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Correlations between Renal Interstitium and Level of Serum Creatinine

Morphometric Investigations of Biopsies in Perimembranous Glomerulonephritis

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Summary. Morphometric investigations in 40 patients suffering from perimembranous glomerulonephritis at different stages showed that there is no certain relationship between the severity of glomerular lesions and the serum creatinine level. In 19 cases in stages I–III, with serum creatinine level less than 1.2 mg/100 ml on biopsy, the renal interstitium was less enlarged than in 21 cases in the same stages, but with serum creatinine level higher than 2 mg/100 ml.

There is a significant positive correlation between the relative interstitial volume and the level of serum creatinine. The best congruence was demonstrated in the lin/log-plotting indicating that our values correlate best with an exponential function. We therefore conclude that in perimembranous glomerulonephritis, generally considered to be a glomerular disease, functional impairment cannot be explained by the glomerular lesions alone; interstitial changes have also to be taken into account as a cause of renal insufficiency.

The following hypothesis is proposed; that the increase in renal interstitium and possible shrinking of collagen fibres may lead to a narrowing of intertubular capillaries. This may result in slowing of glomerular blood flow and may lead to renal insufficiency.

Key words: Perimembranous GN – Glomerular lesions – Interstitium – Interstitial fibrosis – Renal insufficiency

Introduction

During the investigation on the relationships between renal structure and function in different renal diseases (acute renal failure, chronic transplant rejection, different glomerulonephritides with nephrotic syndrome) (Bohle, 1972; Bohle and Fischbach, 1974; Bohle et al., 1976; Fischbach, 1976) we realized that renal function, esp. glomerular filtration rate, is influenced by interstitial fibrosis.

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Results obtained by Risdon et al. (1968) and de Wardener (1973) lead to similar conclusions. These authors reported that in certain chronic glomerular diseases with proteinuria or nephrotic syndrome renal function was influenced by the severity of tubular rather than glomerular lesions.

The morphological part of these studies was not based on measurements, but on visual impressions gained by a systematic study of the histological sections. Furthermore most cases of GN's in the study were not classified.

We therefore thought it would be worthwhile to evaluate the importance of extended interstitium in renal function using morphometric methods. We chose peri- (synonyms epi- or extra-) membranous GN as a well defined disease in which all glomeruli are equally affected. In addition the stages of this glomerular disease can be exactly specified (Ehrenreich and Churg, 1968; Gärtner et al., 1976).

Material

40 adult patients of both sexes were investigated (see Table 1 for details).

Table 1. Distribution of the patients investigated

	Creatinine in serum	
	$\leqq 1.2$ mg/100 ml	$\geqq 2$ mg/100 ml
Normal kidneys $n=20$	20 Group N	0 Group N
Perimembranous GN $n=40$	19 Group A	21 Group B
Nephrotic syndrome present at time of biopsy $n=22$	9	13

The biopsies were fixed in a 4% formalin solution (pH 7.4) and embedded in paraffin; 5–8 µm thick sections were examined with the light microscope. Each tissue specimen contained at least 5 glomeruli.

For determination of the severity of glomerular lesions, methyl-acrylat fixed semi-thin sections stained by silver impregnation were studied. For this purpose only stages I–III of the perimembranous glomerulonephritis were evaluated.

Stages IV and V – healing stages in our classification (Gärtner et al., 1976) – were ignored.

Different *morphometric methods* have been used:

1. Point-counting
2. Tubulometry

1. *Point-counting* to discriminate between interstitium, epithelia and lumina was carried out with a Reichert Visopan (objective 10/0.2, magnification 125:1). Using a lattice of 1 cm, five projection fields of the cortex per kidney were examined, ignoring big blood vessels. The relative volumes, their means and standard deviations in different groups and in the different stages of the disease were calculated. Correlations between interstitium and serum creatinine values gained at the time of biopsy were evaluated graphically by plotting corresponding measurements. Finally we calculated

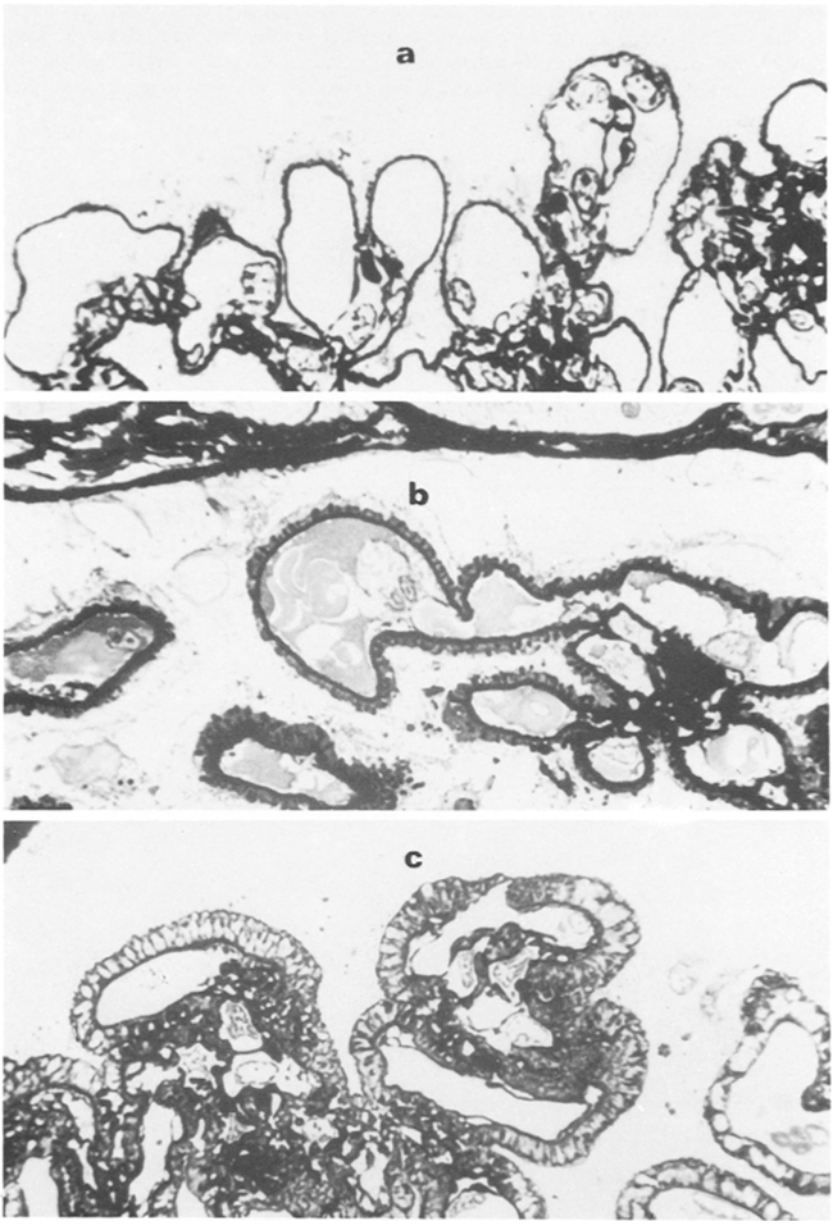

Fig. 1a–c. Perimembranous glomerulonephritis. **a** stage I, **b** stage II, **c** stage III, according to Ehrenreich and Churg, 1968. Semithin-sections 0.5 µm, MOVAT silver impregnation, 1150:1

the correlation coefficient r for linear, exponential and parabolic functions. The error probability α was determined from the t-value.

2. *Tubulometry:* 8–13 periglomerular proximal tubules cut in cross section were measured in each tissue specimen. We evaluated microphotomagnifications (780:1), using an OTT-planimeter. The

resulting areas were described as total tubular area (T.A.), luminal area (L.A.) and epithelial area (E.A.). The values of each particular area were analyzed statistically, computing for each kidney as well as for each group (N,A,B) the mean and standard deviation. As a control, the quotient T.A.:E.A. was analyzed in each kidney and group. The *t*-test was used to check significant differences.

Results

1. In control kidneys, the relative volume of renal interstitium is $9 \pm 1.5\%$.

Table 2. Mean values $\pm$ standard deviation of creatinine and renal interstitium in diff. stages of perimembranous GN. (Significance $p \leqq 0.05$)

	Creatinine in serum (mg/100 ml)	Significance	Interstitium Vol.%	Significance
Normals	1.2		9.0± 1.5	
Perimembranous GN				
stage I	1.3±0.7	∅	11.5± 2.9	
stage II	1.6±0.9		20.1±12.9	
stage III	3.5±3.5		32.5±13	

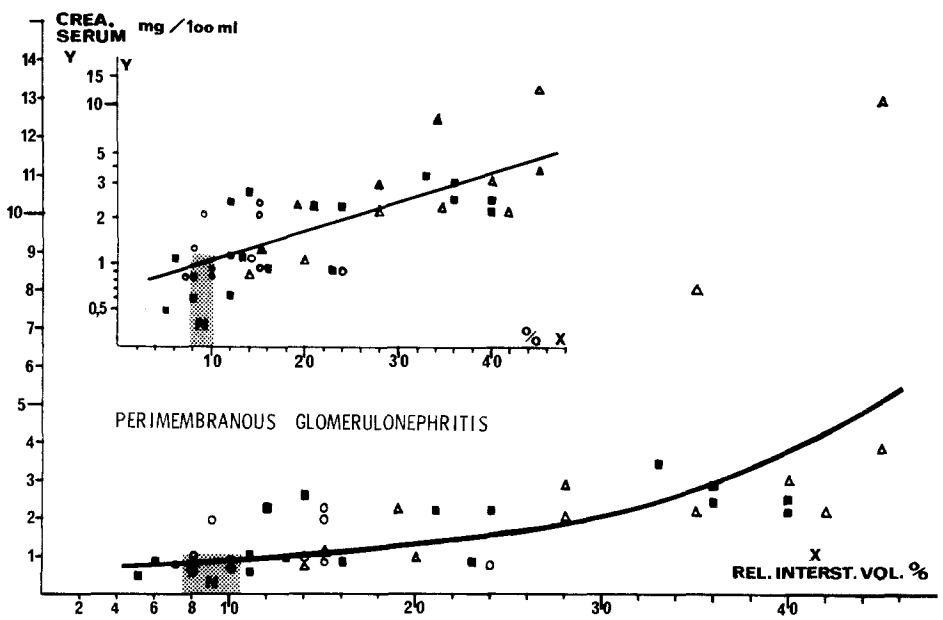

Fig. 2. Correlation between interstitium (*x*-axis) and creatinine in serum (*y*-axis) in lin/lin and lin/log (left above) plotting. Histological stages: ○ = I, ■ = II, △, ▲ = III. *N* = Range of normal kidneys. Function: $y = 0.65\, e^{0.041 x}$

2. In perimembranous glomerulonephritis however, the volume of renal interstitial tissue exceeds that in control kidneys. In stage III of the perimembranous glomerulonephritis the increase of interstitial volume is significant when compared to the control group or to stage I.

3. The serum creatinine in different stages of perimembranous glomerulonephritis is higher than in control groups. This difference is, however, not significant.

4. The relationship between the relative volume of renal interstitium, the corresponding serum creatinine level, and the stage of the disease are plotted in Figure 2. The diagram shows no significant relationship between serum creatinine and the corresponding stage of the disease. On contrast, there is an obvious connection between the increase in serum creatinine and the increase in relative interstitial volume. All above mentioned functions of the x- and y-axis showed an ensured correlation between the parameters interstitium and serum-creatinine.

Table 3. Results of calculation of correlation between interstitium (x) and creatinine (y)

x/y	lin/lin	lin/log	log/lin	log/log	parabolic
Correlation coefficient r	+0.56	+0.73	+0.53	+0.71	+0.62
Error probability α	<0.001	<0.0001	<0.001	<0.0001	<0.0001

Our values are represented best by an exponential curve ($y=0.65\ e^{0.041x}$). Using the transformed y-axis (Fig. 2) a straight line is obtained. The statistical error probability for this correlation is less than 0.0001.

As can be seen in Figure 3, the total area and the epithelial area of the proximal tubules of kidneys with perimembranous GN are not smaller than

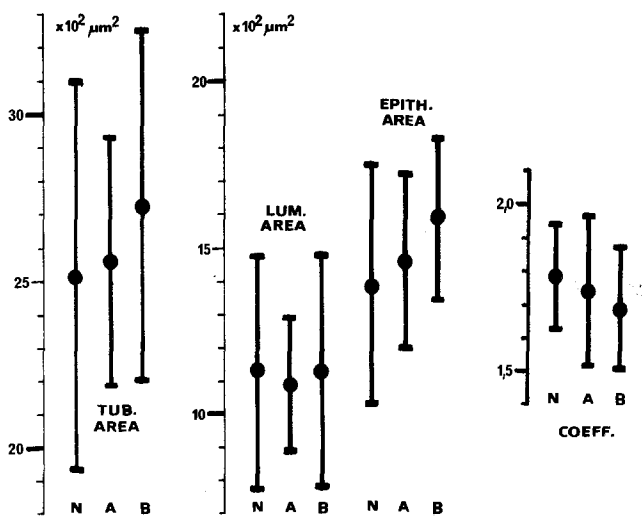

Fig. 3. Results of tubulometry ($\bar{x} \pm s_d$) in the groups N (=normals), A (creatinine in serum $\leqq 1.2$ mg-%) and B (crea. $\geqq 2$ mg-%). *Tub. area*=tubular area inside bas. membrane, *Lum. area*=area of the tubular lumen, *Epith. area*=pure epithelial area, *Coeff.*=quotient Tub. area:Epith. area

in the control kidneys. On the contrary, a tendency towards an increase of these areas can be observed, more marked in group B than in group A.

Discussion

In perimembranous glomerulonephritis there is no significant correlation between the severity of glomerular lesions and the level of serum creatinine. Al-

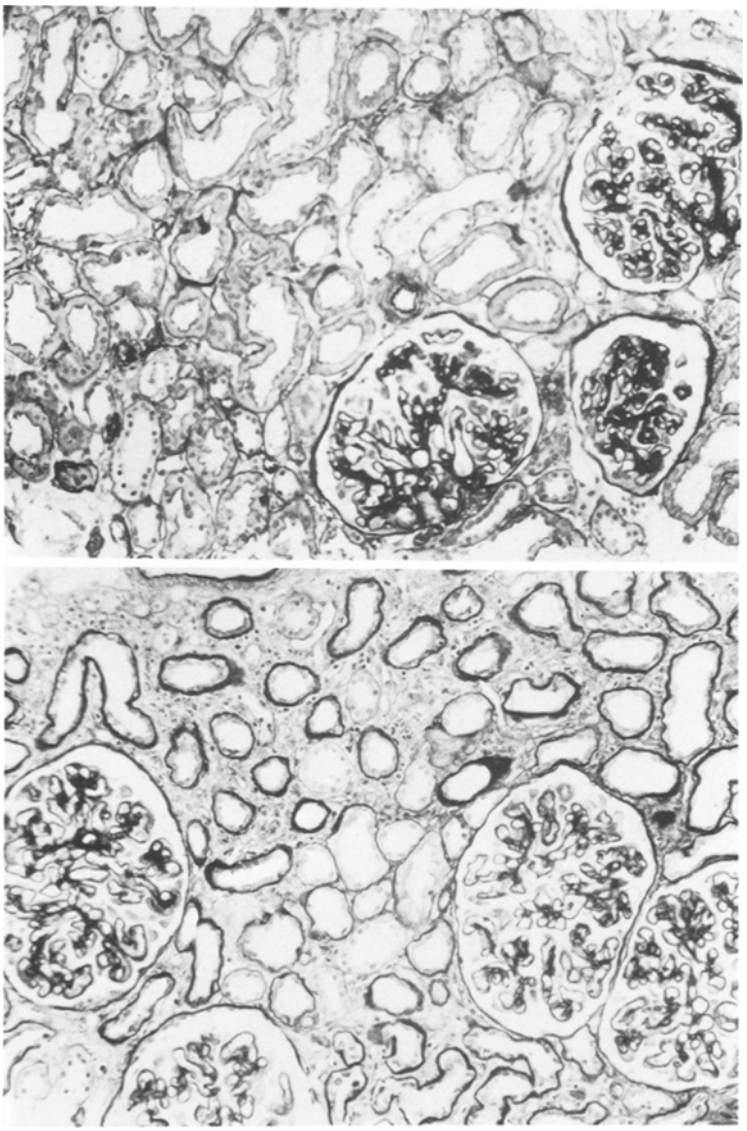

Fig. 4. **a** Perimembranous gn stage II with normal tubules and normal amount of interstitium (crea. in serum: 1.07 mg/100 ml). **b** Perimembranous gn stage II with broad interstitium (crea. in serum: 3.5 mg/100 ml) (PAS reaction, 144:1)

though serum creatinine increases with the severity of glomerular disease, normal values can infrequently be observed even in stage III, if the interstitium is not expanded. On the other hand an increase of serum creatinine can be observed in patients with perimembranous GN stage I, where spikes are barely visible between subepithelial depositions of immune precipitates in silver impregnated semithin sections. Elevated serum creatinine is only observed in cases with increased interstitial volume.

An exponential increase of serum creatinine has been shown to correlate with an expanding renal interstitium. The first part of the curve ist flat however, so that initially the increase in interstitium leads to gradual elevation of serum creatinine. In general, the serum creatinine level remains below 2.5 mg/100 ml as the interstitium increases up to 25% of the total kidney volume, whereas a further enlargement of the interstitium is followed by a considerable increase in serum creatinine (Fig. 2).

In stage III with the severest glomerular lesions, the average serum creatinine reaches its highest level. This is not caused by glomerular lesions alone, but also by the significant increase of interstitium at this stage, significant not only when compared to the controls but also to stage I and II.

The enlargement of the interstitium in stages II and III of perimembranous GN is not accompanied by tubular atrophy. On fact the epithelial area grows with increasing creatinine. The absolute luminal area of the proximal tubules remains unchanged, however, despite alterations of serum creatinine and relative interstitial volume.

On agreement with the results of Risdon et al. (1968), the present findings show that in renal diseases characterized as glomerular, functional disturbances cannot be explained by glomerular lesions alone. Risdon et al. (1968) and de Wardener (1973) suggested that lesions of tubular epithelium would cause functional disturbances. Risdon et al. (1968) examined cases of persisting glomerulonephritis with decreased creatinine clearance and described atrophied tubules with flat epithelium, occasionally dilated and filled with colloidal material. The segment affected was not defined. These authors found that the tubules were usually narrowed and separated from each other by connective tissue. Pyknotic epithelial nuclei and mitoses were observed.

Initially we were under the impression that in kidneys with an enlarged interstitium the tubules were atrophic. Closer morphometrical analysis did not support this impression. It must be taken into account nevertheless that the ascending loop of Henle, the distal convolution and the collecting tubules were not studied in our investigation, since cross sections of these segments seldom appear in needle biopsies.

From our results we think that the increase of serum creatinine in perimembranous glomerulonephritis cannot be explained in the way that Risdon et al. (1968) and de Wardener (1973) suggest. They attribute the decrease of the creatinine clearance in mild glomerular disease to the diffusion of creatinine through the damaged tubular basement membrane into the blood, thus simulating a depression of the creatinine filtration rate. The authors also considered the filtration process might be impaired by blocking casts in the tubular lumina. The failure of glomerular function in the persisting glomerulonephritides is

believed to be caused by tubular lesions similar to those in acute renal failure. The argments for or against the diffusion – or obstruction theory have been published elsewhere (Bohle et al., 1964; Bohle, 1967; Bohle and Thurau, 1974; Bohle et al., 1976).

In the present investigation we found no tubular morphological findings that could explain a spurious filtration rate. Glomerular lesions are not likely to be responsible for the increase in the serum creatinine level of patients with perimembranous GN. On view of the positive correlation between the extend of the renal interstitium and the level of serum creatinine however, it is conceivable that enlargement of the interstitium together with shrinking of collagen fibres leads to narrowing of intertubular capillaries. This may cause slowing and decrease of blood flow in the renal cortex and subsequently may rise blood pressure in glomerular capillaries. The question of whether this depression of renal cortical blood flow is sufficient to explain an elevated serum creatinine level cannot be answered at present, but we think it is possible. The object of further studies will be to determine what kind of factors play a role in the pathogenesis of increase in the interstitium and the resulting renal insufficiency.

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