

ORIGINAL PAPER

Histopathologic and immunohistochemical aspects of the renal parenchyma in patients with glomerulonephritis developed in locked-up spaces

D. GH. MĂLĂESCU¹⁾, M. MOTOCU²⁾, LETIȚIA ADELA MARIA STREBA³⁾,
ADRIANA BOLD⁴⁾

¹⁾General practitioner, Targu Jiu

²⁾Faculty of Medicine, University of Oradea

³⁾Department of Internal Medicine 1,
"Filantropia" University Hospital of Craiova

⁴⁾Department of Histology
University of Medicine and Pharmacy of Craiova

Abstract

A number of 38 renal biopsies and 13 necroptic pieces removed from 51 prisoners were available for our study. From 51 cases, 21 patients were diagnosed with actual chronic glomerulonephritis, 19 patients with non-specific chronic glomerulonephritis, four patients with renal amyloidosis, and seven patients with glomerulonephritis lesions associated to pielonephritis.

Keywords: locked-up space, glomerular nephropathies, pielonephritis, immunohistochemistry.

Background

A patient in the locked-up space can be defined as a person with both the physical and the psychic capacity modifications, due to the total changed environment conditions inside the penitentiaries, different from those of the every day normal life outside the penitentiaries, thus making the prisoner vulnerable to different diseases.

Excretory apparatus pathology raises special problems because the prisoners use to come too late to the medical consulting room; consequently, nephropathies and demises frequently occur.

Starting from those observations, the authors suggested investigating some immunohistochemical and histopathologic aspects of the renal parenchyma in the persons clinically diagnosed with glomerulopathies.

Patients and methods

Our histopathologic and immunohistochemical study was achieved by using a number of 38 adults, male patients, aged between 18–57 years, having been locked-up renal puncture was a differential and positive diagnosis necessity, patient's evolution under correct medical treatment was unfavorable. A number of 13 cases were added to that group; histopathologic exam was imposed as consequence of the patient's death inside the locked-up space of the penitentiary.

Fragments of kidneys removed by either puncture biopsy or necropsy were fixed in neuter formaldehyde solution wax embedded, sectioned by a microscope

and adequate Hematoxylin–Eosin, trichrome with Goldner–Szeckelly green or Congo red stained. LSAB technique was used for the immunohistochemical study of the cytokeratins, collagen IV, vimentin and CD31 endothelial antigens.

Results

One of the most frequently identified renal lesion in 21 patients was the extracapillary or crescentic glomerulonephritis which is microscopically characterized by the proliferation of the parietal membrane epithelium belonging to Bowman's capsule, and formation of some epithelial crescents in a ring disposition around the glomerular ball.

Mentions must be made that the microscopic lesions observed at the renal parenchyma level were extremely various from one case to another and even from one area to another one in the kidney of the same patient, which made us stating the glomerulonephritis identified on the histologic pieces were of a diffuse type.

We also noticed that periglomerular cell proliferation was for many times inhomogeneous, around the same ball of renal glomerular capillaries revealing intense proliferatory areas made up of 5–8 overlaid cell rows and some areas were the "crescent" was made up of 2–3 cell layers.

Areas with hipercellularity were observed as more frequent at the level of the urinary pole of the renal glomerulus while areas with reduced cellularity were

observed around the vascular pole of the glomerulus. That histologic aspect we had observed, demonstrated that the most reactive area of Bowmann's casual epithelium was around the urinary pole (Figure 1).

Cell proliferation of the capsular epithelium led to the periglomerular renal parenchyma compression, therefore determining vascular and tubular changes but also diminishing the glomerular filtration room up to its disappearance; that may explain anuria, which is present in the patients with extracapillary chronic glomerulonephritis.

Sometimes, the component cells of the "crescents" had a various morphology that made us consider that besides the epithelial cells, other cell types may be found into the structure of the periglomerular crescent, such as fibroblasts, lymphocytes, macrophages. Fibroblastic type cells from the crescent structures may be of an extraglomerular origin as the came by the proliferation of the fibroblasts existing into the renal stroma of the glomerulus nearest proximity. Uriniferous tubules appeared with the atrophic epithelium, with the lumen distended by the leucocytary or bleeding cylinders.

The presence of the leucocytary cylinders is a sign of acutization of the inflammatory processes of the chronic glomerulonephritis and the presence of the hematic cylinders (Figure 2) pointed some larger lesions existence of the glomerular filtrating membrane allowing the sanguine cell elements to pass into the filtration space of the primary urine and further on into the intrarenal urinary pathways.

The existence of the chronic inflammatory moderate infiltrate prevalently made up of lymphocytes, plasmocytes, macrophages and sometimes neutrophil polymorphonuclear leucocytes was revealed into the renal interstitium.

The presence of those cells indicated some antigens penetrations into the renal interstitium. Besides those acutized chronic glomerulonephritis forms in a number of 19 patients, we identified images of nonspecific chronic glomerulonephritis clinically expressed by chronic renal failure signs, by microscopic exams, after renal biopsy punction had been performed.

Colligation to the clinical date helped us to identify their causes; that is why we considered that those lesions can be grouped within the chronic primitive glomerulonephritis category. Glomeruli appeared more rarefied probably due to some processes of involution and associated glomerular sclerosis. Frequently we observed a hypertrophy of some restant glomeruli (Figure 3).

That glomerular hypertrophy was accompanied by mesangium proliferation, during the first stage of the glomerulopathy evolution. Mesangium proliferation is a reaction of answering to the presence of some antigens or some mediators of the inflammation penetrating the glomerular capillary walls and going on intercapillary. As they are cells similar to the fibroblasts, mesangial cells have the capacity to synthesize the mesangial stromal components, which, in their turn can be changed in pathologic cases.

Interstitially, in those cases, the presence of a chronic inflammatory process was also pointed. That is why we consider that the persistence of the inflammatory process determined collagen or amyloid synthesis that influenced upon the renal glomerulus leading to the glomerular seize reduction, sclerohyalinosis followed by a hard disturbing of the renal function and the appearance of the clinical manifestations.

As we observed, the process of the renal glomerulus sclerohyalinosis appeared both extra and intra glomerularly. It seemed that the process started at the Bowmann's capsule external membrane level, as concerning the glomerulus level. Here, a thick fibrous band made up of collagen fibers appeared instead of the "cellular crescent" which disappeared; that thick band progressively increased and reduced the size and the numbers of the intraglomerular capillaries (Figure 4).

Finally, glomerulus place is taken by an eosinophil sclerohyaline formation rich in collagen fibers that may also consist of some restant mesangial cells (Figure 5).

Lesions of the uriniferous tubules always accompanied glomerular changes. Most frequently we remarked uriniferous tubules with an extended lumen, atrophic sometimes discontinuous epithelium, hyalinic or hematic cylinders in lumen. Renal glomeruli disappearances were accompanied by tubular necrosis resulting in the rarefiation of the tubular structures form the renal parenchyma. Instead of the parenchimatous structures we often found collagen fibers with an untidy disposition surrounding the parenchimatous rests or they contained other rests belonging to the immunitary system, especially lymphocytes, plamocytes and macrophages (Figure 6).

Those sclerohyalinic changes varied form one patient to another probably according to associated tares, the capacity of the immunitary system, etc. Sclerohyalinic changes of the intersitium are due to the activity of the stromal fibroblasts producing both collagen fibers and uncollagenous matrix filling the spaces created by destroying the glomeruli and the uriniferous tubules.

Another histological form of glomerulopathy met in the patients studied by us was associated to the renal amyloidosis. This type of lesion was established on necroptic pieces that came form four demised patients diagnosed with chronic renal failure. Microscopically, at the renal glomerulus level, there were revealed, by specific staining with red of Congo, some amyloid depositions, a very big quantity through the capillary ansae.

In other seven patients, the most frequent renal lesions were of chronic pielonephritis associated to glomerulonephritis. In those patients, we remarked a great morphologic polymorphism, minimal glomerular and tubular affection areas and other surfaces where the glomeruli, uriniferous tubules and even the interstitium were completely altered. In other areas, we observed many fibrosis bands having a disposition from the renal cortical periphery towards pelvis, with severe glomerular affection.

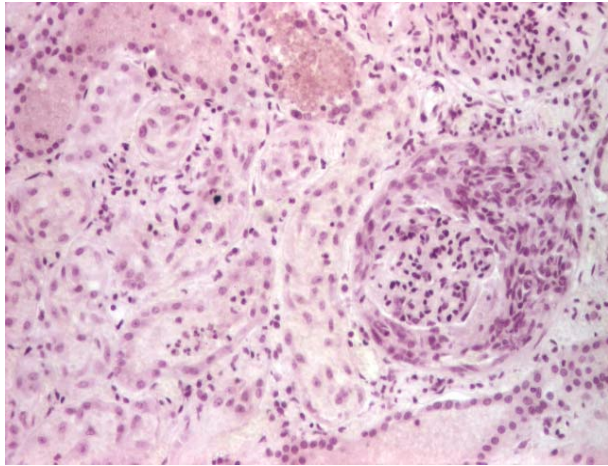


Figure 1 – Crescentic glomerulonephritis with “half-moon” proliferation of the Bowman’s capsule and chronic inflammatory infiltrate from a renal insufficiency deceased patient (Hematoxylin–Eosin staining, ×100)

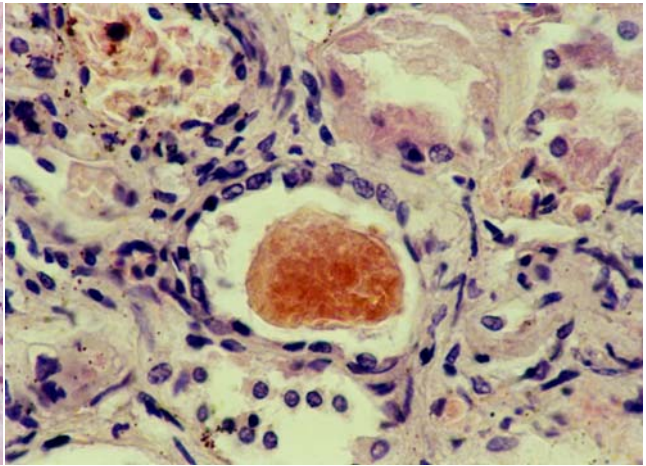


Figure 2 – Chronic primitive glomerulonephritis. Hematic cylinders in the convoluted tubes (Hematoxylin–Eosin staining, ×100)

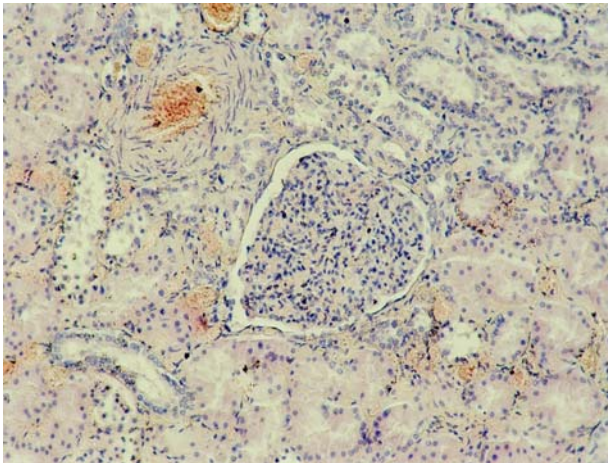


Figure 3 – Microscopic image of a kidney from a patient with non-specific chronic glomerulonephritis, where the glomerular hypertrophy due to mesangial cells proliferation can be observed (Hematoxylin–Eosin staining, ×100)

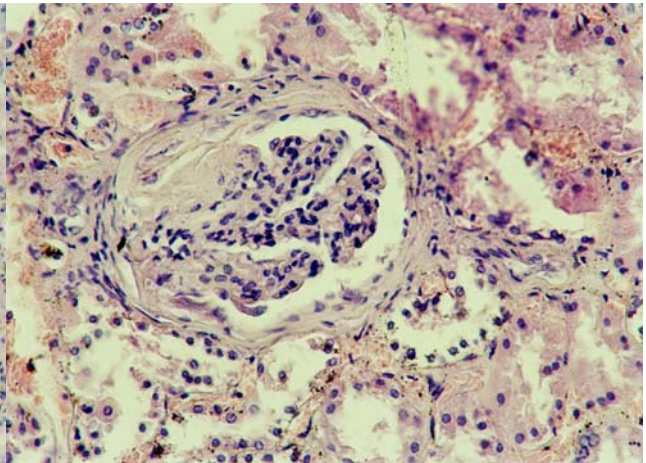


Figure 4 – Glomerulonephritis with the injury mainly of the parietal layer of Bowman’s capsule (Hematoxylin–Eosin staining, ×200)

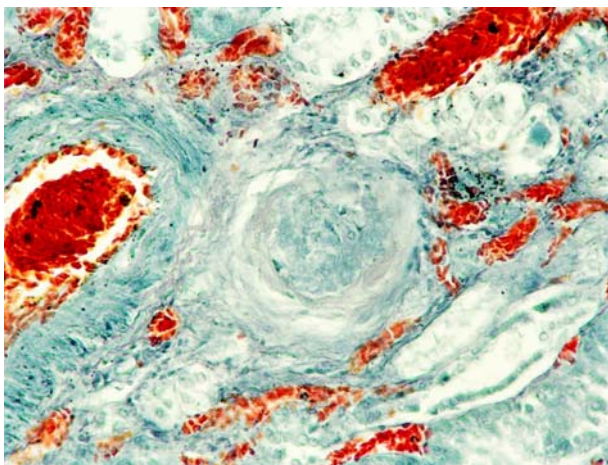


Figure 5 – Complete glomerular sclero-hyalinosis and interstitial vascular congestion (trichromic Goldner–Szeckelly staining, ×100)

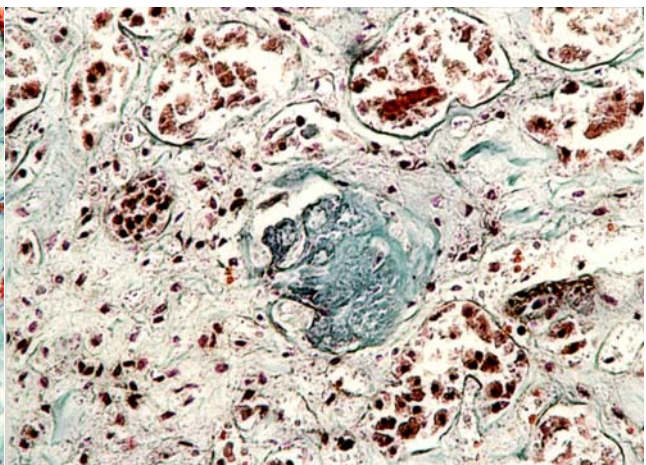


Figure 6 – Glomerular sclero-hyalinosis associated with tubular necrosis and interstitial sclerosis (trichromic Goldner–Szeckelly staining, ×400)

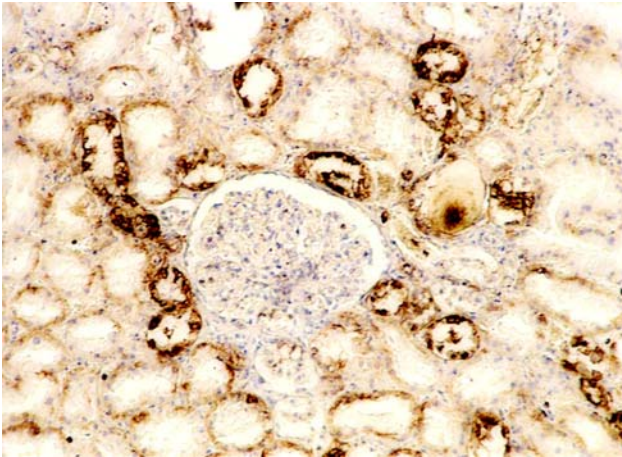


Figure 7 – Positive reaction in distal convoluted tubes and negative in glomerulus and proximal convoluted tubes (AE1/AE3 immunostaining, $\times 100$)

Figure 8 – Renal glomerulus with positive vimentin reaction, $\times 200$

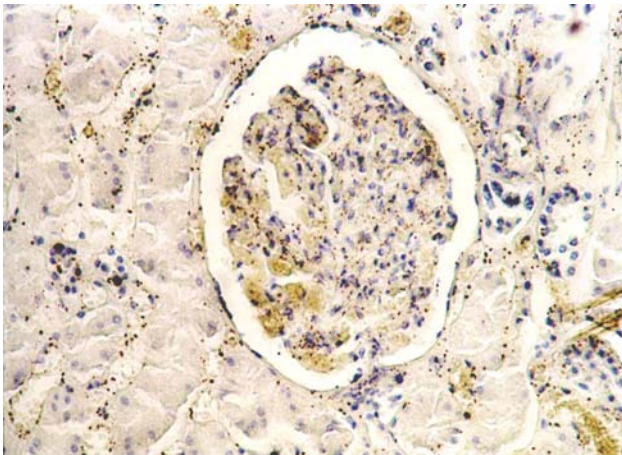
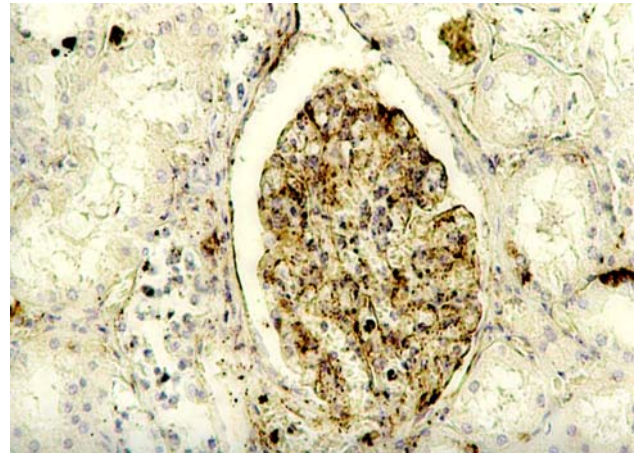
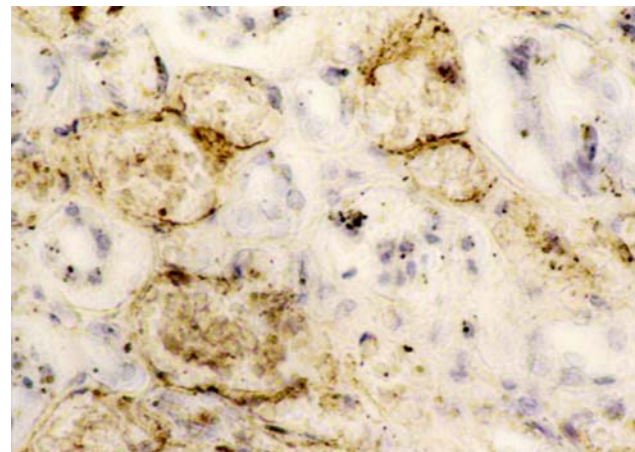


Figure 9 – Hypertrophic renal glomerulus with enlarged capsular space, with unhomogenous reaction at collagen IV, which proves a profound injury of the filtrating membrane (collagen IV immunostaining, $\times 200$)

Figure 10 – Blood vessels with interrupted wall and inconstant reaction at CD31 antibody from a renal medulla with microhemorrhages (CD31 immunostaining, $\times 200$)



One of the characteristic lesions observed in the patients, with chronic pielonephritis was the "pseudotirodization" of the renal parenchyma. Uriniferous tubules appeared with their extended lumini, atrophic flattened epithelium, with hyalinic cylinders in lumen giving a microscopic image similar to that of the tiroidian lobules. Renal interstitium appeared as reduced, with many lymphocytes, plasmocytes and macrophages.

We consider that locked-up spaces in penitentiaries were favorable for such a pathology that could originate from the reduced immunity state of the patient, their lack of medical education and body hygiene and even from the crowded spaces.

Immunohistochemical study was performed on a number of 27 pieces or renal biopsy. It had as an aim to complete the histopathologic study. By this investigation we suggested to reveal the variations of some immunohistochemical markers such as AE1/AE3 cytokeratins, vimentin, collagen IV and CD31 receptors, to add new data to the clinical and histopathological diagnosis, as concerning the change of the nephrocytes cytoskeletons (AE1/AE3) depositions of collagen from the renal sclerohyalinosis and the affection of the basal membrane in some IRC patients (collagen IV) mesangial changes (vimentin) and also the endothelial cell lesions accompanying the changes of the blood vessels.

By using the anticytokeratinic antibodies we were allowed to reveal that the cytokeratins had been absent into the epithelial capsule cells of the renal glomerulus. Into the proximal convoluted tubules, the immunohistochemical reaction had an average intensity, being more increased at the basal pole of the nephrocytes and more reduced at the apical pole.

The cytokeratin depositions into the contort proximal tubule can be explained by that, at the apical pole, nephrocytes skeleton is poor represented, the former having been endowed with microvillus necessary to the process of tubular reabsorption.

By analyzing the microscopic pieces with stronger lens we established an inhomogeneous immunohistochemical reaction of the nephrocytes from the same level of the proximal contort tubule, thus proving that the affection of nephrocytes varied from one cell to another in glomerulopathies. This immunohistochemical aspect is the proof of the clinical fact that the renal failure installed progressively, when more and more nephrocytes under went changes of both organites and cytoskeleton (Figure 7).

Immunohistochemical reaction to vimentin appeared as positive at the level of the renal glomerulus thus marking electively the mesangial cells. In those cases diagnosed by classic staining, with glomerular hypertrophy with mesangial proliferation, immunohistochemical reaction to vimentin was an intense one, as a clear proof for the mesangial cell proliferation.

Vimentin was also positive into the renal glomeruli with advanced involution marking the mesangial cells and even into the completely hyalinised glomeruli that what made us consider that the mesangial cells are

similar to the fibroblasts, having been resistant to hypoxia and capable to synthesize biochemical compounds present into the mesangial fibrosclerous matrix (Figure 8).

Another immunohistochemical marker we were interested in was collagen IV, in order to see if its synthesis increased at the level of the involution or with an intense mesangial reaction renal glomeruli. As we observed on our pieces (Figure 9) collagen IV was revealed in reduced quantities at the level of glomeruli that allowing us to state that collagen IV quantity was that small in patients with glomerulonephritis.

Having into consideration that we remarked vascular changes on the classic histological study, we suggested revealing the endothelial cells immunohistochemically by using CD31 monoclonal antibodies.

The reaction of the endothelial cells to CD1 antibody was variable; it was very intense at the level of the normal or little congested vessels. Endothelial cells from the strong congested vessels had a poorer and inhomogeneous reaction which was given, on one hand, by the increase of the endothelial cell surface and, on the other hand, by the structural change of the endothelial cells undergoing a mechanic stress or other factors which were employed in the glomerular disease etiopathogeny.

There, where we observed hematic extravasats or micro-hemorrhagic on the classic histological material, blood vessels with a broken endothelium presented a poor immunohistochemical reaction as a proof of the endothelial cell affection and of the vascular permeability change (Figure 10).

Discussions

The half-moon shaped epithelial mass formation is considered to be a reaction of Bowmann's capsule epithelium to the fibrin and hematic exudates in the capsular space or to the antigens that pass through the filtrating membrane from blood to the capsular space.

Although the factors involved in organization of cellular to fibrotic crescents have not been fully elucidated, a ruptured Bowman's capsule facilitates the progressive organization of cellular crescents by permitting the entry of activated periglomerular T-cells and fibroblasts into Bowman's space [1, 2].

Recent data show that the half-moon cells are formed not only throughout the proliferation of Bowmann's capsule epithelium but, some part of them, are in fact the monocytes/macrophages from the blood of the glomerulus capillary which arrive in the Bowmann's space passing through the basal membrane.

The extra-capillary cellular proliferation, which conducts to the formation of the half-moon cellular masses around the glomerulus, is considered to be triggered by the appearance of some brakes in the basal membrane of the glomerulus capillary produced by inflammation mediators generated by monocytes and polymorphonuclear leucocytes. If the brakes alter the entire filtrating membrane, the half-moon images are formed mainly by monocytes and T-lymphocytes,

the proliferation of the Bowman's capsule epithelium having less importance [3].

Animal studies showed that the first morphological changes that lead to the appearance of the half-moon cells are the laying down of immune complexes in the glomerulus capillary wall, which activates the cellular adhesion molecules and stimulates the polymorphonuclear leucocytes and the macrophages, which release metalloproteinase type enzymes and O₂ free radicals in the extra-cellular medium. These products generate brakes of the filtrating membrane that allow molecules such as fibrinogen to escape in the urinary space. In addition, the activated monocytes express on their surface a pro-coagulant factor, which produces fibrin polymerization. The appearance of fibrinogen in the urinary space and its polymerization into fibrin is the main event, which produces the extra-capillary proliferation and the thickening of the half-moon cells.

Activation and proliferation of epithelial cells, monocytes, macrophages, fibroblasts, myofibroblasts and mast cells have been implicated in the formation of crescents and their evolution to fibrosis [4, 5].

Other studies demonstrated that the fibroblasts and the mesangial cells are part of the Bowman's capsule epithelium, which forms the majority of the half-moon cells. The appearance of the mesangial cells in the filtrating chamber of the kidney glomerulus is another indicating fact, which demonstrates that the filtrating membrane is altered.

It is also known that the migration of interstitial myofibroblasts into the Bowman's space through holes in the Bowman's capsule may also contribute to the pathogenesis of glomerulosclerosis [6]. These cells have been identified within crescents demonstrating features of fibrocellular to fibrous organization [4].

The appearance of these microscopic formations is a severity maker of the glomerulus disorder but does not indicate the etiology or the pathogenesis of the glomerular injury [7].

It is considered that any glomerular inflammatory process, which produces the brake of the capillaries, determines the entrance of pro-inflammatory factors in the Bowman space, stimulating the formation of the half-moon cells. Fibrinogen and hematic exudates are the most frequent incriminated factors as they are the biochemical elements implicated in cellular proliferation.

Certain proteoglycans are also involved in the development of fibrous crescents via activation of myofibroblasts and TGF- β_1 [8]. Connective tissue growth factor (CTGF) has been recently shown to be involved in the extracellular matrix production in parietal epithelial cells via TGF- β pathway promoting the scarring process in glomerular crescents [9, 10].

The half-moon cells have three evolution stages [7]:

- half-moon cells formed out of epithelial cells and macrophages;
- fibro-cellular half-moons – fibrillar elements appear near the cells;
- sclerous half-moons – the fibrillar tissue replaces the cells, and there is a small number of macrophages.

The change of the glomerular volume due to hypertrophy was often accompanied by hypercellularity as a consequence of mesangial proliferation. The last takes place parallel with the reduction of the capillary number and the fibrosis of the parietal layer of Bowman's capsule, but these changes may not appear.

The change of the glomerular volume was unequal so that, on histological slides, more or less hypertrophic glomeruli, interstitial sclerosis or sclero-hyalinosis was observed, giving the aspect of lesion polymorphism, well highlighted if trichromic staining was used [11].

The immunohistochemical study highlighted important changes of the infra-cellular structure on patients suffering one of the glomeruli diseases.

AE1/AE3 cytokeratin study demonstrated changes of the intensity of the immunohistochemical reaction in proximal and distal convoluted tubes. High intensity reaction was observed in the proximal convoluted tube, especially in the basal part of renal cells, while the cells in Henle's loop and the distal convoluted tube had a very weak reaction. Some cells of the proximal convoluted tube had a medium or weak reaction which explains not only the disorder of the cytokeratin filaments of the cellular skeleton but also a perturbation of the tubular reabsorption processes, which is correlated with the constant proteinuria in all studied patients [12].

Vimentin, a marker of the mesenchymal origin cells, reaction was intense in the glomeruli, which presented mesangial proliferation.

Collagen IV appeared in reduced quantity in the structure of the glomerular basal membrane, which indicates a perturbation of the filtrating processes on glomerulonephritis patients. The collagen IV quantity was much reduced or even absent in sclero-hyalinosis associated with glomerular involution, proving that maybe other types of collagen are synthesized during these processes.

CD31 antibody immunoreaction demonstrated that vascular endothelium cells lesions such as wall lesions with interstitial microhemorrhages accompany the glomerular lesions.

☐ Conclusions

The histopathological study on 38 renal biopsies and 13 necrotic pieces removed from 51 prisoners, revealed that 21 patients were diagnosed with acutized chronic glomerulonephritis, 19 patients with non-specific chronic glomerulonephritis, four patients with renal amyloidosis, and seven patients with glomerulonephritis lesions associated to pielonephritis.

Vimentin immunomarking revealed a proliferation of the mesangial cells in glomerular hypertrophies, and endothelial cell modifications in association with the CD31 monoclonal antibodies that explain the increase of the vascular permeability and interstitial microhemorrhages.

The cytokeratin modifications from the nephrocytes level explain the proteinuria and the hydroelectrolytes variation.

During the process of glomerular involution and sclerohyalinosis, collagen IV was revealed in small quantities at the level of filtrating membrane of the glomeruli, because other types of collagen are synthesized.

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Corresponding author

Dan Gheorghe Mălăescu, MD, Popa Șapcă Street, Bl. 4, Sc. 2, Et. 3, Ap. 5, 210 143 Târgu Jiu, Romania; Phone +40253–217 490, Fax +40253–219 438, E-mail: dmalaescu@dgp.ro

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