ACTA STEREOL 1983; 2: 380 - 383
STEREOLOGY AND MORPHOMETRY IN PATHOLOGY
Proc 2nd Symp Morphometry in Morphol Diagnosis
Collan Y. et al., eds.
Kuopio University Press, Kuopio 1983

MORPHOMETRY OF KIDNEY BIOPSY: NUMBER OF GLOMERULI AND ACCURACY OF MORPHOMETRIC MEASUREMENTS

Yrjö Collan, Esa Jantunen, Jouko Karhunen, and Timo Romppanen

Department of Pathology, University of Kuopio,
P.O.B. 6, 70211 Kuopio 21, Finland

ABSTRACT

Five kidney biopsies with diffuse changes containing 16 - 48 glomeruli were analysed morphometrically. After excluding profiles with a diameter smaller than 60% of the diameter of the largest glomerular tuft in the biopsy the coefficient of variation between glomeruli was 12 - 19 per cent for the following parameters: number of nuclei per glomerular area, surface density of glomerular BM, and the volume density of capillaries. The volume density of mesangium showed a CV of 27%. The biopsy should contain 1-4 glomerular profiles if one would like to keep the relative standard error ($CV/\sqrt{n}$) of these parameters under 15 %. The estimates were tested by letting a computer to sample a glomerular data file in samples of 1 - 10 glomeruli. The probabilities for deviations larger than 10 - 20% of the unbiased value were calculated. Cellular proliferation (nuclear density) in the glomeruli can be estimated in biopsies with 2 glomeruli with a probability of 12% for a deviation of more than 20%. In samples with 6 glomeruli no nuclear density figures deviated more than 20% of the unbiased estimate.

INTRODUCTION

Pathologists interprete findings on histological sections. At times there is lots of tissue available and under those circumstances the sample is sufficient for interpretation. At times the sample is very small, often too small for interpretation. When looking at the section pathologists make intuitional decisions about what sample is too small and what sample is sufficient for diagnosis. In kidney biopsy diagnostics one could say that the sample is large enough for reliable interpretation if the biopsy contains 10 glomeruli. Such recommendation is based on experience in reporting kidney biopsy findings but is also an intuitional estimate. But could the adequate sample size be estimated through calculations? In this paper we show how it is possible to estimate the adequate sample size by applying morphometric methods and that the accuracy linked with each sample size can also be estimated (Romppanen et al. 1982).

Table 1. The number of glomeruli investigated in the biopsies after glomeruli with a diameter under an exclusion limit (0.4, 0.5, 0.6, or 0.7 times the diameter (D) of the largest glomerular profile) have been excluded from calculations.

Biopsy	No limit	0.4 D	0.5 D	0.6 D	0.7 D
A	34	30	24	21	8
B	18	16	14	14	12
C	30	26	24	18	8
D	16	15	14	9	
E	48	44	38	34	28

Table 2. Mean coefficient of variation (in percent) of selected morphometric parameters of individual glomeruli. Values are given separately for different exclusion limits.

Morphometric parameter	No limit	0.4 D	0.5 D	0.6 D
Volume fraction of mesangium	30.7	29.0	28.1	26.9
Number of nuclei per glomerular profile	44.6	36.0	28.3	23.8
Number of nuclei per glomerular area	17.6	16.7	16.6	15.5
Surface density of the glomerular BM	23.2	18.3	17.7	17.0

MATERIAL AND METHODS

Five kidney biopsies with 16 - 48 glomeruli in sections were analysed with a morphometric method (Romppanen and Collan 1981, Collan et al. 1982). The biopsies were from cases with diffuse glomerular diseases (minimal change, mesangioproliferative, and membranous glomerulonephritis). First we wanted to estimate whether the results were more accurate if a size limit was applied. We first estimated 11 different glomerular parameters and based the estimates on all glomerular profiles in the section. We then estimated the variation of results between individual glomerular profiles. Corresponding calculations were made after exclusion of glomerular profiles with a diameter smaller than 0.4, 0.5, 0.6, 0.7, 0.8 or 0.9 times the diameter of the largest glomerular profile. The variation was estimated by the coefficient of variation (CV = SD/mean x 100). Thereafter it was estimated how many glomeruli were necessary for the analysis when the relative standard error of measurements (CV/ $\sqrt{n}$) was kept under a desired predetermined limit.

The above calculations were tested by letting our computer to sample the file of the biopsy containing 48 glomerular profiles, the sample varying from 1 to 10 glomeruli. The proportion of these samples deviating more than 10, 15 or 20% of the unbiased value (which was based on all glomerular profiles in the biopsy) was estimated.

RESULTS

Table 1 shows how the application of an exclusion limit affects the

Table 3. The number of glomeruli at the relative standard error level of 10 or 15 percent.

Exclusion limit>	No limit		0.4 D		0.5 D		0.6 D	
	10%	15%	10%	15%	10%	15%	10%	15%
Volume fraction of capillaries	1	1	1	1	1	1	1	1
Volume fraction of mesangium	10	5	9	4	8	4	8	4
Number of nuclei per profile	20	9	14	7	9	4	6	3
No. of nuclei per profile area	4	2	3	2	3	2	3	2
Surface density of glomerular BM	6	3	4	3	4	2	3	2

number of glomeruli contributing to the results. In the five biopsies analysed, more than 50% of the glomeruli were included at the limit of 0.6 D. The limit 0.7 D already caused a dramatic drop in the number of glomeruli. It seems reasonable to apply an exclusion limit smaller than 0.7 D.

Table 2 shows that the coefficient of variation is larger with smaller exclusion limits. It seems reasonable to choose the exclusion limit which shows the smallest variation, in this case 0.6 D.

Table 3 shows the number of glomeruli which was necessary for a relative standard error of 10 or 15 per cent. With the exclusion limit of 0.6 D 1 - 4 glomeruli are necessary for reaching the relative standard error of 15% or less on the listed parameters.

Table 4 then shows the proportion of computer samples which deviated (at the exclusion limit of 0.6 D) more than 10, 15 or 20 per cent of the unbiased value. The results vary with the size of the sample. If a deviation of 15% is taken as a limit and 5 per cent of samples are allowed to deviate above that limit, we can give the number of glomeruli necessary for certain morphometric measurements. Volume fraction of capillaries can be estimated from 3 glomeruli, volume fraction of mesangium from 8 glomeruli, the number of nuclei per profile area from 6 glomeruli and the surface density of glomerular BM from 5 glomeruli which are larger than the exclusion limit (here 0.6 D).

DISCUSSION

The number of points counted determine the accuracy and variation of measurements in point counting. Because glomerular mesangium has a reasonably small volume fraction the variation is larger than in capillary volume fraction estimation. The variation is not only dependent on this aspect, however. Also the variation between glomeruli need be considered. We could show that by applying an exclusion limit variation can be made smaller. This suggests that results can be achieved which are independent of the variation caused by the levels of sectioning. No doubt this principle should be applied in diagnostic morphometry of kidney biopsies.

Could we apply the results for traditional histopathology diagnostics? In case of absolute figures we might. One example is the number of nuclei per glomerular profile area. Our study suggests that a sample of 6 glomeruli (after the glomeruli smaller than 0.6 D are excluded) gives a deviation of

Table 4. The proportion (in percent) of computer samples of glomeruli deviating above 10, 15, or 20 percent of the unbiased value. Separate values are given for sample sizes of 1-8 glomeruli. Exclusion limit of 0.6 D was applied.

Parameter tested	Deviation from unbiased value	Size of the sample (number of glomeruli)					
		1	2	3	4	6	8
Volume fraction of capillaries	10	36.8+-14.5	16.1+- 3.3	7.2+- 4.4	5.7+- 9.8	0.5+- 1.0	0
	15	17.0+- 5.1	7.5+- 7.9	0.7+- 1.6	0	0	0
	20	9.2+- 8.7	1.8+- 2.9	0	0	0	0
Volume fraction of mesangium	10	75.9+- 8.6	62.8+- 7.3	55.5+-10.9	45.0+-16.3	28.6+-17.8	20.0+-13.9
	15	66.3+- 4.0	49.1+- 9.2	40.5+-13.9	27.5+- 8.9	17.2+-10.8	4.6+- 8.5
	20	56.4+-13.0	35.4+-10.0	25.7+- 8.9	19.4+- 8.9	5.9+- 3.9	2.2+- 3.3
Number of nuclei per profile area	10	54.0+- 6.3	45.5+- 8.5	31.7+-10.7	26.8+-11.6	17.5+-10.9	3.4+- 4.8
	15	39.4+- 6.8	23.8+- 8.6	17.6+- 7.9	9.2+- 7.0	3.1+- 3.0	0
	20	25.9+- 5.1	11.9+- 8.7	9.3+- 6.1	0.7+- 1.5	0	0
Surface density of glomerular BM	10	60.2+- 7.5	45.7+-15.3	28.5+-25.5	20.9+-19.2	9.6+-12.8	4.4+- 6.5
	15	44.9+- 3.3	25.3+-12.4	12.5+-12.1	5.5+- 8.1	2.5+- 4.1	0.7+- 1.7
	20	30.7+- 5.5	14.5+- 5.7	6.3+- 6.6	1.1+- 1.5	0.5+- 1.0	0

less than 15% of the unbiased value in less than 5% of samples (of 6 glomeruli). Such sample size could be considered reliable also in diagnostic histopathology.

REFERENCES

Romppanen T, Karhunen J, Jantunen E, Collan Y: Interpretation of kidney biopsy: representative number of glomeruli for evaluation of various glomerular parameters. Abstract. Int Soc Stereol Meeting, Sheffield 1982

Romppanen T, Collan Y: Morphometrical method for analysis of kidney biopsies in diagnostic histopathology. Contemporary Stereology, Proc 3rd Eur Symp Stereol, Ljubljana. Stereol Iugosl, Suppl.1 1981; 3: 435 - 442

Collan Y, Romppanen T, Seppä A, Karhunen J, Jantunen E: Interobserver variation in morphometric kidney biopsy interpretation. In: Morphometry in morphological diagnosis. Collan Y, Romppanen T (eds.). Pp. 155 - 162. Kuopio University Press 1982