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A multivariate morphometric analysis of the glomeruli in the normal and pathologically changed human kidney

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Summary. Morphometric studies have shown several significant differences in certain features of the kidney of normal individuals, those with minimal changes disease (MC), mesangioproliferative glomerulonephritis (MPGN) or diabetic glomerulosclerosis (DGS). However, there is a considerable overlap. As this could prevent the application of morphometry in diagnostic kidney pathology, we have applied multivariate analysis.

In total, material from 89 different patients was studied (13 normals, 30 MC's, 13 MPGN's and 33 DGS patients).

A two-step approach has been used because of the pattern of deviations between the different groups. First, the normals and MC's as one group were distinguished from the MPGN's and DGS's as another. With 6 features 90.5% of all the patients were correctly classified (sensitivity 95.6%, specificity 84.6%).

For the distinction between the normals and MC's, three features (mesangial cell percentage, total glomerular cells and endothelial cell percentage), was the best discriminating combination. Using 0.75 as a numerical classification probability threshold (for doubtful or inconclusive) none of the minimal changes were misclassified, and only two of the normal patients (16%). Four of the normals were inconclusive (33%) as were four of the minimal changes (14%). This result should be considered with the initial selection criteria in mind (no observable histological changes after careful subjective evaluation, in the presence of a clinical nephrotic syndrome in the minimal change patients). This emphasizes the possibility of morphometry to *detect* differences, which escape qualitative observations.

An even better discrimination can be obtained between the MPGN's and DGS's. Only one of the MPGN's was misclassified, but in contrast

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to all the other cases, the numerical classification probability of this patient was low (0.65 in comparison with 0.79 to 1.0).

It is concluded that in kidney pathology, multivariate analysis of morphometric data gives a better discrimination between different groups than single variate analysis.

Key words: Kidney – Morphometry – Multivariate analysis

Introduction

Pathological changes in the kidney are usually expressed in a subjective way. In contrast, morphometry has been used to asses objectively changes in both normal and pathologically changed human kidneys (Wehner 1968 and 1974; Wehner et al. 1980; 1983). These morphometric studies showed that there are significant differences in the morphometric features of the kidnies of normal individuals, in minimal change disease, mesangioproliferative glomerulo-nephritis, and diabetic glomerulosclerosis. Although the differences between these groups were significant, there was a considerable overlap in all the features investigated. This could prevent the application of morphometry in diagnostic kidney pathology.

With multivariate analysis (Cooley and Lohnes 1971) a better discrimination of two or more groups can be obtained. The essential point in this technique is the *simultaneous* consideration of different features in order to come to a certain diagnosis. For the discrimination of different groups, it has been succesfully applied to morphometric features of human endometrium (Baak et al. 1978, 1981a, and 1982a), ovary (Baak et al. 1981c), breast cancer (Baak et al. 1981b, and 1982b), stomach (Boon et al. 1982a), the thyroid (Boon et al. 1982b) and other pathological deviations in different organs.

We have therefore investigated whether multivariate analysis of morphometric features of the human kidney enhances their discriminating power.

Materials and methods

The primary morphometric study has been described by Wehner (1974). Briefly, kidney material was available from 13 normal individuals (N) (i.e. sudden explained death, the cause of which did not interfere with the kidney features studied), 30 minimal changes (MC), 13 mesangioproliferative glomerulonephritis (MPGN) and 33 diabetic glomerulosclerosis patients (DGS). The diagnosis "normal" was established if the material was obtained within a few hours after death, and if careful subjective investigation did not reveal any sign of abnormality, using very strict criteria. The diagnosis "minimal changes" was made in the presence of a clinical nephrotic syndrome without detectable histological abnormalities in the glomeruli after careful subjective evaluation. Classification to the MPGN-group was made if mesangial cell proliferation was clearly and exclusively present, without considering the clinical data. The diagnosis of diabetic glomerulosclerosis was made in patients with a clinically confirmed diabetes mellitus and a diffuse or focal, PAS-positive increase of the mesangium. So, in the MC- and DGS-group clinical features were also considered. Renal tissue with at least 10 glomeruli (also necessary for histological diagnosis) is divided into blocks of 1–2 mm border length, fixed for 24 h in 4% buffered formalin, refixed for 2 h in 2% buffered osmic acid and embedded in methacrylate. Sections 1.0 mμ thick are cut on an ultramicrotome and silver impregnated using the

Table 1. Features used in the analysis

Feature	Abbreviation	Dimension
1. Total glomerular cells	Tglom	
2. Mesangial cell percentage	Meceper	%
3. Total glomerular cells/1,000 $m\mu^2$ total area	Tgcar	number
4. Mesangial area percentage	Mearper	%
5. Mesangial cells/1,000 μ^2 mesangial area	MC/MA	number
6. Endothelial cells	Endoce	number
7. Mesangial cells	Mesace	number
8. Epithelial cells	Epice	number
9. Endothelial cell percentage	Endoper	%
10. Epithelial cell percentage	Epiper	%
11. Total glomerular area	Garea	area
12. Total mesangial area	Mesarea	area

procedure of Movat (1961). From each block only one section is used as this guarantees the investigation of different glomeruli in each case. If material is already embedded in paraffin it must be deparaffined and treated as described above. From these sections the following variables were determined: mesangial area as a percentage of the total glomerular area, the number of glomerular cells, and the glomerular diameter and area.

Quantitative determination of the mesangium

Mesangial area percentage was assessed by point counting, using a projection microscope. As a reference system the total glomerular area, limited by the Bowman's capsule, with nearly circular glomerular sections is employed. The measurements are performed on a projection microscope at 750 × magnification, with a superimposed test-grid (inter-point distance approximately 15 μ). The minimum number of glomeruli investigated in each case of biopsy material is 10 (Iidaka et al. 1968; Wehner 1968).

Determination of the number of glomerular cells

The determination of glomerular cell number (i.e. nuclear profiles in the section) and their differentiation ensued from direct counting in the microscopic field, using the grid-lines of a grid-ocular as a guide-line (such ocular grids are widely available commercially). These values yielded the percentage of the cell types in relation to the total number of cells. The glomerular cells were differentiated in endothelial, epithelial and mesangial cells. Differentiation ensued from their localisation in the glomerulus. Cells localized outside, but in direct contact with the glomerular capillary wall were denoted as epithelial. Classification "endothelial" was made, if cells or nuclei were localized inside the glomerular capillaries, but only if in close connection with the wall. Mesangial cells were cells localized inside the mesangium.

Glomerular diameter and area

Only nearly round glomerular profiles were used. The mean diameter of these sections is determined on the projection screen as the arithmetic mean of the larger of the horizontal and vertical diameter of approximately circular glomerular sections. From these values the mean absolute area of the investigated glomerular sections is determined. Knowing the mesangial portion of this area expressed as a percentage, the absolute mesangial area can be evaluated. Because of the differences between these areas in the investigated groups and in consequence of the dependence of cell number on the glomerular size, glomerular cell number is determined in a fictious uniform area of 1,000 $m\mu^2$ (glomerular cell density). The same procedure is applied to mesangial cells using a uniform area of 1,000 $m\mu^2$ (=mesangial cell density). The features investigated are summarized in Table 1.

Short definition of the variables

All values per case are the means of counts made in at least 10 glomeruli.

1. Total glomerular cells = total number of cells within one glomerular profile.
2. Mesangial cell percentage = number of mesangial cells divided by the total number of glomerular cells times 100.
3. Total glomerular/cells/1,000 $m\mu^2$ total area = the total number of glomerular cells in a unit area of 1,000 $m\mu$.
4. Mesangial area percentage = mesangial area divided by the total glomerular area times 100.
5. Mesangial cells per 1,000 $m\mu^2$ mesangial area = number of the mesangial cells in 1,000 $m\mu^2$ mesangial area.
6. Endothelial cells = absolute number of endothelial cells in a glomerular profile.
7. Mesangial cells = absolute number of mesangial cells in a glomerular profile.
8. Epithelial cells = absolute number of epithelial cells in a glomerular profile.
9. Endothelial cell percentage = endothelial cell number divided by the total glomerular cell number times 100.
10. Epithelial cell percentage = epithelial cell number divided by the total number of glomerular cells times 100.
11. Total glomerular area = total glomerular area calculated from the horizontal and vertical diameters in $m\mu^2$.
12. Total mesangial area = the mean mesangial area in a glomerular profile in $m\mu^2$.

Statistical analysis

Wilcoxon's test was used to establish significant differences between single features of the different groups.

The techniques for multivariate analysis were patterned after Cooley and Lohnes (1971) and programmed on a PDP 11/44 computer. The Kolmogorow-Smirnov test was used to assess normal distribution, and equality of variance was investigated also. As these were satisfactory for all features used, no further transformations of the data were applied. Discriminant analysis was carried out to distinguish the different groups, after selection of the most optimal discriminating set with stepwise regression analysis. This set was selected for the following reasons: 1) significance of the model, 2) increase of squared correlation coefficient (r^2) in comparison with the preceding step, 3) significance and number of the constituting features of the model.

Numerical classification probability thresholds

Apart from "black-white" classification, the classification probability of each individual patient has also been calculated. The maximum value of this probability (P) is: 1.0 (indicating a maximum probability for a certain group), and 0.0 indicates a very low provability. In the "black-white" model 0.50 is taken as the decision threshold for classification ($0.50 <= P \leqq 1.00$) or not ($0.0 < P < 0.50$).

A slight change then in the quantitative data could change the classification of a patient. Therefore, the usefulness of a "grey-zone" is investigated also, with 0.25 and 0.75 as threshold values. A case with a classification probability $0.25 < P < 0.75$ is then regarded as doubtful (or inconclusive). Apparently, the better the discriminating power of a certain feature, or combination of more features, the less cases will be classified as "doubtful". The use of numerical probability statements and threshold values has recently been advocated by Pauker and Kassirer (1980); Schwarz et al. (1981) and Sappenfield et al. (1981).

Results

Figure 1 shows the means and ranges (minimum and maximum) of the four groups and the significance of the differences between the normal and

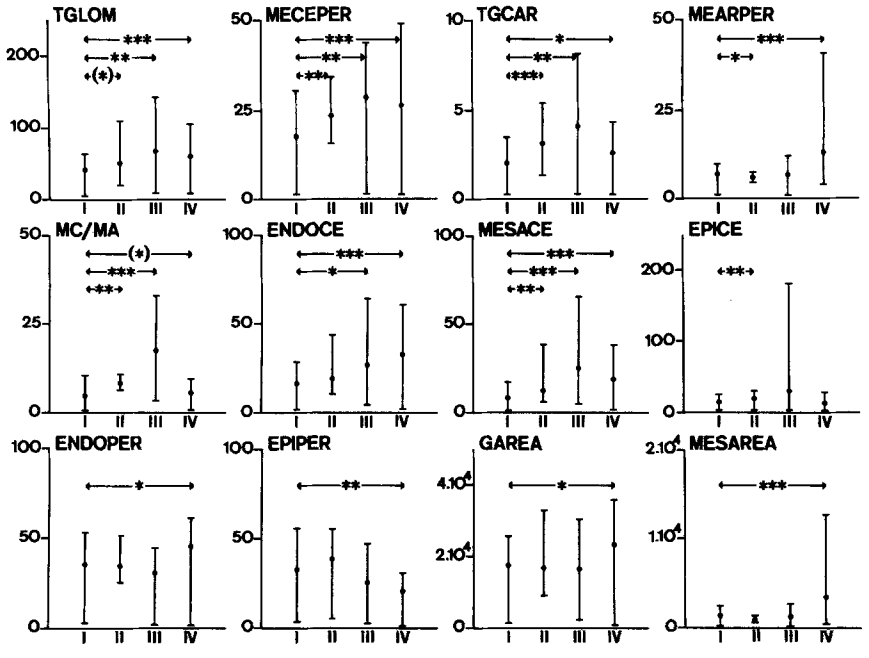

Fig. 1. Mean and range (minimum and maximum), of 12 morphometric features of the 4 groups (*I* = normal; *II* = minimal changes; *III* = mesangioproliferative glomerulonephritis; *IV* = diabetic glomerulosclerosis), with the differences between the normal group and the other groups (Wilcoxon's test, two-sided; (*): $0.10 < P < 0.05$; *: $0.05 < P < /= 0.01$; **: $0.01 < P < /= 0.001$; ***: $P < /= 0.001$)

the other groups. In agreement with earlier results (Wehner 1974), there are many significant differences, but also considerable overlaps between the different features, and none of them discriminates absolutely between any of the four groups. The mesangial variables and the glomerular cell density (TGCAR) are the most promising morphometric features for discriminating the different classes of diseases if single variate analysis is used. In addition, the mean age of the patients differs, as the patients with diabetic glomerulosclerosis are significantly older than those in the three other groups ($P < 0.001$).

The multivariate analysis was hampered, because in the MC- and MPGN-group the features mesangial area percentage, mesangial cell density and absolute mesangial area were missing in several cases and some other features in other cases. This problem can be solved in two ways. First, the cases in which these values are missing can be left out, but this would result in a decrease of the number of patients available. Another possibility is to replace the missing data by the value of mean of the remaining group. As the number of missing values was too high, this solution could not be adopted. Therefore, the analyses were performed without these features, as pilot studies suggested that other features were equally good discriminators. A total of 84 cases remained.

Table 2. Confusion matrix of discriminant analysis of groups 1 and 2 (normals and minimal changes) and 3 and 4 (mesangioproliferatives and diabetics)

Actual groups	*n*	Computer classification groups	
		1+2	3+4
1+2	39	33	6
3+4	45	2	43
		Overall efficiency = 90.5%	

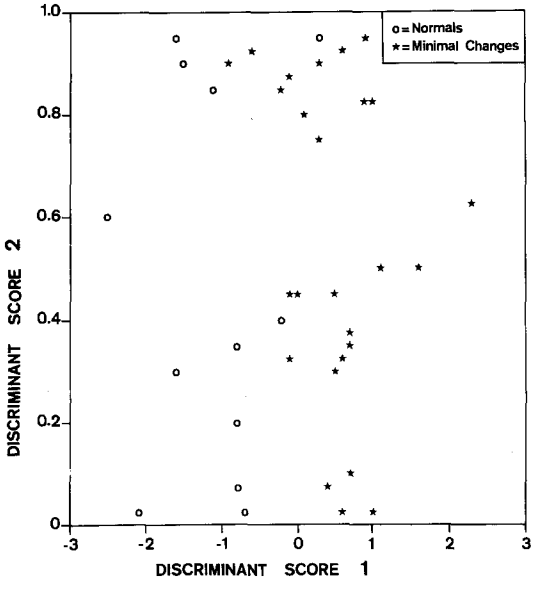

Fig. 2. Discrimination of the normals (*open circles*) and minimal changes (*stars*) with three features (see text). On the X-axis are the results of the first discriminant function, on the Y-axis those of the second one. The discriminant functions were obtained with linear discriminant analysis

A closer consideration of the results of the discriminant analysis showed that it would be difficult to discriminate all four groups simultaneously. We therefore tried to distinguish the normals and MC's together, from the MPGN's and DGS together. With 6 features (total glomerular cells, mesangial cell percentage, total glomerular cells per 1,000 $m\mu^2$, endothelial cells, epithelial cells, and epithelial cell percentage) 90.5% of all 84 patients was correctly classified (sensitivity: 95.6%, specificity: 84.6%) (see Table 2).

For the distinction of the normals and MC's from each other, a combination of 3 features (mesangial cell percentage, total glomerular cells and endothelial cell percentage) turned out to be the best discriminating combination (r^2: 0.46, $P < 0.0001$), (see Fig. 2). Using 0.75 as a numerical classification probability threshold (for "doubtful" or "inconclusive") none of

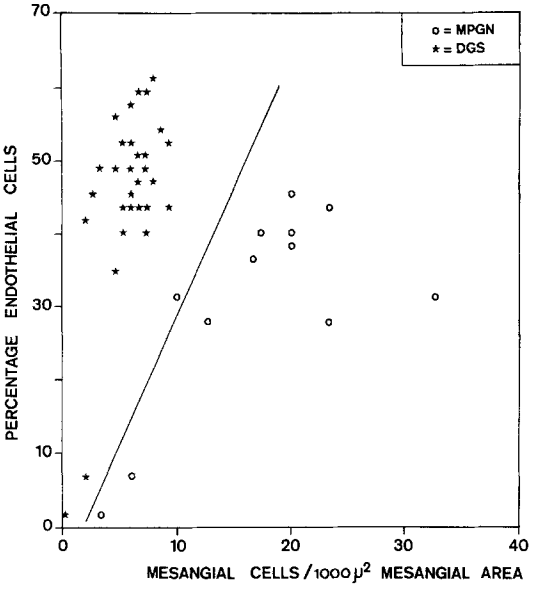

Fig. 3. With the features numerical mesangial cell density and percentage endothelial cells, 98% of all MPGN's and DGS cases are correctly classified

the minimal changes was misclassified, and two of the normal patients (16%). Four of the normals were inconclusive (33%) and four of the minimal changes (14%).

For the discrimination of the 45 MPGN's and DGS's, the mesangial cell number per 1,000 $m\mu^2$ mesangial area and the endothelial cell percentage was the best discriminating combination. Only one of the MPGN-cases was misclassified. However, the numerical classification probability of this case was very low (0.65). In contrast, all the real diabetics had classification probabilities between 0.79 and 1.0. Figure 3 shows the results.

Discussion

The present data show that the discrimination between the different subclasses of kidney diseases with morphometry is possible with an acceptably low degree of misclassifications. We should remember, that the differentiation between the different subclasses with subjective evaluation is often difficult. Therapeutic and prognostic considerations require as exact a definition as possible.

There is one essential condition in the application of morphometry in diagnostic pathology. We emphasize that the morphometric techniques should be applied to areas and cells, which are carefully *selected* by the pathologist. Thus, selective diagnostic morphometry is by no means suitable for a random statistical approach (Baak and Oort 1983). Secondly the results of morphometry always should be *interpreted* by the pathologist. It is the pathologist who selects the cells and nuclei of interest, using strict diagnostic criteria. Therefore, the morphometric techniques are selectively applied. This

type of selective morphometry is a diagnostic tool in the hands of the pathologist and should not be used autonomically. There is thus a two-stage-procedure. First, the diagnostic selection of relevant objects is made by a skilled pathologist; secondly the confirmation of the diagnosis, or indication of the most probable disease class, is carried out using morphometry.

In practical diagnostic pathology, the distinction between the normals and MC together from the MPGN's and DGS is of little value for the pathologist. This distinction is usually easily performed with subjective evaluation if the pathologist is well-trained and is used to judge many kidney biopsies. If this is not the case, morphometric analysis can be used as a pre-screening method to *support* rather than to *make* the diagnosis. The sensitivity (positivity in disease) is then the factor which determines the value of the investigative procedure. This is high (95.6%, 2 misclasssifications out of 45 cases, see Table 2), and thus morphometry can be considered for this purpose.

Cases of MPGN and DGS are usually easily discriminated by conventional light microscopy. However, borderline cases do occur and here, morphometry could be of help. Together with other clinical data, such as the age of the patient; it can put the pathologist on a certain track.

In the cases mentioned sofar, morphometry mainly has an adjunctive function to support the diagnosis. In contrast, the present results emphasize the *detection* possibilities of morphometry in the distinction of kidney biopsies from normals and MC patients. It should be stressed that with conventional careful investigation, no differences were detectable between these groups. It is frustrating not to find any microscopical differences in the presence of a clinical nephrotic syndrome. Here, morphometry can be used to lighten this burden of the pathologist in a certain number of cases. Morphometry will slightly overdiagnose if used in an autonomic way, as none of the MC's is misclassified as normal, and two (=16%) of the normals are regarded as MC. This is precisely what most pathologists prefer.

Thus, we conclude that especially in cases of doubt, morphometry can be used to assist the diagnosis in an objective way.

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