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Tubulo-Interstitial Changes in Glomerulopathy. II. Prognostic Significance.

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In the first part of this report mechanisms that lead to the development of tubulo-interstitial changes in glomerulonephritis (GLN) were presented. This issue is of a great practical importance, since explaining renal function in GLN solely by glomerular parameters yields to unreliable and inconsistent results. When the prognosis is based on the glomerular lesions only, the risk of progression to chronic renal failure is often underestimated. In routine biopsy material occurs that no glomerular sclerosis is seen, although the clinical signs of renal failure are present. On the other hand, one can see advanced glomerular lesions, which are not necessarily associated with deteriorated renal function when no interstitial fibrosis is noted. Although in the light of the published reports these facts are not surprising, they are often neglected in everyday nephropathological practice.

Interstitialium and increased interstitial volume

Among extraglomerular parameters, the key position is occupied by the relative interstitial volume. In numerous reports this variable has shown the strongest association with both renal function at the time of biopsy and with the risk of chronic renal failure. As early as 1968, Risdon, Sloper and Wardener studied the association between morphological and functional parameters in the course of long lasting glomerulonephritis. To their surprise, they saw much stronger association between the renal function and tubular atrophy and interstitial fibrosis than between renal function and glomerular lesions [49]. Starting in mid-seventies, Bohle et al. demonstrated the correlation between interstitial fibrosis and renal function in the majority of primary glomerulonephritis cases [e.g. 10 - 13, 38]. Yet the concept of the decisive value of extraglomerular changes for the prognosis in kidney diseases took a long time to become generally accepted. Other lesions, accompanying interstitial fibrosis and by some authors believed to be of prognostic value, include inflammatory infiltration of the interstitium and tubular atrophy.

The phenomena triggered by interstitial processes and resulting in renal failure most likely consist in decreased blood flow in consequence of mechanical pressure, an increased vascular resistance, inhibited autoregulation and tubulo-glomerular balance. Interstitial changes lead also to an abnormal interstitial osmolarity and impaired water and sodium reabsorption. The emergence of atubular glomeruli also may be of some importance in the development of renal failure [12, 13, 45, 55]. Howie stated that a decisive role in the development of chronic renal failure in glomerulonephritis was played not by interstitial changes themselves, but rather by concomitant tubular damage, especially involving the proximal tubules. According to this author, the damage to the tubular epithelial cells, which is manifested by a decreased tubular cross-section surface area, was statistically associated with the interstitial volume [24].

In progressive glomerular disease such phenomena as damage and loss of peritubular capillary vessels are observed, what is supposed to lead to impaired glomerular blood flow. The loss of peritubular capillaries in glomerulopathies was observed by Mackensen-Haen et al. [35]. Also Seron et al. detected a significant drop in the number of peritubular capillaries in the course of various glomerulopathies. This decrease was significantly correlated with impaired renal function, the degree of interstitial fibrosis, tubular loss and intensity of inflammatory infiltration [53].

An increased interstitial volume, as well as tubular loss constitutes a uniformly observed phenomenon in the evolution of glomerular diseases. These factors are significantly correlated with biochemical symptoms of renal function loss and with the risk of progression to chronic renal failure. The highly significant association between interstitial changes and creatinine levels was confirmed in IgA nephropathy, post-streptococcal glomerulonephritis, mesangial proliferative glomerulonephritis, membranoproliferative glomerulonephritis, focal segmental glomerulosclerosis, membranous nephropathy and other diseases [9 - 17, 22, 24, 26, 35, 36, 38, 51, 52, 55, 56]. The processes occurring within the interstitium are so important that some authors regard

glomerulonephritis as "a bizarre interstitial process" [45]. In numerous reports, the increase of interstitial volume in the renal cortex is the most important or even the sole morphological parameter of any prognostic value. Despite the fact that in glomerular diseases the process primarily involves the glomeruli, in many reports interstitial changes show no firm and significant association with the degree of glomerular changes. For example Bohle et al. [11] failed to find such an association in focal segmental glomerulosclerosis. According to Mackensen-Haen et al., renal interstitial lesions in membranoproliferative glomerulonephritis showed no association with the progression of glomerular changes. In the material collected by that author even the most advanced glomerular changes were not associated with impaired renal function if they were not accompanied by an increased interstitial volume and interstitial fibrosis [38]. Danilewicz et al. found only a weak and non-significant correlations between the progression of glomerular changes and interstitial volume in membranous glomerulopathy [15]. Woźniak et al. did not observe any significant associations between early glomerular hyalinization and interstitial changes [58]. According to Roberts, in membranous glomerulopathy, neither the percentage of sclerosed glomeruli nor the progression of glomerular lesions showed association with renal function and with the prognosis. On the other hand, the interstitial volume and the number of myofibroblasts in the interstitium were demonstrated to be significantly associated with renal function [50]. Nevertheless, in patients with various types of nephropathy, extra- and interglomerular changes may be of different and complementary importance for the progression to renal failure. Katafuchi et al. suggested that in proliferative glomerulopathies both the increased interstitial volume and the degree of glomerulosclerosis can be of prognostic significance. In mesangial proliferative glomerulonephritis the authors found the presence of significant associations between interstitial and glomerular changes [32]. Widstam-Attorps et al. observed an association between the development of renal failure and both glomerulosclerosis and interstitial lesions. An increase in the interstitial volume was, however, associated with the presence of interstitial inflammatory infiltration. The interstitial lesions belonged to the components of the histological picture that had the greatest impact on the prognosis. The authors observed that mesangial and interstitial changes were independent of each other [57].

Chronic renal failure is supposed not to occur in cases where glomerular lesions are not accompanied by secondary interstitial and tubular phenomena. A typical example of such a condition is minimal change disease [13]. A severe proteinuria is typical, yet it does not progress to chronic renal failure and the long-term prognosis is excellent. In view of the available information on the role of proteinuria in the development of renal failure, a good prognosis and the absence of extraglomerular changes in minimal change

glomerulopathy may seem amazing. The reason for such a phenomenon is believed to lie in the high selectivity of proteinuria. And indeed, also in other glomerulopathies a high selectivity of proteinuria in the course of nephrotic syndrome is generally associated with good nephrologic prognosis. The degree of proteinuria selectivity may be even helpful in classifying patients to groups characterized by a good or poor response to steroid therapy. Another factor resulting in good prognosis in minimal change disease may be the fact that steroid therapy usually results in disappearance of proteinuria, what as a rule diminishes the possibility of tubular and interstitial damage. In contrast, in other, progressive glomerulopathies, proteinuria is usually non-selective and may persist for a long time despite therapy [4, 6, 46, 47, 48, 55]. However, an argument against explaining the good prognosis in minimal change disease by a good therapeutic response can be found in papers demonstrating interstitial lesions in glomerulonephritis to be present soon after the symptoms onset [25]. Hruby et al. studied cases of glomerulonephritis in their early clinical stages, i.e. in patients in whom biopsies were performed less than 2 months after the onset of symptoms. The investigators demonstrated in their material a significant association between the increased interstitial volume and tubular loss on the one hand, and subsequent renal function on the other, the latter being assessed 6 and 24 months after the diagnosis [25].

In chronic kidney diseases, the key component of the interstitium, necessary for the connective tissue matrix production, is the myofibroblast. In IgA nephropathy Ando et al. demonstrated that the presence of myofibroblastic cells in the interstitium exerted a negative effect on the prognosis; steroid therapy combined with heparin administration decreased both the number of myofibroblasts and the rate of renal function loss [3]. In membranous glomerulopathy Roberts et al. found that an increase in the number of myofibroblasts is significantly associated with the degree of interstitial fibrosis and decline of renal function. Both these factors manifested a significant prognostic value [50]. Danilewicz et al. also investigated membranous glomerulopathy and observed a significant increase of smooth muscle actin expression in the renal interstitial cells. The author also noted the significant positive correlation between the presence of myofibroblasts and lymphohistiocytic infiltration and fibrosis in the interstitium [19].

Associations with proteinuria

As it has been mentioned, a major cause of tubulointerstitial lesions in glomerulonephritis is the effect of high protein levels in renal tubule contents, what results in the activation of the tubular epithelial cells, which produce various mediators, and finally in the production of extracellular substance components by interstitial myofibroblasts.

As has been suggested in numerous papers, the level of proteinuria may directly affect the prognosis. According to many authors, this is one of the most important laboratory indicators of unfavorable clinical evolution of glomerular disease. The composition of urinary proteins may be also of prognostic significance. It is long known that in progressive kidney diseases, including glomerulonephritis, low-protein diet results in improved renal function. Most likely it also slows down the progression of the disease. An explanation of the good effect of low-protein diet in glomerulonephritis may be found in the decreased production of TGF- β .

Bazzi et al. demonstrated that electrophoretic determination of the composition of urinary proteins may be used for establishing the prognosis in glomerulonephritis. In the material analyzed by the above authors, the presence of low molecular weight proteins (below 10kD) in the urine was associated with an almost 50% risk of chronic renal failure as compared to 12% in the remaining cases. This association remained statistically significant when the investigated group was limited to patients with low creatinine level. According to Bazzi's report, the composition of urinary proteins was only slightly associated with degree of interstitial fibrosis [7]. On the other hand, the same team later reported significant association between the degree of proteinuria, selectivity and the progression of interstitial scarring [6]. Berg et al. believed an increased urinary IgG level to be a particularly sensitive index of the risk of renal failure progression [9]. In turn Remuzzi and Bertani found that the ratio of proteinuria to the creatinine level was an excellent indicator defining the risk of chronic renal failure. When this ratio was below 1, the risk of an unfavorable course of the disease could be regarded as negligible [48].

The question arises whether there is an association between the level of proteinuria and the progression of interstitial changes. No such association was observed by Jepsen and Mortensen [26]. In membranous glomerulonephritis Roberts found a significant association between the presence of myofibroblasts in the interstitium and the level of proteinuria. On the other hand, the latter was not associated with increased interstitial volume [50]. Magil studied patients with membranous glomerulonephritis and demonstrated a significant correlation between the level of proteinuria and the progression of tubulo-interstitial changes. The univariate analysis also revealed an association between interstitial changes and plasma protein levels, which, nevertheless, was not seen in the multivariate regression model. We might assume that the effect was fully explained by the level of proteinuria. In addition, this author did not observe any association between creatinine level and the intensity of interstitial lesions. He explained this findings by the selection bias effect, as less advanced cases were chosen for his study.

Since Magil excluded from his set of data cases with pronounced vascular changes and fibrosis involving more than 10% of the glomeruli, this might also be an argument confirming the opinion that interstitial changes constitute an epiphenomenon, while vascular changes play a decisive role in preserving renal function. On the other hand, numerous publications stressed that the association between the interstitial volume and creatinine level was seen only when the relative interstitial volume was higher than 20%. In the Magil's material, when the analysis was repeated in the entire set of cases, the expected correlation between the relative interstitial volume and renal function reappeared [39].

In the context of the importance of proteinuria for the pathogenesis of interstitial fibrosis, the above quoted ambiguous results may appear surprising. Yet, when we explore the problem thoroughly, we may specify possible reasons responsible for the failure in demonstrating an association between an increase in the relative interstitial volume and proteinuria level. Firstly, the relation between proteinuria and interstitial fibrosis is complex and other factors contribute to the development of interstitial lesions, such as substances produced by inflammatory cells within the very glomerulus or an immune reaction occurring in the interstitium. The composition of proteins excreted to the urine may be of significance in inducing changes in the tubular epithelium and thus in the interstitium. Secondly, the time span when chronic tubulo-interstitial changes develop may be prolonged and determinations of proteinuria levels at a given time point, i. e. at the time of the biopsy may not necessarily reflect the significant proteinuria level influencing tubulointerstitium. This explanation is not in full agreement with the results of Danilewicz et al. [15]. These authors observed a distinct and significant increase in the interstitial volume in patients who were biopsied in the early stage of membranous GLN, i.e. approximately six months on the average following the onset of symptoms. Similar conclusions may be drawn from the report by Hruby et al., who detected an increase of the relative interstitial volume already in the very early phase of glomerulonephritis [25].

The associations between the interstitial volume and renal function are not specific for glomerulonephritis. Akin associations between increased interstitial volume and impaired renal function were demonstrated in renal amyloidosis [37]. In this case, contrary to GLN, a highly significant association between renal function and degree of glomerular lesions (i. e. amyloid deposition) was found. Also in kidneys allograft recipients, interstitial fibrosis and the increased interstitial volume are associated with renal function and constitute an important cause of graft function loss. These lesions may be due to chronic rejection, but also can be evoked by adverse effects of drugs, especially calcineurine inhibitors.

Interstitial changes - but why?

The explanation of why an increase in the relative interstitial volume is the most effective prognostic factor in glomerular diseases is neither easy nor self-evident. The extent and the anatomical continuity of the interstitium within the kidney may facilitate the spread of the pathological process over the entire organ, including regions that were spared by primary processes [44]. In routine renal biopsies, the increase of interstitial volume is often not uniform. Various levels of scarring seen in various regions of the interstitium reflect the variable activity and proportion of changes within the nephrons situated in neighbouring region. Thus, data on interstitial lesions may provide information on an area of the kidney larger than nephrons, and especially glomeruli physically present in the investigated histological material. Impaired renal function may to a greater degree depend on the generalized impaired function of the preserved nephrons than on a simple decrease of their number. A high usefulness of interstitial changes in establishing the prognosis may also result from the fact that their measurement is to a much lesser degree subject to sample bias as compared to glomerular changes. The percentage of fully hyalinized glomeruli assessed by a biopsy is highly susceptible to the random factors. In fact, the number of nephrons in each normal kidney is 800,000 - 1,200,000, while the number of glomeruli in a biopsy specimen rarely exceeds 20 - 30, so histological examinations is performed on 1/27,000 - 60,000 of all glomeruli at the most [32, 35]. Sclerosis involving some glomeruli in individuals who do not manifest any symptoms of kidney diseases and the age-dependent interstitial volume increase are well documented both in humans and in experimental animals. In healthy men below 40 years of life the percentage of sclerosed glomeruli may equal 5%, to reach as high as 30% in subject above 70 years of life [26, 28, 29]. Thus the mere presence of single hyalinized glomeruli - even in a representative biopsy specimen - does not necessarily indicate a kidney disease, and to an even greater degree does not allow for establishing a prognosis. The age-dependent emergence of sclerosed glomeruli in the kidney of otherwise healthy persons is explained by vascular changes that spontaneously progress with age. This explanation was confirmed by Kasiske, who stated that the degree of angiosclerosis was associated with the number of hyalinized glomeruli [27]. Nevertheless, his observation was not confirmed by McLachlan et al. [41].

Counter-arguments

Despite a number of evidence confirming that extra-glomerular changes, and especially an increased relative interstitial volume, are significant for renal function and the prognosis, this concept is at times questioned. In particular,

some authors question whether the cause-effect relation indeed occurs here.

Katafuchi et al. studied patients with Berger disease and compared the prognostic value of a semiquantitative assessment of glomerular changes with the complete index of histological changes, including both interstitial and vascular changes. The authors achieved the most reliable prognosis estimate when their assessment was limited only to changes within the glomeruli. On the other hand, in their previous report, the same team stated that when a multivariate analysis was employed, the increase of the interstitial volume and interstitial fibrosis of the kidney constituted significant indicators of the risk of organ failure [30, 31]. Soma et al. found that contrary to Berger disease, in membranoproliferative glomerulonephritis tubulo-interstitial changes did not contribute to the progression of the disease. The authors compared changes in the relative interstitial volume in these two diseases. They observed that in IgA nephropathy in patients with an unfavorable clinical course a significant increase in the interstitial volume occurred. On the other hand, in membranoproliferative glomerulonephritis no such association was noted [54].

Another argument raised against the theory that interstitial lesions would be a significant factor of renal failure progression is that the interstitial volume increases spontaneously with age, both in the population of people free of kidney disease symptoms and in patients with documented nephropathy. The mechanism of "physiological" interstitial fibrosis is said to be associated with the activity of TGF- β , and thus analogous to that underlying "pathological" fibrosis [20, 21]. Parallel to the interstitial expansion, a decline in renal function is observed. Thus Danilewicz et al. observed significantly higher values of the relative interstitial volume in IgA nephritis patients older than 45 years as compared with younger [18]. Similar results were published by Mackensen-Haen et al. [36]. One might then argue that the increase of interstitial volume is a phenomenon that accompanies the development of chronic renal failure, but bears no relation to the disease. To contradict this reproach, it is necessary to include the patients' age in the analysis. An alternative explanation of the above results may be that elderly patients may have gone through a longer period of an asymptomatic disease. Particularly in the case of Berger disease such an explanation seems plausible, in view of the prolonged and often relatively asymptomatic clinical course.

The discrepancies in published results concerning prognostic significance of interstitial changes may be explained in several ways. According to Ong and Fine [47], one of the reasons is that some of the observed morphological changes are reversible. Mackensen-Haen et al. [36] noted that the association between the interstitial volume and creatinine level was stronger when patients with acute renal failure were excluded from the analyzed group. In addition, as stated

by Nath, at least in some cases of serially repeated biopsies, degree of histologically determined interstitial lesions and biochemical symptoms of renal failure were not fully parallel [43]. It may even happen that in some cases an increase of the interstitial volume is accompanied by an outright drop in creatinine levels. Bennett et al. found that in IgA nephropathy in repeated biopsies there was no direct relationship between renal failure and increased interstitial volume [8]. As it has been already mentioned, in some cases this discrepancy may be the result of acute renal failure. And indeed, this is known to occur in Berger disease.

Cellular infiltration in the renal interstitium

As was said before, we know from the pathogenetic studies that the appearance of inflammatory cell infiltration within the interstitium is important for development of extraglomerular lesions. Such foci of more or less intensive inflammatory infiltration are seen in the interstitium in several cases of glomerulopathies. An exception is again minimal change disease. Interstitial cells include predominantly lymphocytes and macrophages, as well as granulocytes and mast cells. Lymphocytes that are present in inflammatory infiltration mostly belong to the T population; the CD4/CD8 cells ratio is increased [2, 14].

Intensive cellular infiltration is most often seen in glomerular diseases of high activity and considerable clinical aggressiveness, such as active proliferative forms of lupus nephritis or membranoproliferative glomerulonephritis. In the light of such observations, as well as in view of the pathogenesis of interstitial fibrosis, it is interesting whether the presence of cellular infiltration within the interstitium may exert a negative effect on the prognosis. According to the majority of reports, this question is answered positively. Remains to be clarified to what degree this effect is an independent prognostic factor [14].

Lee et al. found that in Berger disease the number of inflammatory cells in the interstitium was positively correlated with creatinine level. The number of CD3, CD4 and CD8-positive cells was also associated with the mean blood pressure. However, the authors did not observe similar correlations in membranous glomerulopathy [34]. Roberts et al. also did not observe a significant association between renal function and the prognosis in membranous glomerulopathy and the increased intensity of inflammatory infiltration in the interstitium [50]. The significant prognostic value of interstitial inflammatory infiltrates in IgA nephropathy was demonstrated by Freese et al., where it was the most important prognostic factor in addition to tubular loss and interstitial fibrosis [22]. Similarly as glomerular and extraglomerular fibrosis, also the number of cells in interstitial inflammatory infiltration is said to better correlate with renal function loss than does the number of cells within a glomerulus [2]. In the

opinion of Naiker et al., in the course of membranoproliferative glomerulonephritis the number of cells within the interstitium significantly affects renal function [42]. Of the investigated types of inflammatory cells, T lymphocytes were the most important for renal function, while B lymphocytes and macrophages demonstrated weaker, but still significant associations with it. The authors stated that the number of cells, mostly of T lymphocytes, within the renal interstitium in membranoproliferative glomerulonephritis was significantly higher than in other glomerular diseases. In addition, the investigators found a significant correlation between creatinine level and the number of inflammatory cells. Nevertheless, such results might have been a consequence of the fact that in this highly aggressive disease the inflammatory infiltration is usually pronounced. Thus the conclusion describing a significant association between the number of inflammatory cells and creatinine level might be only a consequence of natural differences between various diseases. Also in the case of ANCA-associated glomerulonephritis, the extent of interstitial infiltration is a prognostic factor. Other prognosticators include necrosis of the tubular epithelium and tubular loss. The histological parameter that shows the strongest association with the prognosis is the percentage of intact glomeruli [5].

Renal tubules

The tubular epithelial cells play a very important role in the pathogenesis of extraglomerular lesions. Impaired tubular function is assumed to be co-responsible for the development of chronic renal failure in patients with prolonged glomerular diseases; according to some authors this is an outright decisive factor. Changes in the tubular epithelium might potentially be a prognostic factor. In routine biopsy material tubular loss is usually seen in conjunction with interstitial scarring or the presence of inflammatory infiltration. The structure and size of tubular epithelial cells are in a great measure dependent on the method of fixation and the processing of the material. This is easily observed in animal studies when we compare the structure of a perfusion-fixed kidney with an organ that has been fixed by immersion in a fixing solution. Therefore, tubular measurements may be difficult to perform.

Khan and Sinniah analyzed various forms of glomerulonephritis and - apart from the association between creatinine clearance and interstitial volume - demonstrated the existence of an association of renal function and the degree of tubular damage, involving both the proximal and distal tubules. The authors reported that the measurement of the cross-section surface area of the distal tubule was a particularly effective indicator. The fact that changes in the distal tubule appear readily and in the early stage of the disease is supposed to result from its physiological properties. And indeed, in the above quoted paper changes involving the distal tubules were more intense than those noted in the

proximal ones. A strong association with renal function is, in turn, justified by the role of distal tubules in maintaining the tubulo-glomerular balance [33]. On the other hand, Hruby et al. observed that in the early stage of glomerulopathy the changes preferentially involve the proximal part of the nephron [25].

In the report of Mackensen-Haen et al. the interstitial volume in various glomerulopathies showed a strong association with creatinine level, while the surface area of the cross-section of the proximal tubular epithelium and Henle's loop were rather associated with urine osmolarity [35]. Based on material including solely patients with Berger disease, Mackensen-Haen et al. showed also an association between creatinine level and both the interstitial volume and the surface area of the cross-section of proximal tubules. The latter correlation was seen only when patients with acute renal failure were excluded from the investigated group [36]. In their report Freese et al. found that tubular loss was correlated with IgA nephropathy progression. The authors investigated patients with Berger disease who developed end-stage renal failure in the follow-up period and required renal replacement therapy. They observed that interstitial fibrosis, infiltration in the interstitium and tubular loss were significantly more common in such material than in other cases [22].

Summary

As it follows from the above reported data, tubulo-glomerular changes in GLN are of an extreme prognostic importance. The relations between individual components of the kidney are complex and not fully understood. Yet the majority of investigators agree that the relative interstitial volume is the most important prognostic factor. Thus the assessment of this parameter is of a paramount practical importance and should be incorporated in routine renal biopsy evaluations.

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