Topographic Study of Sudanophilic Lesions in Cholesterol-Fed Minipigs by Image Analysis

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A new morphometric technique using image analysis has been developed to express the topographic distribution of atherosclerotic lesions in unambiguous statistical terms. Computer-stored images of opened Sudan IV-stained aortas and iliac and coronary arteries from hypercholesterolemic minipigs (n = 39) were used in this study. The image processing methods included transformation of the data to standard templates, automated image segmentation, and creation of probability-of-occurrence maps. These maps have shown that sudanophilic lesions are localized with a characteristic topography along the aortas and iliac and coronary arteries. Areas of high probability are associated with the entrance regions of vessels and the lateral leading edges of the major flow dividers. Regions immediately distal to large branches were found to be areas of low probability. Despite the association of areas of sudanophilia with entrance regions and branch points, a major portion of sudanophilic lesions was not associated with any orifice region (e.g., ductus scar, dorsolateral surface of abdominal aorta, and ventral surface of terminal aorta). The present study provides the necessary information for the development of a rational sampling strategy for the experimental study of the distribution of localizing factors (e.g., hemodynamic, biochemical, cellular, mass transport, histological) and their relationships to putative atherogenic mechanisms. (Arteriosclerosis 5:415-426, September/October 1985)

The lesions of atherosclerosis appear to occur more frequently at certain sites along the arterial tree than at others.1-6 Efforts to pursue the inferences of these important topographic observations have been hampered by lack of the requisite measuring and analytic methodologies to express such information in unambiguous statistical terms. The present report focuses on the development of a new methodology that uses image analysis to define the spatial occurrence of sudanophilic lesions and to facilitate the correlation of disease patterns with local structure, biochemistry, and fluid mechanics.

Briefly, computer-stored digital images of opened Sudan IV-stained arteries were divided into a number of triangular subsections, with the vertices of the triangles defined by anatomical landmarks, such as ostia. With the use of a simple linear transformation, the data were transformed to a (x-y) coordinate system representing a standard template of the opened arterial tree, thereby removing anatomical variation among individual vessel specimens. Various algorithms were developed to operate on the transformed image data to produce probability-of-occurrence maps of arterial sudanophilic lesions for the specified population. The details of this approach and its application to the topographic study of sudanophilic lesions in a population of cholesterol-fed minipigs constitute the body of this report.

Methods

Experimental Animals

Atherosclerosis was induced in 39 adult minipigs (17 males, 22 females) (Sinclair Research Farms, Columbia, Missouri) by feeding a cholesterol-supplemented diet for 9 months. The diet consisted of a basic semisynthetic chow to which was added 15%...
fat and varying amounts of crystalline cholesterol up to 1.5% by weight.7 In addition, eight minipigs on a normal, basal hog chow diet were used as controls. Each experimental animal was housed individually and fed 2 lbs of the diet per day. Mean serum cholesterol over the 9-month period was 351 mg/dl ± 29 (SEM). The detailed dietary, biochemical, and pathological data for these animals will be presented elsewhere. All procedures used in this study were in accordance with institutional guidelines concerning the use of experimental animals.

Specimen Preparation

Necropsy Protocol

A systemized protocol was developed for necropsy, vessel collection, and opening of all major vessels. At the end of the experimental period (9 months), the minipigs were anesthetized with ketamine (40 mg/kg body weight) via intravenous infusion and were necropsied. The vascular system was removed as a single unit, including the heart, thoracic and abdominal aortas, major portions of the right and left carotid arteries, right and left subclavian, celiac, hepatic, splenic, superior mesenteric, right and left renal, right coronary, left anterior descending and circumflex coronary, right and left internal iliac, right and left iliofemoral, and pulmonary arteries. Of these vessels, the upper, middle, and terminal aortas (including the right and left internal iliac arteries); the right and left external iliac arteries; and the right, left circumflex, and left anterior descending coronary arteries were analyzed in this study.

Standardized Opening of Vessels

Upper Aorta. The upper aorta was defined as the segment of aorta from the aortic valve to the level of the eighth intercostal ostia. The upper aorta (Figure 1) was opened longitudinally from the midportion of the noncoronary sinus of Valsalva and was cut posteriorly and distally so that the free-cut edge was slightly dorsolateral to the origin of the brachiocephalic and left subclavian vessel. This cut was continued along the dorsal aspect of the descending aorta so that the origins of the intercostal ostia were to the right of the free-cut edge when the vessel was opened and pinned flat to reveal the endothelial surface as shown in Figure 1.

Middle Aorta. The middle aorta was defined as that segment of the descending aorta from the origin of the eighth intercostal ostia to the level of the origin of the fourth lumbar artery. This segment was opened longitudinally along the mid-dorsal aspect so...
that the opened, free edge occurred slightly to the left of the line defined by the origin of the intercostal and lumbar arteries. The middle aortic segment (Figure 2) includes the origin of the celiac, superior mesenteric, and right and left renal arteries.

**Terminal Aorta.** The terminal aorta was defined as that portion of the descending abdominal aorta and internal iliac arteries which is distal to the fourth lumbar artery and proceeds for a distance of approximately 6 cm into both the right and left internal iliac arteries. The terminal aortic segment was opened along the dorsal aspect. The opened free-cut edge was slightly to the left of a line defined by the fourth, fifth, and sixth lumbar ostia and then divided so as to cut the right and left internal iliac arteries along their midposterior aspects. The terminal aortic segment (Figure 3) includes the orifices of the right and left iliac, and inferior mesenteric arteries.

**External Iliac Arteries.** The right and left external iliac arterial segments were defined as the vessel segment between the origin of the iliac artery at the aorta and the occurrence of the anterior femoral artery. The iliac segments were opened along the mid-posterior aspect (Figure 3) and include the origin of the circumflex iliac, deep femoral, and the anterior femoral arteries.

**Coronary Arteries.** The right coronary arterial segment (approximately 10 cm in length) was defined as the vessel segment originating at the sinus of Valsalva and proceeding distally to a point at which the circumference of the vessel was less than 4 mm. The left anterior descending and circumflex coronary arteries were defined similarly. The coronary arteries (Figure 4) were opened longitudinally along their mid-pericardial aspect so that the perforating intramyocardial vessels occurred on the midaxial portion of the opened arterial segment.

**Mounting and Staining of Arterial Segments**

The arterial specimens were rinsed with physiological saline, dissected free of excess adventitial tissue, stretched to approximate their in vivo dimensions, stapled at the cut peripheries to vinyl-covered corkboards, and fixed in 10% buffered formalin for 24 hours.
hours. The segments were then rinsed briefly (15 to 30 seconds) with 70% ethanol and placed in Sudan IV dye (5 g Sudan IV in 500 ml acetone and 500 ml 70% ethanol) for 15 minutes. After this, the tissues were placed in 70% ethanol for 20 minutes to remove stain from nonlipid containing areas, i.e., to “differentiate” the staining. Each board was then photographed under saline in a uniformly illuminated field with 35 mm Ektachrome 160 film used for subsequent image processing (Figure 1 B).

### Image Analysis Techniques

The image analysis techniques that were developed include digital image capture, transformation of data to standard templates, editing, image processing for edge detection (lesion identification) and, finally, computation of probability maps.

#### Image Capture

The images of the vessels on the 35 mm slides were digitized at a resolution of 50 μ with an Optronics Revolving Drum Densitometer (Applied Physics Laboratory, Johns Hopkins University, Laurel, Maryland). In the digitization process, a gray level ranging from 1 to 256 was assigned to each point in space. A typical thoracic aorta would be represented by a data matrix that consisted of approximately 100,000 points. The scanned digital images were stored on magnetic tape.

#### Image Processing

Images were read from the magnetic tape and displayed on an Evans and Sutherland Video Frame Buffer with 256 intensity levels and a spatial resolution of 512 by 480. Diseased portions of the vessels, stained red with Sudan IV dye, appeared black or dark gray on the television monitor (Figure 1 B). The raw image was translated to a common coordinate system by using the technique of subdividing the image into triangular sections that can be transformed (Appendices A and B) into corresponding triangles on a standard template. (The standard templates were constructed from the mean location of the anatomical landmarks of the populations being studied.) Anatomical landmarks corresponding to the apices of these triangles were identified manually with a graph pen interfaced to a PDP 11/70 computer (Figure 1 B). The coordinates of the apices of the triangles in the raw images and those of the corresponding triangles of the standardized vessel template were used to compute the requisite transformation coefficients. A demonstration of this process for a single triangle is shown in Figure 5. This process was repeated for each triangle, thereby transforming the entire image to the standardized vessel template (Figure 1 C). Transformation of the entire image required approximately 10 seconds.

#### Editing

After the transformation, the image was edited to remove artifactual features, such as staples and tears, by identifying the artifact with a graph pen and removing these pixels from the image. The intensities of the deleted pixels were replaced by the mean values of the nearest neighbors. (This process is referred to as “local spatial filtering.”) Ostial shadows were removed by subtracting the average intensity field of a standard template previously generated from nondiseased arteries.

#### Threshold Detection

The edited image was separated into diseased and nondiseased areas by using an iterative algorithm for multiple threshold detection, which is described elsewhere.\(^9\) This algorithm was then compared to the average boundary gradient algorithm for image segmentation\(^9\) and was found to yield virtually identical results (unpublished observations, Laboratory of Experimental Atherosclerosis, The Ohio State University). Once the segmentation threshold was identified, the image was converted into a binary image by setting all intensity values below this threshold to one (disease) and values above this level to zero (no disease) (Figure 6). This binary image was stored for future use.

#### Quantitation of Disease

The binary image was used for two types of calculations: 1) percentage of area involvement and 2) probability-of-occurrence maps. Percentage of area involvement was calculated as the ratio of the number of pixels with the value of “1” divided by the total number of pixels in the template. The calculation provides an objective, unbiased measure of the ex-
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Figure 6. Upper aorta transformed to standard template (top); Binary image of aorta with black region representing areas of sudanophilia as determined by the segmentation algorithm.


tent of disease. Probability-of-occurrence maps were calculated by summing the stored binary images for specified arterial segments for the entire population. This summed image (the sum of 0’s and 1’s at each x and y) was then normalized by dividing the sum at each pixel location (x and y) by the number (n) of vessels in the population to obtain the desired probability-of-occurrence maps. These maps can be displayed as incidence-interval-isopleths (0 to 10%, 10% to 20%, etc.) without smoothing or interpolation (e.g., Figures 9–12) or with smoothed probability isopleths (e.g., magnified inserts Figures 9–11). Smoothed probability isopleths were created with the Surface II Graphics System by appropriate smoothing and interpolation. The probability map provides an important guide for the design of experiments to explore physical and metabolic differences between areas of high and low incidence.

Comparison with Subjective Methods

Certain aspects of the results obtained with the present image processing techniques in a blind study were compared to results obtained from the same images by using conventional subjective methods.

Comparison of Percentage of Area Involvement Estimates

Areas judged to have sudanophilia on photographs of the upper aorta from the 39 animals in the present study were outlined and then sketched onto a translucent sheet containing the standard upper aortic template. The areas of disease were blackened and photographed with high-contrast film. The resulting subjectively identified and transformed binary images were digitized as described for the original images, and the percentage of area involvement was calculated. The linear regression between these data and the percentage of involvement computed by the segmentation algorithm is presented in Figure 7 and shows a very significant correlation between the two \( r = 0.98, \ p < 0.0001, \ \text{slope} = 0.95 \). The mean values for the percentage of surface area with disease determined with the computer was 17.7% and using subjective methods was 19.8%. The mean difference between the surface area determination was 2.1% (sd ± 4.5). Five data points of the 39 cases that were examined fell outside two standard errors of the estimate of the linear regression line. These outlying data points were found to occur in cases where the observer tended to enclose large areas of diffuse punctate involvement within a single large perimeter, whereas the computer algorithm determined the numerous discrete areas of sudanophilia separately.

Comparison of Probability Maps

The probability map computed from the subjectively identified and transformed data described above was compared to the corresponding map generated by the automated computer algorithms (Figure 8). General agreement was observed between the two maps. Both illustrate that the areas of high probability of sudanophilia (50% to 75%) occurred on the lateral leading edges of the brachiocephalic flow divider and that areas of moderate probability (25% to 50%) occurred in the ascending aorta, at the ductus scar, and at the intercostal ostia. The probability values at each pixel location in the two maps were

\[
Y = 0.954 \cdot X - 1.18
\]

\[
Y = 0.965 \cdot X - 1.18
\]

\[
\chi^2 = 0.986 \quad p < 0.0001
\]

Figure 7. The correlation between percentage of surface area of the upper aorta with sudanophilic lesion determined by computer algorithm as a function of percentage of surface area with sudanophilic lesion determined manually.
correlated by linear regression and had a $r^2$ value of 0.771 ($p < 0.001$; standard error of the estimate (SEE) = 7.3, $n = 39,884$).

The significant correlation between the subjective and computer-generated probability maps reflects not only good agreement between the two methods of lesion identification, but also the agreement between the processes involved in the transformation of the image to the standard template. Given the difficulty of the latter subjective operation, the close agreement between the two methods is remarkable.

**Comparison of Segmentation Threshold**

The appropriate threshold for dividing the pictures into areas of sudanophilia and nonsudanophilia was determined both by the computer algorithm as presented previously and by adjusting the threshold control on the image analysis system to a point at which the observer believed the threshold best represented the sudanophilic lesions in the vessels. This comparison was made on the 39 vessels of the upper and middle aortas. The mean critical threshold for the upper aorta was 126 (gray level value) for the manual determination and was 125 for the computer determination. In the upper aorta, a linear regression between the two methods of threshold estimation yielded a $r^2$ value of 0.92, a $p$ value of $<0.0001$, and
a slope of 0.97. A similar comparison in the middle aorta produced a critical manual threshold of 122 and a critical computer threshold of 118 with a \( r^2 \) value of 0.92, a \( p \) value of <0.0001, and a slope of 0.93.

**Computer-Generated Probability Maps**

The contoured probability maps for the arterial segments are presented in Figures 9 through 12 and are summarized in Table 1. (Photographs used in Figures 9 through 12 were taken from a Mitsubishi display monitor that was interfaced to a Gould Deanza IP8400 image processor and a Digital Equipment VAX 11/750 computer.) For simplicity of presentation, "the probability of sudanophilic lesion occurrence" will be referred to simply as "probability," "upstream" will be referred to as "proximal," and "downstream" will be referred to as "distal." The probability will be shown to have a well-defined spatial distribution in each vessel segment. It is of interest to examine these probability patterns in more detail. On the basis of chi-squared analysis, statistically significant differences occur when the differences in the probability between two sites is of the order of 15% (i.e., 10% differences, \( p < 0.25 \); 20% differences, \( p < 0.025 \)).

**Upper Aorta**

In the upper aorta, the highest probability is associated with the Sinus of Valsalva, ductus scar, and ostia of the coronary, brachiocephalic, and intercostal arteries (Figure 9). Coronary ostia disease has a high probability completely surrounding each ostia. Disease associated with the brachiocephalic ostium has the highest probability lateral to the leading edge of the flow dividers, with the second highest probability in the proximal inflow tract (Figure 9, insert). Areas immediately distal to the brachiocephalic and left subclavian ostia have a low probability. The outer curvature of the aortic arch, the portion from which

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these larger arteries originate, has a higher probability than does the inner curvature; the probability over most of the inner curvature is less than 20%. The probability associated with the intercostal ostia is less well defined but tends to be somewhat higher lateral and proximal to the ostia. The probability in the descending thoracic aorta distal to the ductus scar lesion was generally uniform at a value of between 10% to 20%. The mean percentage of surface area with sudanophilic lesions was 18% ± 4 (SEM).

**Middle Aorta**

The highest probability in the middle aorta tends to occur on the dorsal and lateral surfaces of the distal half of the segment (abdominal portion) (Figure 10). The regions of the abdominal aorta immediately distal to the celiac and superior mesenteric ostia have a low probability. The proximal inflow tract of the celiac ostia exhibits a significant anticipatory lesion which extends several ostial diameters cephalad, whereas the inflow tract to the superior mesenteric ostia is free from sudanophilic lesions (Figure 10, insert). The regions lateral to the leading edge of the flow dividers of these vessels have a high probability of producing the so called “butterfly” lesions, similar to the high probability regions observed at the same locations at the origin of the brachiocephalic and left subclavian ostia. The portion of the middle aorta distal to the renal ostia has moderate probability of sudanophilia with only small areas of low probability. The mean percentage of surface area with sudanophilic lesions was 13% ± 2 (SEM).

**Terminal Aorta**

In the terminal aorta, the highest probability of sudanophilia occurs on the ventral surface of the vessel proximal to the origin of the right iliac (RI) and left iliac (LI) arteries (Figure 11). The areas between and lateral to the origin of the iliac ostia also have a high probability of sudanophilia. The areas immediately distal to the origin of the RI and LI arteries were relatively spared of disease (Figure 11, insert). Little sudanophilia was seen in the distal portion of the terminal aorta in the region of branching into the internal RI and LI arteries. Some sudanophilia was located proximal and lateral to the branching of the umbilical artery from the internal iliac arteries. The mean percentage of surface area with sudanophilic lesions was 15% ± 2 (SEM).

**External Iliac Arteries**

The highest probability of sudanophilia in the external iliac arteries, which are mounted on either side

![Figure 10. Probability-of-occurrence map of sudanophilia in the middle aortic segment. The banded incidence isopleths are the same as in Figure 9. The insert presents a magnified, smoothed contour map of the region at the level of celiac (C) and superior mesenteric (SM) ostia. Two intercostal ostia (I) are illustrated.](image-url)
of the terminal aorta in Figure 11, occurred on the ventral surface of the proximal portion of the vessels leading to the circumflex ostia. Additional high probability areas were associated with the branches of the deep femoral and anterior femoral arteries from the RI and LI arteries. These focal areas of high probability were also located in regions lateral to, or immediately proximal to, the origins of these vessels. The areas distal to the flow dividers were spared from any significant sudanophilic disease. The mean percentage of surface area with sudanophilic lesions in the RI and LI arteries was 9% ± 2 (SEM) and 11% ± 2 (SEM) respectively.

**Coronary Arteries**

In the coronary arteries (right, left anterior descending, and circumflex), the highest probability of sudanophilia occurred at the origins of the vessels and decreased as one proceeded distally (Figure 12). Specific areas of high probability of sudanophilia were observed at the bifurcation of the main left coronary artery and the left anterior descending and circumflex coronary arteries. The disease in the right coronary artery tended to be distributed along the length of the vessel, while that in the left coronary system was more discrete and localized at the origin of the vessels. No significant spiral distribution of lesions was observed. The mean percentage of surface area with sudanophilic lesions in the right, left anterior descending, and circumflex coronary arteries were 21% ± 4 (SEM), 22% ± 3 (SEM), and 18% ± 3 (SEM), respectively.

**Discussion**

This paper describes a new morphometric technique of image analysis that provides a general methodology for the probable description of certain structural features of interest in a set of homologous images. Although application of this new methodology in the present work has dealt with the study of sudanophilic patterns along the opened arterial trees of cholesterol-fed minipigs, the modular design of the method (i.e., image capture, transformation to a

![Figure 11](image-url). Probability-of-occurrence maps of sudanophilia in the terminal aortic segment (middle) and right (bottom) and left (top) external iliac arteries. The banded incidence isopleths are the same as in Figure 9. The insert presents a magnified smoothed contour map of the region of the terminal aortic segment proximal to the right iliac (RI), left iliac (LI), and posterior mesenteric (PM) ostia.
Figure 12. Probability-of-occurrence maps of sudanophilia in the right coronary (top), main left—left anterior descending coronary (middle) and left circumflex coronary (bottom) arteries. The banded incidence isopleths are the same as in Figure 9.

standard template, automated image segmentation of features of interest, and creation of probability-of-occurrence maps) makes this general analytic approach flexible and easily adapted to other classes of problems in which the data are captured as an image, e.g., photomicrographs, ultrasonic B scans, nuclear magnetic resonance images, and so forth. For example, this methodology has been adapted for the study of the spatial incidence of intimal lipid deposition in histological sections of coronary arteries (unpublished observations). The method of transformation of spatial data used in this study is similar to the method of biorthogonal grids that has been applied to the study of the crania and other structures.11 This methodology overcomes the limitations of the subjective and spatially limited polar coordinate technique12-13 and the time-consuming, subjective, spatially distorted, manually generated, probability-of-occurrence maps.3-5

In the present application, we generated probability-of-occurrence maps that define for the first time in unambiguous statistical terms the probability that sudanophilia will occur at any specified point along the surface of the opened arterial tree. Although some geometric distortions occur with vessel removal, opening, fixation, and staining, the methods of vessel normalization overcome much of this problem and allow the probability at a particular site to be presented with respect to fixed anatomical landmarks. These maps have shown that sudanophilic lesions are localized in a characteristic topographic distribution along the aorta and iliac and coronary arteries. The maps show that areas of high probability are frequently associated with the entrance regions of vessels (e.g., ascending aorta, origins of the RI and LI arteries, and origins of the right and left anterior descending and circumflex coronary arteries). Areas of high probability are also associated with the lateral leading edges of the flow divider at the major branches from the aorta (e.g., brachiocephalic, left subclavian, celiac and superior mesenteric arteries). The regions immediately distal to the large branch points (e.g., brachiocephalic, left subclavian, celiac, superior mesenteric, RI and LI) are shown to be areas of low probability. The regions immediately proximal to the major branch vessels present a less consistent picture with regard to the localization of sudanophilic lesions. For example, areas of relatively high probability occur proximal to the origin of the brachiocephalic, celiac, and iliac arteries. However, the regions proximal to both the left subclavian and superior mesenteric ostia are regions of low probability.

In contrast to the foregoing, a significant (if not major) portion of sudanophilic lesions was not associated with any particular orifice region. Some of the highest probabilities observed in the study were associated with the ductus scar, the dorsolateral surface of the abdominal aorta, and the ventral surface of the terminal aorta.

Although the association of the disease with specific geometric entities, such as entrance regions,
branch points, and major bends, suggests some relationship to hemodynamic events, the correlation is not sufficiently strong to point to any specific localizing hemodynamic factor, e.g., high or low shear stress. Although hemodynamic forces probably play a role in the localization of some of these lesions, this role is probably played in concert with many other important localizing biochemical, cellular, mass transport, and structural factors.

The study provides the necessary information for the development of a rational sampling strategy for experimental studies of the distributions of these localizing factors and their relationship to putative atherogenic mechanisms. Probability-of-occurrence maps, such as those that have been presented, are essential to provide the spatial information with which workers now can locate experimental regions of both high and low probability for the study of the putative factor. Accordingly, the probability-of-occurrence map provides a powerful tool to reduce the uncontrollable variables in experimental designs and to greatly strengthen the associated statistical correlations in studies of atherogenesis.

Appendix A

Image Transformation Relationships

The image of an arterial segment of interest was divided into triangular regions using anatomical landmarks as illustrated in Figure 1. Each point on each triangular region was mapped onto a corresponding point in a homologous triangular region of a common arterial template of the arterial segment so that all images could be viewed and processed in the same coordinate system. This was done by simple linear spatial transformation (Equations 7 and 8 below) of the intensity data from the original triangles to the homologous (standard) triangles. The coordinates of the apices of the triangle in the raw image \((X_a, Y_a)\), \((X_b, Y_b)\), and \((X_c, Y_c)\) and those of the corresponding triangle in the standardized coordinate system \((X'_a, Y'_a)\), \((X'_b, Y'_b)\), and \((X'_c, Y'_c)\) were used to compute the necessary transformation coefficients \((A, B, C, D, E, F)\). These transformation coefficients were calculated using the following six equations:

\[
A = X'_a B X_a C Y_a \tag{1}
\]
\[
B = X'_a X'_b C(Y'_a-Y_a) \tag{2}
\]
\[
C = \frac{(X'_c X'_a X_a)(X'_a X'_b X_b)}{(Y'_a-Y_a)(X'_a X'_b X_b)} \tag{3}
\]
\[
D = Y'_a E X_b X'_a \tag{4}
\]
\[
E = Y'_c X'_a X'_b \tag{5}
\]
\[
F = \frac{(Y'_c X'_a X'_b X'_a)(Y'_a Y'_b X'_b X'_a)}{(Y'_a Y'_b X'_b X'_a)(Y'_a Y'_b X'_b X'_a)} \tag{6}
\]

With the values of these coefficients, each point in the data triangle \((X, Y)\) may be transformed to its new coordinate system \((X', Y')\) by the following equations:

\[
X' = A + BK + CY \tag{7}
\]
\[
Y' = D + EX + FY \tag{8}
\]

This procedure was repeated for each triangle with the calculation of the six transformation coefficients for that triangular section. The \(x\) and \(y\) coordinates were altered in this transformation, while the intensity or gray level of each point in the matrix was maintained. This method of linear transformation of triangular sections is shown schematically in Figure 5.

The subdivision of the upper aortic segment into 38 triangular sections and the resulting transformation to the standard template system is illustrated in Figure 1. The standard template was determined from the mean \(x, y\) position of the anatomical landmarks (e.g., ostia, ductus scar, sinus of Valsalva, and so on) of the population being studied. The specific identification of these landmarks is presented below.

Appendix B

Definition of Landmarks

The transformation of individual vessel data to a standard template required the definition and identification of specific anatomical landmarks or fiducial points. These fiducial points were selected to create triangular subsections over the entire arterial segment. The spatial configuration of fiducial points was chosen in accordance with the need for a higher density of triangles in areas of rapidly changing probability of disease, to prevent triangle inversion during transformation, and to minimize fiducial points not associated with specific anatomical entities.

The fiducial points were determined either on the basis of specific anatomical structures or by the division of the distance between anatomical structures (e.g., halfway between two landmarks). The anatomical structures used to define these points were the midostial flow divider, the ductus scar, the commissures of the aortic valve, as well as standard points on the periphery of the opened segment. A description of the anatomical landmarks used in each arterial section is given below. It should be pointed out that this approach may be used in any structure in which specific anatomical fiducial points may be identified.

Upper Aorta

The upper aortic segment was divided into 38 triangular subsections by using 31 fiducial points (Figure 1). The major anatomical landmarks are Points 1 through 4, which define the origin of the aorta at the aortic valves; Points 6, 7, and 8, which are the distal commissures of the aortic valve leaflets; Points 11 and 16, which are the midpoints of the distal flow dividers of the brachiocephalic and the left subclavian arteries, respectively. Point 20 is the proximal edge of the ductus scar, and Points 27, 28, and 31 are the midpoints of the distal flow divider of the 6th, 7th, and 8th intercostal arteries respectively.

Middle Aorta

The middle aortic segment was divided into 25 triangular sections as defined by 23 fiducial points (Figure 2). Points 2, 3, 6, 7, and 10 were defined by the midpoints of the distal flow dividers of the eighth through 12th intercostal arteries. Points 11 and 14 were the midpoints of the distal flow dividers of the celiac and superior mesenteric arteries,
respectively. Points 17 and 18 were defined as the midpoints of the distal flow dividers of the right and left renal arteries respectively.

**Terminal Aorta**

The terminal aortic segment was divided into 26 triangular sections as defined by 25 fiducial points (Figure 3). Points 3, 4, and 9 were defined by the midpoints of the distal flow dividers of the fourth, fifth, and sixth lumbar arteries. Points 6, 7, and 8 were the midpoints of the distal flow divider of the posterior (inferior) mesenteric, LI and RI arteries, respectively. Point 12 was the apex of the bifurcation of the internal RI and LI arteries. Points 14 and 15 and Points 20 and 21 form a line at the level of the left and right umbilical arteries respectively.

**External Iliac Arteries**

The RI and LI arterial segments were divided into six triangular sections by using eight fiducial points (Figure 3). Landmarks 3 and 4 were placed at the level of the origin of the circumflex iliac artery. Landmarks 5 and 6 were placed at a level of the origin of the deep femoral artery. Landmarks 7 and 8 were placed at levels of the anterior femoral arteries.

**Coronary Arteries**

The right coronary arterial segment was subdivided into eight triangular sections using 10 fiducial points (Figure 4). Landmarks 1 and 2 were at the origin of the right coronary artery at the aorta, and Landmarks 5 and 10 were placed at the distal end of the left anterior descending coronary artery. The left circumflex coronary artery was divided into 10 triangular segments by using 12 fiducial points (Figure 4). Landmarks 1 and 2 were placed at the origin of the left coronary artery at the aorta, and Landmark 4 was at the level of the origin of the circumflex coronary artery. Points 11 and 12 were placed at the distal end of the left anterior descending coronary artery. The left circumflex coronary artery was divided into eight triangular sections by using 10 fiducial points (Figure 4). Landmarks 1 and 2 were placed at the origin of the left circumflex coronary artery from the main left coronary artery. Points 9 and 10 were placed at the distal end of the left circumflex arterial segment.

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