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Interstitial, Tubular and Vascular Factors in Progression of Primary Glomerulonephritis

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Glomerulonephritis is one of the diseases leading to chronic renal failure and need of renal replacement therapy. Changes in extraglomerular compartments, especially in the interstitium, are thought to play a major role in progression. However, the exact relationships between renal interstitium, tubules and vessels and their prognostic impact are less well understood.

The material consisted of 111 biopsies with primary glomerulonephritis. Normal renal tissue from surgically removed kidneys served as controls. Relative interstitial volume (RIV), its variability, volume of interstitial infiltrate, cross-sectional tubular area were measured with the point-counting method. A number of vascular parameters were also measured.

The assessed interstitial and tubular parameters were strongly correlated to creatinine level. The strongest correlation was seen for RIV, also on multiple regression. In patients with renal failure, increased RIV, more pronounced vascular lesions and interstitial infiltrates were seen. Survival analysis showed that interstitial expansion is the most important factor leading to renal failure.

Tubulointerstitial and vascular factors are interrelated and linked to renal function. RIV has strongest impact on renal function and survival, even taking into account other factors.

Introduction

Glomerulopathy, or glomerulonephritis (GN) is relatively rare. Indeed, diabetes pandemics and increase in vascular nephropathies become the main challenge for nephrologists [2]. GN frequency remains stable or decreases, though it can be still important etiology of chronic renal failure [3, 14]. It remains interesting, because of a high progression rate, and difficulty in prognostication. In the diagnosis of GN renal biopsy remains an essential tool. The term GN implies that the disease involves glomeruli. However, prognosis based only upon glomerular lesions is not reliable [18]. This is of practical importance, as early treatment may significantly decrease the rate of progression towards end-stage renal disease and delay the need for renal replacement [13]. Various phenomena, mainly immunological occurring in GNs are increasingly being understood, especially with respect to basic mechanisms in experimental models. The extrapolation of such data to human disease may be difficult. However it can be concluded that understanding purely glomerular changes does not suffice to explain the clinical course of GN and its prognosis [18]. Similar mechanisms may be acting in other nephropathies [25].

Material and Methods

Material consisted of 111 renal biopsies of patients diagnosed in the Chair of Pathomorphology between 1992 and 1997. Cases of systemic disease, diagnoses other than glomerulonephritis and nonrepresentative biopsies were excluded, as well as minimal change disease. The biopsy was regarded as representative, if immunofluorescence and electron microscopy were performed and non less than 6 non-sclerosed glomeruli were available for light microscopy. All

cases were reclassified according to WHO criteria [4]. The controls consisted of unaltered renal tissue of surgically removed kidneys; of these in 18 cases nephrectomy was due to renal tumour, 1 to traumatic rupture and 1 to adrenal tumour. Only cases without significant autolysis or pathological lesions were included. All specimens were fixed in 10% buffered formalin, routinely processed and embedded in paraffin. Paraffin blocks were cut into 4μm sections; the thickness of sections was controlled with method described by Weibel [27].

Image acquisition was performed using a Zeiss Axioscop microscope (Zeiss GmbH, Germany) with ZVS-47DE CCD camera (Optronics Inc, USA) connected by a RGB line to GraBIT PCI frame grabber (Soft Imaging System GmbH, Germany) installed on a standard PC. Image acquisition, processing and measurements were performed with AnalySIS 3.0 pro image analysis system (Soft Imaging System GmbH, Germany) and applications custom-made by one of the authors (K. O.).

The measurements were done in a blinded manner, i. e. without knowledge of diagnosis or clinical data. Measurements of relative interstitial volume (RIV), interstitial infiltrates relative volume (RCV), mean cross-sectional area of proximal and distal tubule were made by point counting method. Silver methenamine – trichrome contrasted stained preparations were used (Fig. 1). The image was entered into the system and displayed on its screen. A 72-elements grid was superimposed on it, and the operator clicked on grid elements hitting measured structures. The number of hits was recorded by the system. As a measure of variability of interstitial volume and infiltrates their variance between the fields of vision was used.

For the measurement of arteries orcein stained sections were used. The slides were scanned at low magnification and the well preserved vessel profiles chosen. The image was taken with lens magnification appropriate for vessel size, and displayed on the image analysis system screen. A linear measurement of the lumen diameter, intima and media thickness was taken interactively. The lumen, lamina elastica interna and externa contours were automatically recognised. The system calculated indexes of intima (IIT, RIT) and media (MIT, RMT) thickness, defined as:

$$RIT = \frac{4\pi S_1}{L_I^2}$$

$$RMT = \frac{4\pi S_M}{L_E^2}$$

$$IIT = \frac{S_W - S_L}{S_W}$$

$$IMT = \frac{S_Z - S_W}{S_Z}$$

where:
RIT – ratio of intimal thickening
RMT – ratio of media thickening

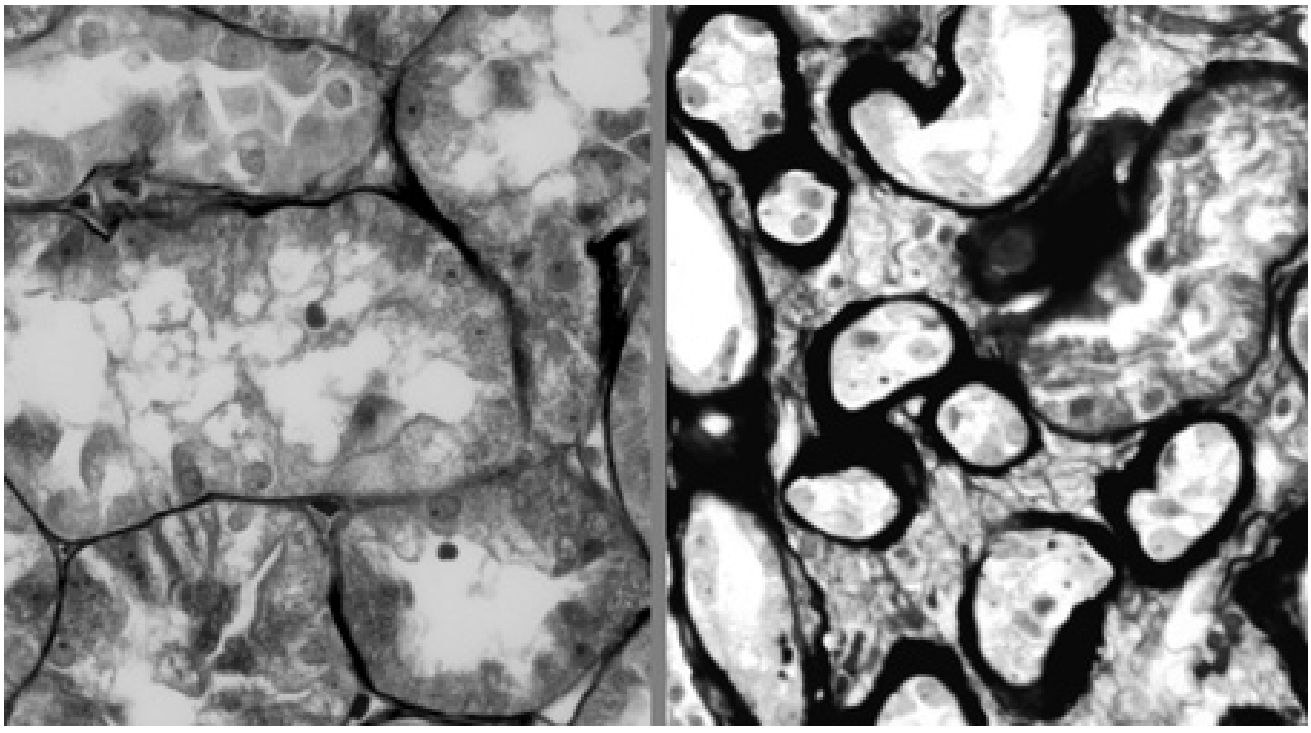

Fig. 1. Silver methenamine – trichrome contrastained stained kidney. Both in the normal (left) and expanded (right) interstitium the borders of the canaliculi can clearly be seen. Silver methenamine – trichrome stain. Lens magnification 60×.

IIT – index of intimal thickening
IMT – index of media thickening
S_I – surface area of intima
L_I – perimeter length of lamina elastica interna
S_M – surface area of media
L_E – perimeter length of lamina elastica externa
S_W – surface area enclosed by lamina elastica interna
S_L – luminal surface area
S_Z – surface area enclosed by lamina elastica externa

The PAS stained slides were used to count and categorise small vessels. Small vessel hyaline index was defined as:

$$SVHI = \frac{n_1 + 2n_2 + 3n_3 + 4n_4}{n_1 + n_2 + n_3 + n_4}$$

where:
SVHI – small vessel hyaline index
n_1 – number of vessels without hyaline change
n_2 – number of vessels with hyaline change involving <50% of the circumference
n_3 – number of vessels with hyaline change involving >50% of the circumference
n_4 – number of vessels with hyaline change involving the whole circumference

Of the clinical data, creatinine level, daily proteinuria and arterial blood pressure were included. As a final point, the date of the start of chronic renal replacement treatment or disappearance from observation was noted.

Statistical analysis was performed with Statistica 5.5 software (StatSoft Inc, USA). Differences between variables were assessed using Kruskall-Wallis ANOVA, Mann-Whitney U-test and Wald-Wolfowitz run test. Correlations were assessed using Spearman and Pearson's correlation coefficients. For identification of factors influencing the prognosis, survival analysis was performed by Cox method. The significance level was set to 0.05.

Results

The population under study consisted of 111 patients, of whom 70 were men and 41 women. Mean age was 38.06 years, standard error of mean (SE) 1.55, range from 15 to 77 years. The diagnoses were: IgAN – 34 cases, membranous nephropathy – 24, membranoproliferative glomerulonephritis – 14 (type I 11, type II 1, type III 2), mesangioproliferative glomerulonephritis – 14, focal segmental glomerulosclerosis – 5, postreptococcal glomerulonephritis – 4, crescentic glomerulonephritis – 1, fibrillary glomerulonephritis – 1, chronic glomerulonephritis – 7, focal proliferative glomerulonephritis – 1; 6 cases did not fit into any category. The control group consisted of 20 cases; there were 9 women and 11 men. Mean age 54.9 years, SE 3.9 and range from 24 to 79 years.

The relative interstitial volume in the study group was 17%, range of 1 to 44% and SE of 0.008. In the control group RIV was 6.6%, range 3.2 to 14.2 and SE 0.006. The difference was statistically significant (<0.001). RIV did not correlate with age in the experimental population nor in controls. Relative volume of cell infiltrate in the study group was 3.3%, range 0.6 to 10.7 and SE of mean 0.001. Such infiltrates were not present in the controls.

The biopsies contained a total of 134 complete, well-preserved cross-sections of arteries. 49 of them (36.56%) had less than 100 μm in diameter; 55 (41.05%) 100 to 200 μm; 30 (22.39%) over 200 μm. Table 2 summarises the results of measurements. The mean hyaline change index was 1.93, range 1 to 3.5 and SE 0.056. In the control group mean small vessel hyaline index was 1.48, range 1.1 to 2.2 and SE 0.066.

RIV showed a negative correlation with proximal epithelial cross-sectional area (R=–0.37, p<0.001). Also RCV correlated negatively with proximal epithelial cross-sectional area (R=–0.26, p<0.006).

TABLE 1
Cross-sectional area of tubules, expressed as mean (standard error of mean). p is significance level of Mann-Whitney test. NS means non-significant

	proximal tubule		distal tubule	
	biopsy	control	biopsy	control
total	2.66 (0.09)	2.95 (0.15)	1.09 (0.06)	1.80 (0.17)
	p=0.14 (NS)		p<0.001	
lumen	0.35 (0.02)	0.34 (0.07)	0.39 (0.02)	0.56 (0.06)
	p=0.48 (NS)		p<0.009	
cells	2.31 (0.08)	2.62 (0.16)	0.70 (0.04)	1.24 (0.12)
	p=0.09 (NS)		p<0.003	

TABLE 2
Vascular parameters

	biopsy		control	
	mean	(SE)	mean	(SE)
luminal diameter	57.34μm	(3.31)	90.10μm	(7.46)
intimal thickness	19.25μm	(1.70)	29.67μm	(2.37)
media thickness	19.49μm	(0.90)	29.66μm	(2.42)
RIT	0.29	(0.01)	0.33	(0.02)
IIT	0.49	(0.03)	0.38	(0.05)
RMT	0.37	(0.01)	0.31	(0.02)
IMT	0.36	(0.02)	0.15	(0.04)

Mean creatinine level was 147.96 μmol/l, range 35.4 to 1038, SE 21.30. Mean daily proteinuria was 3.49g, range 0 to 20 and SE 0.44. Mean systolic blood pressure was 143.1 mmHg, range 100 to 200 and SE 3.29. Mean diastolic blood pressure was 88.93 mmHg, range 60 to 170 and SE 2.10.

Creatinine level correlated significantly with age of the patients (R=0.36, p<0.002). Proteinuria differed significantly between the types of glomerulopathy (p<0.001). Age was positively correlated to arterial blood pressure, both systolic and diastolic (R=0.34, p<0.007; R=0.26, p<0.04 respectively). Data on follow-up was available in 66 patients. Mean follow-up length was 1064 days. Seven patients are on chronic dialysis.

Creatinine showed correlation to RIV (R=0.46, p<0.000003), variance of RIV (R=0.36, p<0.003), relative infiltrate volume (R=0.20, p<0.09) and its variance (R=0.29, p<0.015). On multiple regression only RIV and RIV were related to creatinine (β=0.48, p<0.013 and β = –0.34, p<0.061 respectively). Small vessel hyaline index correlated significantly with age (R=0.21, p<0.04) RIV (R=0.31, p<0.001), RIV variance (R=0.24, p<0.012), RCV (R=0.18, p<0.05), and proteinuria (r=–0.41, p<0.001). No relationship was found between small vessel hyaline index and changes in larger arteries.

A correlation was found between age and intima thickness (r=0.36, p<0.001), media thickness (r=0.27, p<0.01), RIT (r=0.41, p<0.01) IIT (r=0.53, p<0.001) and small vessel hyaline index (R=0.33, p<0.001). RMT showed a weak, negative correlation to age (r=–0.20, p<0.056); the relationships between IMT and age were similar (r= –0.21, p<0.04). Media thickness showed correlation with diastolic blood pressure (r=0.26, p<0.04). Intima and media thickening indexes showed positive correlation with diastolic and systolic blood pressure (RIT r=0.28, p=0.018 and r=0.28, p=0.018, IIT r=0.28, p=0.017 and r=0.36, p=0.003, IMT r=0.25, p=0.036 and r=0.27, p=0.024). Small vessel hyaline index was not related to blood pressure.

TABLE 3
Survival analysis by the Cox method, p – significance level

	$\chi^2 = 19.35$ p < 0.00025	
	Wald statistics	p
relative interstitial volume	10.75	<0.0011
infiltrate variability	8.56	<0.04
interstitial volume variability	7.09	<0.01

In order to preliminarily estimate the associations of increased risk of chronic renal failure, the patients on chronic dialysis were compared with the others by U Mann-Whitney test. Of clinical data creatinine (p<0.003) and systolic blood pressure (p<0.08) were most discriminating the groups. The largest differences of morphological parameters were observed with respect to relative interstitial volume (p<0.006) and then interstitial infiltrate (p<0.09). Differences in IIT (p<0.04), RIT (P<0.05), and IMT (p<0.008) were also present. The results of Cox survival analysis are shown in Table 3.

Discussion

The mechanisms underlying interstitial and tubular changes in GN are now relatively well known. Proteinuria is thought to be the main initiating factor. The proteins in tubular lumen cause tubular epithelial cells to expose surface antigens (as MHC-II), adhesive molecules (as integrins) and produce mediators (as TGF-β). Macrophages and lymphocytes are attracted into interstitium. Signals from both tubular epithelial and inflammatory cells causes increase in number and activation of myofibroblasts. Myofibroblasts produce extracellular matrix components, leading finally to widening and fibrosis of renal interstitium [19, 21].

Although GN involves primarily the glomeruli whereas extraglomerular lesions are secondary, several reports demonstrate that interstitial lesions are independent of glomerular lesions, but a strong correlation is seen between interstitial volume, total of myofibroblasts and renal function [5, 15, 21, 22, 26]. Katafuchi et al. suggested that both interstitial volume and degree of glomerulosclerosis might have prognostic value [8]. The prognostic value of increased RIV could be due to anatomic continuity of the renal interstitium favouring spreading of the disease all over it. Interstitial lesions might provide information about a larger renal area than glomeruli physically present in the biopsy

[17]. In our study, a negative correlation between the variability of interstitial volume and symptoms of renal failure was seen on multivariate analysis. Thus uniform increase in the interstitial volume is associated with more significant renal function deterioration than focal changes. The prognostic significance of increased interstitial volume may also result from lower susceptibility to sampling errors [8, 15].

The present study validates significance of interstitial expansion in relation to a set of other variables. RIV was strongly correlated to creatinine level at the time of biopsy, both in univariate and multivariate analysis. More importantly, RIV was also the most significant morphological feature predicting progression to chronic renal failure. In the present study none of variables could match its significance.

Inflammatory cells participate in the pathogenesis of extraglomerular lesions, however, the relationship of such infiltrates to actual renal function is unclear. Roberts did not find such an effect in membranous GN [21], however Lee et al. demonstrated that in IgAN inflammatory infiltrate is related to creatinine level [12]. The same was shown in membranoproliferative GN [16] and ANCA-associated GN [1]. The technique of measuring interstitial infiltrates implemented in the present study is easy and quick, but it is only a rough estimate. In spite of this, a significant relationship between the severity of infiltration and renal impairment was seen. However there was no significant relationship between renal survival and extent of interstitial infiltration.

According to some authors, the main factor in causing renal failure in GN would be tubular atrophy [7]. In the present study proximal tubular epithelial atrophy was significantly associated with renal function. Similar results were obtained by Khan and Sinniah study [11].

Changes in renal arteries consist of hyaline change and intimal fibroplasia. The former is seen in arterioles and terminal arteries, the later in larger vessels. Interestingly, hyaline change was recently linked to loss of renal autoregulation [6]. The etiology of vascular damage in GN is unclear. Arterial hypertension is thought to be an important for renal damage in GNs. In multivariate models blood pressure accounts for 1/3 of the rate of renal failure progression [26]. Quantitative assessment of vascular changes in primary nephropathy has not been extensively studied. Risdon et al. [20] demonstrated a correlation between intimal thickening and tubular atrophy. Hyaline changes were in his study correlated to glomerular lesions. Katafuchi et al. [10] found a correlation of small vessel thickening with glomerulosclerosis rather than with age or blood pressure.

In the present study there was a strong positive correlation between age and intimal thickening. This relationship was strongest for larger (>200 μm) vessels (data not shown). These results correspond to the findings of Katafuchi et al. [8, 10]. Intimal thickening was also associated with daily proteinuria. Though the correlation with renal function was not significant, intimal thickness was significantly more severe in patients on chronic dialysis (mean RIT 0.26 versus 0.47; p=0.038). This indicates that intimal thickening could be an unfavourable prognostic sign also in GN. Intimal thickness of larger arteries (>200 μm) was strongly correlated with blood pressure. In contrast smaller arteries (<200 μm) showed no significant correlation to blood pressure. These findings differ from that obtained by Katafuchi and Takebayashi and Tracy et al. in patients with essential arterial hypertension [9, 24]. This might be due to differences of biology of the studied nephropathies. However the contrast between the small vessels and arterial lesions is similar to that found in other studies [23].

Conclusions

- GNs are characterised by significant interstitial, tubular and vascular lesions.
- These lesions, especially increased relative interstitial volume, show a significant correlation with renal function parameters.
- The effect of renal interstitial expansion is stronger than that of other lesions; however other factors, as focality of lesions, tubular atrophy or arterial changes modifies this effect.

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