increasing concentrations of alcohol, clearing in xylene and embedding in paraffin. The photographed surface of the embedded vessels were cut into 6 μ thick sections and mounted on glass slides, stained with Verhoeff's elastic stain and counterstained with Van Gieson's connective tissue stain. Multiple sections were reviewed. Those in which there were no tears or distortion of the vessel by folds or wrinkles were rephotographed at 7.5 x magnification as done previously with the fresh, post-fixation and decalcified specimens.

**Measurement technique.** Photographs of all technically adequate coronary artery segments (n = 61) were further analyzed. There were photographs (n = 199) of the 61 coronary artery cross sections in their fresh, post-fixation, post-decalcification and post-processed state. Two independent observers performed planimetry (Numonics industrial planimeter) in duplicate to measure the total area included within the vessel's outer border and the luminal cross-sectional area enclosed by the vessel's inner border. Vessel wall area was calculated by subtraction of the luminal area from the total area. The dimensional changes induced by various steps in the processing procedure were studied by comparison of planimetrically determined values for the prefixation (fresh), post-fixation, post-decalcification and post-processing specimens. All measurements were made from enlarged photographs at identical magnification (7.5 x), with all values expressed in square centimeters (cm²).

**Statistical analysis.** Inter- and intraobserver reliabilities for total area, luminal area and wall area during each processing stage were assessed by the intraclass correlation coefficient (24). The mean area changes due to fixation and processing were analyzed by a mixed model analysis of variance (ANOVA), with segments nested within patients and both segments and patients nested within groups (minimal or moderate to severe atherosclerosis). For this analysis, segments and patients were considered to be random effects, while groups and processing were viewed as fixed effects (25,26). The data were analyzed using the Statistical Analysis System's (SAS) General Linear Model (GLM) program (27). Significant (p ≤ 0.05) main and interaction effects were further analyzed using Scheffe's multiple comparison procedure with alpha equal to 0.05. Sixteen segments from patients in the group with moderate to severe disease also underwent decalcification. A separate one way ANOVA was conducted, which included the post-decalcification stage for this group. The statistical model employed was similar to that just outlined, but without the grouping factor.

**Results**

**Inter- and intraobserver reliability.** The resultant intraobserver reliability coefficients ranged from 0.963 to 0.998, while the interobserver reliability coefficients ranged from 0.962 to 0.999. These coefficients indicate that the measuring process is reproducible for the same observer at different times as well as for two independent observers. Dimensional changes are shown in detail in Table I and Figure 1 and schematically in Figure 2.

**Dimensional changes in vessels with minimal disease.** Fixation caused no significant dimensional changes. However, processing resulted in a reduction in both luminal and total cross-sectional areas, while wall area did not change. As a consequence of these changes, the calculated percent luminal cross-sectional area (luminal/total cross-sectional area) decreased from a prefixation value of 47.6 ± 8.5% to 36.2 ± 7% after processing (p ≤ 0.05) (Fig. 1 and 2).

**Dimensional changes in vessels with moderate to severe disease.** No significant dimensional changes occurred with fixation or decalcification. After processing, there was a reduction in both total area and wall area, but no change in absolute luminal area. As a result of these changes, the calculated percent luminal cross-sectional area increased from 21.1 ± 10.1% before fixation to 28.7 ± 9.7% after processing (p ≤ 0.05). There was no statistical difference in
the percent luminal cross-sectional area of the segments with mild compared with segments with moderate to severe disease (36.2 ± 7 versus 28.7 ± 9.7%, p = NS) post-fixation.

Thus, in the fresh state, the luminal areas were different in the vessels with minimal versus the moderate to severe disease (47.6 ± 8.5 versus 21.1 ± 10.1%, p ≤ 0.05), but after fixation and processing there was no difference (36.2 ± 7 versus 28.7 ± 9.7%, p = NS).

Discussion

Discrepancy between angiography and pathologic findings. Several reasons have been cited for the discrepancy between the results from coronary arteriography and the necropsy evaluation (4–19). These factors include the technical limitations of arteriography (which may be the result of a slow injection of contrast medium, poor filling of the vessel, problems with X-ray resolution and photographic processing), errors resulting from foreshortening or overlap of vessels, long arterial segments with disease, the diffuseness of the atherosclerosis resulting in an arteriogram.

Figure 2. This schematic figure (not drawn precisely to scale) demonstrates the observed dimensional changes in human coronary arteries after fixation and processing. In minimally diseased segments from the prefixation state to post-fixation, there is mild swelling (p = NS) of the wall, with an increase in wall area and a mild increase in total vessel area, with a resulting decrease in cross-sectional luminal area. With shrinkage associated with processing, there is a further disproportionate reduction in the cross-sectional luminal area. In moderately to severely diseased segments, there is no change in dimensions with fixation. There is a decrease in wall and total area with processing without a change in luminal area. As a result, the percent luminal cross-sectional area increases after processing.

Figure 1. The effects of fixation and processing on human coronary artery dimensions. In vessels with minimal atherosclerosis, total (cross-sectional) area and luminal area diminish after processing, whereas wall area does not change. In vessels with moderate to severe atherosclerotic narrowing, total area and wall area decrease; however, luminal area remains unchanged after processing. Brackets indicate mean values ± standard deviation.
that underestimates the extent of disease and the presence of eccentric lesions or lesions that are recanalized. While there are certainly technical and anatomic limitations to coronary arteriography, there also appears to be significant limitations to the methodology applied to postmortem morphologic evaluation of the severity of coronary artery narrowing.

**Effects of fixation and tissue processing.** This study of coronary artery segments with both mild and moderate to severe atherosclerotic changes demonstrates that fixation and processing alter the total cross-sectional, wall and luminal areas of coronary artery segments. Further, different dimensional changes occur in coronary segments with different degrees of atherosclerosis. As a consequence of these differential changes, the percent luminal cross-sectional area of eccentric lesions or lesions that are recanalized. While limitations to the methodology applied to postmortem morphologic evaluation of the severity of coronary artery narrowing.

**Role of intramural distending pressure.** In this study, we did not evaluate the effect of nondistension on the morphologic assessment of arterial narrowing. In contrast to the in vivo state, at autopsy the intraluminal coronary artery pressure is zero. Some investigators use perfusion fixation to distend the coronary arteries before morphologic study (5,28); many others, however, examine nonperfused vessels (6–9,11,14,16,21–23,28–30). Wolinsky and Glagov (33) documented the limitation of studying dimensions of vessels that do not undergo perfusion fixation. They demonstrated in rabbit aortas that radius increased and wall thickness decreased as intraluminal distending pressure increased from 5 to 80 mm Hg. From 80 to 200 mm Hg, there was little change in vessel dimensions. All coronary arteries, however, must undergo fixation and processing before histologic assessment of the extent of coronary luminal narrowing. Therefore, in this study, we chose to examine only the effects of tissue fixation and processing on vessel wall thickness and luminal cross-sectional area.

**Role of fixation and processing.** In 1941, Stowell (23) demonstrated that formalin fixation of rabbit renal cortex resulted in swelling and that routine processing (dehydration in alcohol, clearing in xylene and embedding in paraffin) lead to tissue shrinkage. These findings have been confirmed by others (34–36). It has been shown that the concentration of formalin affects the amount of tissue swelling and that the addition of macromolecules to the formalin may prevent tissue swelling (34). Similarly, the stages at which tissue shrinkage occurs has been determined to be during dehydration with alcohols, clearing with xylene and embedding in paraffin (35). In addition, Falk (36) reported that the lumen of coronary arteries decreases with processing. In our study, it was also found that differential changes occur during fixation and processing of coronary vessels depending on the extent of coronary atherosclerosis.

**Implications of study.** Recent diagnostic advances, such as digital angiography (37), measurement of coronary artery gradients (38) and quantitative angiography (39), as well as the development of the new therapeutic modalities of intracoronary thrombolysis and coronary angioplasty underscore the need for accurate standards that can be used to validate techniques for assessing coronary artery luminal dimensions. Our study of nondistended human coronary artery segments with both mild and moderate to severe atherosclerotic plaques suggests that histologic quantification of coronary artery dimensions (as currently routinely performed) may not accurately reflect the degree of luminal narrowing because of disproportionate artifactual changes in luminal cross-sectional areas relative to the total vessel size which occur with fixation and processing of tissue. The degree to which fixation of vessels in distension or other alterations in tissue processing will compensate for the observed artifactual changes remains to be determined.

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**References**

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