Morphometry Error Due to Non-Perpendicular Section of Microvessel Characterized by Two-Dimensional Isotropic Arrangement

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Formulas for calculation of the section non-perpendicularity error in estimation of the perimeter and cross-sectional area of microvessel profiles are proposed for the two-dimensional isotropic approximation. A relationship was established between the bias and random error associated with non-perpendicularity corresponding to θ_{min} for microvessel profiles meeting the condition $\theta_{min} \leq B/C \leq 1$, where B and C are the minimum and maximum axes of the microvessel profile, respectively.

Key words: *morphometry; microvessels; measurement error*

Ultrastructural morphometry is widely used in the study of microvessels (MV), the primary geometric characteristics being the basement membrane perimeter (P_{MV}) and cross-section area (S_{MV}) of MV. However, true values of these parameters are usually overestimated due to non-perpendicular section orientation.

There are some approaches minimizing the this section non-perpendicular error (SNE) [1-4]. The most widespread method is based on non-perpendicularity error, i.e. the minimum axial ratio (θ_{min}). According to this method, measurements are performed only for MV profiles, in which the minimum (B) and maximum (C) axes meet the condition $\theta_{min} \leq B/C \leq 1$. Other profiles are rejected as non-representative.

The accuracy of the morphometric analysis made by this method largely depends on factor θ_{min}. There is no particular guidance in the literature how to choose the optimal θ_{min}. We calculated SNE for S_{MV} and P_{MV} measurements at different θ_{min} under conditions of two-dimensional isotropic arrangement of vessels. In this approximation, MV are randomly oriented on a plane or a curved surface. Two-dimensional isotropic arrangement is a good approximation for MV laying in superficial layers of the derma, in the cornea, peritoneum, pleura, arachnoid membrane, *etc.* Sections are cut at the random angles perpendicularly to the plane in which MV are arranged [5].

Estimation of SNE for S_{MV} measurement. Let us approximate a short segment of MV basement membrane by a cylinder with radius B. Assume also that the radius of longitudinal curvature and characteristic length of MV diameter variations far surpass B. Then the ellipse area on the section plane inclined by angle α (Fig. 1) is:

$$S_{MV} = \pi BC = \pi B^2 \times C/B = \pi B^2/\cos\alpha.$$

Theoretically, α varies from $-\pi/2$ to $\pi/2$ and its values are distributed uniformly over this interval. The condition $\theta_{min} \leq B/C \leq 1$ imposes a restriction on possible α deviations. As $\cos\alpha_{max} = \theta_{min}$, α can vary in the range $[-\arccos\theta_{min}; \arccos\theta_{min}]$. Thus, the admissible interval of α deviations is $2\arccos\theta_{min}$, and the density of MV distribution over angle α [D(α)] and the portion of MV profiles meeting the above condition (Q) can be determined from the formulas:

$$D(\alpha) = 1/2 \arccos\theta_{min},$$
$$Q = 2 \arccos \theta_{min}/\pi.$$

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The mean area and the mean squared area for profiles which complying with the requirement $\theta_{min} \leq B/C \leq 1$ are defined by the formulas:

$$\bar{S}_{MV} = \int_{-\arccos\theta_{min}}^{\arccos\theta_{min}} S_{MV}(\alpha) D(\alpha) d\alpha = D(\alpha) \times \int_{-\arccos\theta_{min}}^{\arccos\theta_{min}} \frac{\pi B^2}{\cos\alpha} d\alpha = \frac{\pi B^2}{\arccos\theta_{min}} \times \int_{0}^{\arccos\theta_{min}} \frac{1}{\cos\alpha} d\alpha \quad (1),$$

$$M(S_{MV}^2) = D(\alpha) \times \int_{-\arccos\theta_{min}}^{\arccos\theta_{min}} M(S_{MV}^2(\alpha)) d\alpha = \frac{\pi^2 B^4}{\arccos\theta_{min}} \times \int_{0}^{\arccos\theta_{min}} \frac{1}{\cos^2\alpha} d\alpha \quad (2),$$

As the real cross-sectional area of MV is πB^2, one can calculate the correction coefficient, by which the mean value of S_{MV} obtained from morphometrical measurements must be divided in order to compensate the bias component of SNE. The values of $K(S_{MV})$ for different θ_{min} are given in Table 1.

Using formulas (**1**) and (**2**), we determined the standard deviation for S_{MV}:

$$Var(S_{MV}) = M(S_{MV}^2) - (\bar{S}_{MV})^2 \quad (3),$$

The variation coefficient characterizing the random component of SNE can be calculated as follows:

$$CV(S_{MV}) = \frac{\sqrt{Var(S_{MV})}}{\bar{S}_{MV}}.$$

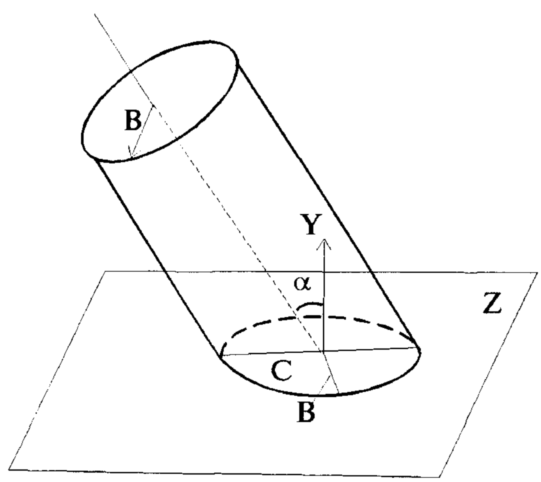

Fig. 1. Scheme of a microvessel (MV) sectioned with a nonperpendicular plane Z. The true MV radius is equal to B. The profile of MV on the plane Z is an ellipse with the small axis B and great axis C. α is the angle between the microvessel axis and perpendicular Y to the section plane. The cylinder approximation was made for a short fragment of MV (much shorter than B). In the Figure this condition was violated for the purposes of clearness.

The values of the random error due to non-perpendicular section calculated for single S_{MV} measurement are given in Table 1. For estimation of the standard error for a group of measurements, $CV(S_{MV})$ should be divided by a factor of $\sqrt{n}$, where n is the number of analyzed MV profiles.

Estimation of SNE for P_{MV} measurement. Perimeter of an elliptic profile is calculated by the formula:

$$P_{MV} = B \int_{0}^{2\pi} \sqrt{\cos^2\varphi + \left(\frac{C}{B}\sin\varphi\right)^2} d\varphi,$$

where φ is the angle corresponding to a certain point on the elliptic curve with respect to the major axis. Let B=1 and α is distributed uniformly over the interval [$-\arccos\theta_{min}$; $\arccos\theta_{min}$]. Then the formula for the mean perimeter of MV is:

$$\bar{P}_{MV} = \int_{-\arccos\theta_{min}}^{\arccos\theta_{min}} P_{MC}(\alpha) D(\alpha) d\alpha = \frac{1}{\arccos\theta_{min}} \times \int_{0}^{\arccos\theta_{min}} \int_{0}^{2\pi} \left(\cos^2\varphi + \frac{1}{\cos^2\alpha} \times \sin^2\varphi\right)^{\frac{1}{2}} d\varphi d\alpha,$$

and for the mean squared P_{MV} is:

$$M(P_{MV}^2) = \int_{-\arccos\theta_{min}}^{\arccos\theta_{min}} M(P_{MV}^2(\alpha)) D(\alpha) d\alpha = \frac{1}{\arccos\theta_{min}} \times \int_{0}^{\arccos\theta_{min}} \int_{0}^{2\pi} \left(\cos^2\varphi + \frac{1}{\cos^2\alpha} \times \sin^2\varphi\right) d\varphi d\alpha.$$

As the real perimeter of MV profile is $2\pi B$ (the perimeter of radius B), one can calculate the correction coefficient, by which the experimentally measured P_{MV} must be divided in order to compensate for the SNE bias. The values of $K(P_{MV})$ corresponding to different θ_{min} are presented in Table 1.

Similar to formula (**3**), standard deviation for P_{MV} is calculated by the formula:

$$Var(P_{MV}) = M(P_{MV}^2) - (\bar{P}_{MV})^2.$$

Thus the random error of a single P_{MV} measurement due to section non-perpendicularity is:

$$CV(P_{MV}) = \frac{\sqrt{Var(P_{MV})}}{\bar{P}_{MV}}.$$

TABLE 1. The Biased and Random Components of Section Non-Perpendicularity Error for S_{MV} and P_{MV} Measurements at Certain θ_{min} and Corresponding Portion (Q) of MV

θ_{min}	Q	$K(S_{MV})$	$CV(S_{MV})$, %	$K(P_{MV})$	$CV(P_{MV})$, %
0.01	0.994	3.380	208.99	2.434	182.33
0.10	0.936	2.035	79.54	1.592	60.71
0.20	0.872	1.674	52.59	1.374	36.88
0.30	0.806	1.480	38.29	1.260	25.16
0.40	0.738	1.352	28.64	1.187	17.82
0.50	0.667	1.258	21.40	1.135	12.71
0.60	0.590	1.185	15.62	1.096	8.90
0.65	0.550	1.154	13.12	1.079	7.33
0.70	0.506	1.126	10.82	1.065	5.94
0.75	0.460	1.100	8.70	1.051	4.69
0.80	0.410	1.077	6.72	1.039	3.57
0.85	0.353	1.056	4.88	1.028	2.55
0.90	0.287	1.036	3.16	1.018	1.62
0.92	0.256	1.028	2.50	1.014	1.26
0.94	0.222	1.021	1.85	1.010	0.94
0.96	0.181	1.014	1.22	1.007	0.62
0.98	0.128	1.007	0.60	1.003	0.30

The calculated random error for a single P_{MV} measurement is presented in Table 1. The standard error for a group of measurements can be calculated by dividing $CV(S_{MV})$ by a factor of $\sqrt{n}$, where n is the number of measurements.

Example. Let us estimate the bias and random components of SNE for measurement of the mean cross-section area and the mean perimeter of peritoneal microvessels at θ_{min}=0.75 and n=25 (25 profiles). Table 1 shows that the portion Q of MV complying to the non-perpendicularity limit constitutes 46%, and that the measured mean values of S_{MV} and P_{MV} surpass the real values by factors of 1.100 and 1.051, respectively. After dividing the $CV(S_{MV})$ and $CV(P_{MV})$ by 25 we determine the contribution of SNE to the standard errors of mean S_{MV} and P_{MV} values (1.740 and 0.938%, respectively).

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