

Tubulo-Interstitial Injury in Glomerulonephritis: Causes and Consequences

Ljubica Djukanovic, Neda Bankovic-Calic, Stevan Pavlovic, Miodrag Sindjic, Jovan Dimitrijevic, Radomir Naumovic

Clinic of Nephrology, Clinical Center of Serbia, Belgrade

Glomerular diseases present the main cause of end-stage renal disease (ESRD) in many European countries and the third main cause of ESRD, after diabetes and hypertension, in North America. The high prevalence of glomerular diseases has resulted in ongoing investigations into the pathogenesis and treatment, especially in the factors that contribute to the progression of the renal failure. These factors are considered the targets of therapeutic measures. Since the clinical parameters present at the time of renal biopsy revealed limited prognostic value in some studies (1,2), the investigations were directed at the pathohistological characteristics as the prognostic indices.

Tubulointerstitial lesions – the major determinant of the course and outcome of primary glomerulonephritides.

In the late seventies of the results of clinicomorphological comparisons undertaken by several authors' groups indicated that the intensity of glomerular lesions did not have a significant effect on the course and survival of patients with the most types of GN (1,3). At the same time Bohle's group published several papers that first suggested the significance of tubulointerstitial (TI) changes for the prognosis of various types of GN based on the correlation between kidney function and TI changes measured morphometrically (1,4,5). In one of the first papers they ascertained that the serum creatinine correlates with increased relative interstitial volume, regardless of intensity of glomerular lesion (4). Later numerous studies of the same authors' group, as well as many others, confirmed significant correlation between severity of TI lesions and kidney function, the course and outcome of both primary and secondary GN (6-11).

Predictive value of clinical and histological factors in the progression and the outcome of primary glomerulonephritis.

Analysis of renal biopsy proven glomerulonephritis at Clinic of Nephrology, Clinical Center of Serbia

In the period of 1988 to 2001, 1369 kidney biopsies were performed at Clinic of Nephrology, Clinical Center of Serbia. The diagnosis of primary GN was confirmed in 655 patients with the following incidence: minimal change nephrotic syndrome (MCNS) in 52 (8%), focal segmental glomerulosclerosis (FSG) in 72 (11%), membranous nephropathy (MN) in 97 (15%), IgA nephropathy (IgAN) in 85 (13%), mesangioproliferative GN (MZGN) without IgA in 32 (5%), membranoproliferative GN (MPGN) in 59 (9%).

The majority of patients were treated and regularly followed-up in the Outpatients Department of the Clinic. Using this clinicopathological material several studies were designed to review the course, outcome and the predictors of the prognosis of particular types of GN. The kidney biopsy samples of all patients involved in these studies were investigated morphometrically and following semiquantitative scores were determined: glomerular index (12), vascular index, interstitial infiltration, interstitial fibrosis and tubular atrophy indexes (13).

Factors predicting prognosis of GN.

The first study included 160 patients (104 males, aged 35±11 years) with primary GN (26 with MCNS, 31 with FSG, 26 with MZGN, 28 with IgAN, 37 with MN and 12 with MPGN) followed-up for three years after kidney biopsy. Using mathematical model of discriminatory analysis, the discriminatory power of 19 clinical and morphological parameters present at the time of the kidney biopsy were evaluated for various degrees of renal function impairment at the time of kidney biopsy and three years later. The analysis revealed that the pivotal discriminatory variables were endogenous creatinine clearance, proteinuria, nephrotic syndrome and semiquantitative scores of glomerular, tubular and vascular injury. In Table 1 discriminatory power of selected parameters for kidney function after three years of kidney biopsy is presented. Creatinine clearance and proteinuria at the time of kidney biopsy has the highest discriminatory power among clinical parameters. Among morphological parameters, the index of interstitial fibrosis has the highest discriminatory power in all type of GN while in FSG, IgAN and MN glomerular index has equal discriminatory power for the renal function three years later after biopsy (14,15). Discriminatory power in this study is numerical information of "value" that certain factor present at the time of biopsy have for later outcome of primary GN.

Artificial neural network in prediction of membranous nephropathy prognosis.

Retrospective clinicomorphological study of 75 patients (48 males, aged 15-65 years) with primary MN was undertaken with the aim to investigate the applicability of artificial neural network (ANN) in prediction of therapy response and prognosis of the disease. At the time of kidney biopsy 70 patients had nephrotic syndrome, 27 hypertension and 12 mild chronic renal failure. Significant linear correlation was

found between kidney function, blood pressure and age of the patients and glomerular and TI index at the time of biopsy.

The patients were regularly followed from 72 to 144 months after kidney biopsy. Initial steroid treatment was applied in 49 patients; in 30 steroid-resistant patients cyclophosphamide was added; Ponticelli's protocol was applied in 17 patients. Complete remission was achieved in 12 out of 47 steroid treated patients, in 7 of patients treated with steroids and cyclophosphamide and in 6 patients on Ponticelli's protocol. During the follow-up period 18 patients developed CRF and four of them started hemodialysis. Clinical, laboratory and semiquantitative histomorphometric variables were used as input for previously developed and instructed ANN. It was shown that the age, glomerular index and index of interstitial fibrosis had the highest predictive value for prognosis of MN. Using these variables in ANN unambiguous predictions of therapy response were made for 79% patients (16). These results indicated that the use of ANN for prediction of the therapy selection and response as well as prediction of the course and prognosis of MN could be reliable but the model should be tested in the prospective study.

Factors influencing outcome of focal segmental glomerulosclerosis.

The long-term retrospective study, which included 38 patients (26 males, aged 15-69 years) with FSG was undertaken with the aim to find out the factors influencing the course and outcome of the disease. During follow-up period of 36-120 months (averagely 72 months) 16 patients developed ESRD, while in remaining complete or incomplete remission of nephrotic syndrome with stable renal function was achieved. The most powerful influence on patients and kidney actuarial survival had intensity of tubular atrophy (Figure 1). Intensity of interstitial fibrosis, glomerular sclerosis and impaired creatinine clearance at the time of biopsy had also significant influence on the outcome of FSG (17).

The results of three studies summarized here are in accordance with many others clinicomorphological studies showing the significant influence of TI lesions on kidney function, course and outcome of primary GN but the intensity of glomerular lesion should not be underestimated.

Mechanisms of tubulointerstitial injuries

TI inflammation and fibrosis and tubular atrophy characterize most forms of progressive nephropathies regardless the primary pathology is glomerular, tubular or vascular. As TI changes present the common pathway for the ESRD, numerous studies have been investigating underlying mechanisms.

Induction of local chemotactic and adhesive factors that leads to the local influx, accumulation and activation of leukocytes presents the first events of TI injury. Different factors can induce these processes: proteinuria, direct effects of infective agents, toxins, drugs, immune mechanisms, renal ischemia, increased intratubular pressure (18).

Proteinuria, one of the main factors causing TI injury in GN, involves several different pathways of actions. Endocytosis of proteins by the proximal tubule with lysosomal "overload" and leakage of lysosomal enzymes presents one of the causes of TI injury (18). Besides, endocytic uptake of proteins disturbs collagen homeostasis in proximal tubular cells enhancing collagen synthesis and reducing collagen degradation (19). On the other hand, proteinuric urine may contain cytokines, transferrin, membrane attack complex (C5b-9), free fatty acids and all these compounds can mediate TI injury (18-20). At the same time, expression of leukocyte adhesion molecules by vascular endothelium contributes to influx of leukocyte, predominantly monocyte-macrophages and T cells. Different factors stimulate proliferation of the macrophages accumulated in TI space. The most significant influence on proliferation and survival of macrophages has macrophage colony stimulating factor (MCSF), which expression is up regulated in human GN (21). Besides, these accumulated cells release oxidants and cytokines and some of them (IL-2, γ -interferon) stimulate macrophages to produce fibrogenic cytokines causing fibroblast proliferation and collagen synthesis (18,21,22). Numerous evidences over the last decade indicate that infiltration of TI by mononuclear cells play a key role in the evolution of TI inflammation and fibrosis.

Role of the tubular cells in tubulointerstitial injury.

Any of above mentioned factors may activate tubular cells leading to expression of nuclear factor- κ B and nuclear transcription factors and production of osteopontin (OPN), cytokines and growth factors. Production of these mediators by tubular cells contributes to attraction of inflammatory cells. Besides, fibrogenic cytokines and growth factors activate fibroblasts to proliferate and secrete collagens (18,22). Some tubular cells may transdifferentiate into myofibroblast partly explaining tubular atrophy and the local increase of interstitial fibroblast (23). Regardless of their origin, interstitial fibroblasts contribute to extracellular matrix synthesis and development of TI fibrosis. There are many cytokines, growth factors and adhesion molecules that are involved in these processes but TGF- β is a key regulator of epithelial-myofibroblast transdifferentiation and fibrosis development (18, 23, 24).

Vascular changes and ischemia

A loss of peritubular capillaries has already been described in the first papers on the relationship between TI changes and kidney function (5). Recent studies have explained that these capillaries loss develops due to increased endothelial cell apoptosis and decreased endothelial cell proliferation. Factors mediating this impaired angiogenic response are described in several papers and can be divided in pro-angiogenic (VEGF, nitric oxide, TGF-B, angiopoetin, fibroblast growth factor, hepatocyte growth factor, interleukin-8) and anti-angiogenic factors (inhibition of nitric oxide, thrombospondin-1, uric acid, TNF-A, TNF-B, angiostatin, endostatin) (18, 22,24). In addition to peritubular capillary loss the thickening of preglomerular vessels develops due to

increase in vascular smooth muscle cell number, infiltration of macrophages and deposition of extracellular matrix. Both these vascular lesions result in ischemia, which contribute to TI injury development.

Conclusion

TI injury is recognized as the major determinant of the course and outcome of primary GN. Different factors may induce interstitial inflammation characterized by infiltration of leukocytes, predominantly monocyte-macrophages. These accumulated cells proliferate and produce different mediators, especially fibrogenic cytokines causing fibroblast proliferation and collagen synthesis. Tubular cells may also transdifferentiate to fibroblasts contributing to extracellular matrix synthesis and development of TI fibrosis. In addition, peritubular capillary loss and thickening of preglomerular vessels cause ischemia and aggravate TI injury. Elucidation of mechanisms inducing and stimulating development of TI injury may provide new insight into therapy of different chronic renal diseases.

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