CASE REPORT

RJME
Romanian Journal of Morphology & Embryology
http://www.rjme.ro/

The association of polymorphonuclears with humps in acute postinfectious glomerulonephritis

E. MANDACHE, M. N. PENESCU

Department of Nephropathology,
"Carol Davila" Clinical Hospital for Nephrology, Bucharest

Abstract

It is currently considered that hump dense deposits developed during an acute poststreptococcal glomerulonephritis become finally dissolute by three hypothetical mechanisms: loosing their electron density, internalization and processing by podocytes and by incorporation in the glomerular basal lamina (GBM). Analyzing ultrastructurally the association of polymorphonuclear leukocytes and hump deposits, we emphasized features endorsing the hypothesis that the immune complexes of dense deposits are discharged in the circulation under the leukocytes activity. The active polymorphonuclear cells are melting the GBM in the area of contact by complement activation and by the NAPlr bound plasmin. The reversed flow of immune complexes from humps towards the blood circulation leaves fading, wrinkled shaped humps, before total dissolution.

Keywords: hump, dissolution, leukocyte, acute postinfectious glomerulonephritis.

Introduction

Proliferative postinfectious glomerulonephritis is caused by an acute bacterial or viral infection localized somewhere else in the body. Most common it is a consequence of a streptococcal infection. In this case, the condition is defined as acute poststreptococcal glomerulonephritis. The histologic pattern of the injury during the acute phase is a diffuse proliferative endocapillary glomerulonephritis. The most affected age by this condition is from 5 to 20 years of age. The clinical usual features are hematuria, proteinuria, oliguria, edema and renal failure. Hypocomplementemia and rising antistreptococcal antibody titers are also frequent. For documenting a streptococcal pharyngitis, the evaluation of rising antistreptolysin O (ASLO) titers is very indicative [1].

The most frequent histologic pattern of poststreptococcal glomerulonephritis (APSGN) is an acute diffuse proliferative glomerulonephritis characterized mainly by numerous neutrophil leukocytes and endocapillary hypercellularity involving all glomeruli with variable intensity. The leukocytes are placed within the capillaries, adherent to the capillary walls. About half of patients develop crescents. With special light microscopy techniques, large subepithelial immune deposits may be identified in some cases. By immunofluorescence, the common finding in the acute phase is a granular capillary and mesangial staining for complement alone, or complement and immunoglobulins, while in the resolving phase there is only mesangial staining. The common intense staining is for the C3 complement component, the membrane attack complex included. The patterns of this immunostaining have been categorized as: starry sky, garland and mesangial [2, 3].

The hallmark of acute postinfectious glomerulonephritis is the occurrence of electron-dense large subepithelial deposits, known as humps. These deposits are prominent and contiguous, without adjacent spikes and can be several per capillary loop. The humps placed on the capillary wall tend to resolve first. Thus, during the late resolving stage the hump deposits can be found only on the paramesangial glomerular basement membrane (GBM). Initially, humps are denser then the lamina densa of GBM. During the acute and resolving phases, there may be thinning and lamination of GBM beneath the humps [4]. Subepithelial dense deposits are sometime continuous with intramembranous dense deposits [5, 6]. The epithelial cytoplasm overlaying the deposits contains condensed cytoskeletal elements, mostly actin. The endothelial layer of capillaries is often swollen and focally denuded in areas where neutrophils become marginated, sometimes adjacent to the subepithelial dense deposits [4].

Two major antigens have presently been identified as the potential causes of APSGN: a nephritis strain-associated protein (NSAP), and the nephritis-associated plasmin receptor (NAPlr) [7]. The localization of these two antigens was observed in the mesangial matrix and on the endothelial side of GBM [8]. Once bound to glomeruli NAPlr may capture plasmin activated by streptokinase. Bound plasmin can cause tissue destruction by direct action on the GBM, or by indirect activation of procollagenases and other matrix metalloproteinases. NAPlr can also activate the complement pathway, leading to accumulation of polymorphonuclear cells and macrophages and developing a local inflamemation. In addition, the circulating immune complexes can readily pass through the altered GBM and accumulate in the subepithelial space as humps [9].

ISSN (print) 1220–0522 ISSN (on-line) 2066–8279

Both the evolution and the resolution of hump dense deposits are still under consideration. Beyond one month since the disease onset, the humps electron density becomes fading. It was stated by electron microscopy that all humps do finally disappear, but the mechanism of dissolution is a field of several interpretations [1, 6, 10].

Our findings in this field are trying to contribute with ultrastructural details in this direction.

Materials and Methods

An 8-year-old girl has been admitted with nephritic syndrome. Proteinuria was 1.47 g%, macroscopic hematuria and slight generalized edema were present, ASLO antibody titer was 319 u, the blood C3 fraction was low (21 mg%) and C4 was normal (29 mg%). Leukocytes and thrombocytes were in the normal range (21 000 and 449 000/mm^3 respectively).

Four weeks later, the patient was submitted to kidney biopsy. The biopsy was performed with a 16 G GBL guillotine needle, under anesthesia and ultrasound control. The resulting sample was a 2 cm long kidney fragment including the whole cortical layer.

The sample was divided in two, for immunofluorescence and for electron microscopy. The first fresh fragment was snap frozen and sectioned in a cryostat. The 4 μm thick sections were reacted with ten FITC-conjugated antibodies including anti-C3c.

The sample for electron microscopy was fragmented in several 1-mm^3 blocks in a few drops of 4% buffered glutaraldehyde and fixed for several hours. After over night washing in buffer solution, the samples were post-fixed in buffered 1% osmic acid for one hour. This post-fixation was then followed by the classic procedure of dehydration in graded ethylic alcohols and embedded in a Maraglas epoxy resin. The following procedure was cutting thick and then thin sections. The 70 nm thin sections obtained with a diamond knife were targeted on glomeruli. Six glomeruli were selected and processed according to this procedure and finally double stained with uranyl acetate and lead citrate.

The thin sections were thoroughly examined with a JEM 1011 transmission electron microscope and pictures were obtained with a CCD Megaview G2 camera.

Results

The main finding in immunofluorescence was a C3c labeling of glomeruli according to the so-called "starry sky" pattern, characteristic for the acute postinfectious glomerulonephritis.

The electron microscopy provided essential arguments for this diagnostic. Dense deposits were very frequent, mainly epimembranous and some subendothelial. Glomerular capillaries were stuffed with polymorphonuclears almost exclusively (Figure 1). The epimembranous deposits, usually named "humps", were round or ovoid mildly dense structures, a little more dense then the GBM (Figure 2). Some humps looked like round independent deposits surrounded only by podocyte cytoplasm (Figure 3). On the other side of GBM, most humps were faced by leukocytes suggesting some kind of affinity between these two elements. Some of these leukocytes were in close contact with the thin endothelial cytoplasm also known as lamina fenestrata (Figure 4). From place to place, short leukocyte cytoplasmic processes removed the endothelial layer, and penetrated through it in the subendothelial space, in direct contact with the GBM (Figures 5 and 6). In such areas, the humps and the leukocytes were separated only by GBM. Furthermore, we found similar features were the GBM became thinner, irregular and sometimes more or less disappeared (Figures 7 and 8). In such instances, the leukocytes were in fact in direct contact with the hump deposits (Figure 9). Sometime we have noticed few short cytoplasmic processes stretched by the leukocyte towards the hump (Figure 7).

Some features of the direct contact hump-leukocyte showed smaller, irregular humps, looking like being deflated or sucked of content in the capillary lumen direction (Figures 10–12).

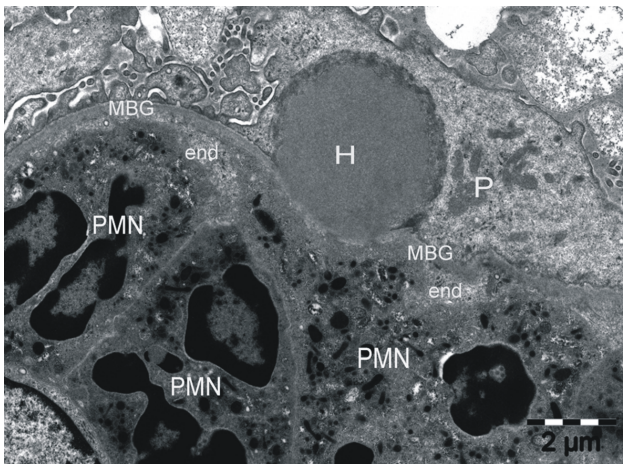

Figure 1 – ***Glomerular capillary stuffed with polymorphonuclears (PMN) showing an epimembranous dense deposit (H). Glomerular basement membrane (GBM), capillary endothelium (end) and a podocyte (P) surrounding the hump.***

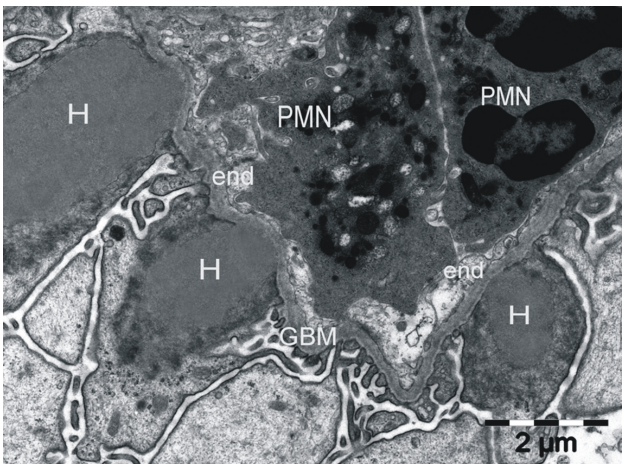

Figure 2 – ***Glomerular capillary containing two polymorphonuclears (PMN) and supporting three round or ovoid mildly dense humps (H). Glomerular basement membrane (GBM) and the thin endothelium (end) known as lamina fenestrata.***

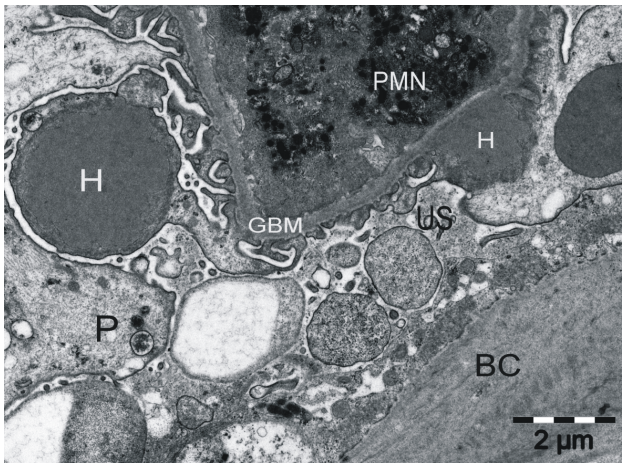

Figure 3 – ***Glomerular capillary loop with glomerular basement membrane (GBM) and two humps (H). One hump is attached to the capillary, and the one in the left side is surrounded only by podocyte cytoplasm (P). Polymorphonuclear (PMN), urinary space (US) and Bowman capsule (BC).***

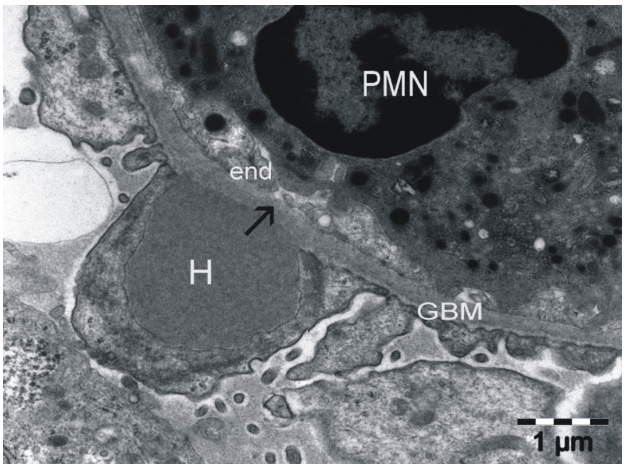

Figure 4 – ***Glomerular capillary wall with a hump (H) denser then the basement membrane (GBM), and lamina fenestrata (end). The intracapillary polymorphonuclear (PMN) has a cytoplasmic short process oriented toward a large fenestra (arrow).***

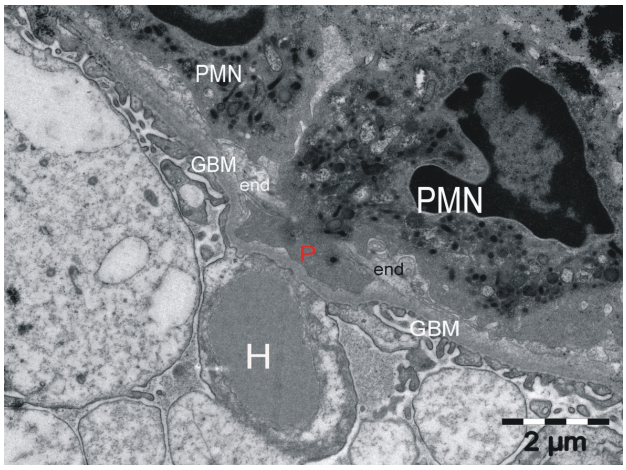

Figure 5 – ***Blood capillary wall with hump (H) attached. Two intracapillary polymorphonuclears (PMN), one showing a cytoplasmic process (red P) protruding in the subendothelial space, in direct contact with the basement membrane (GBM) and facing the hump on the opposite side. The lamina fenestrata of endothelium (end).***

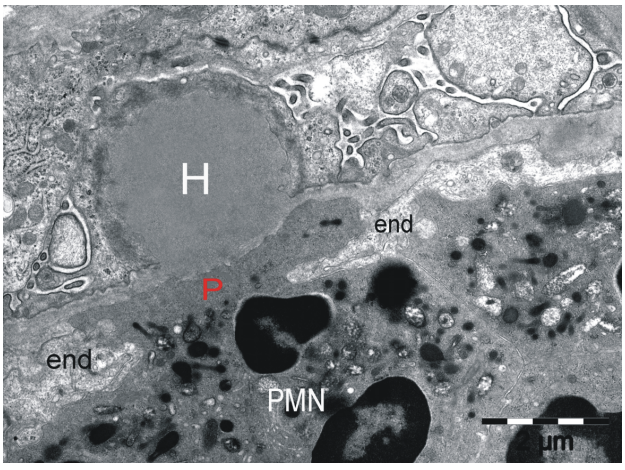

Figure 6 – ***A polymorphonuclear (PMN) cytoplasmic process (red P) penetrating the lamina fenestrata at the base of a hump (H). The endothelium (end) is removed and the basement membrane is thinner then normal.***

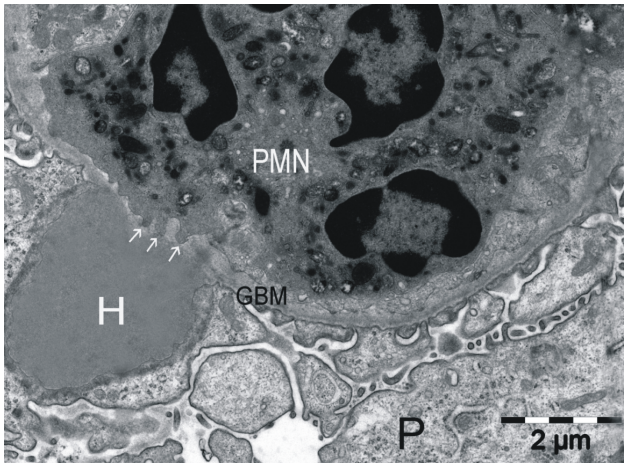

Figure 7 – ***Glomerular capillary wall with hump (H) and three opposite polymorphonuclear (PMN) cytoplasmic processes (arrows). The GBM looks disorganized in this area. Podocyte (P).***

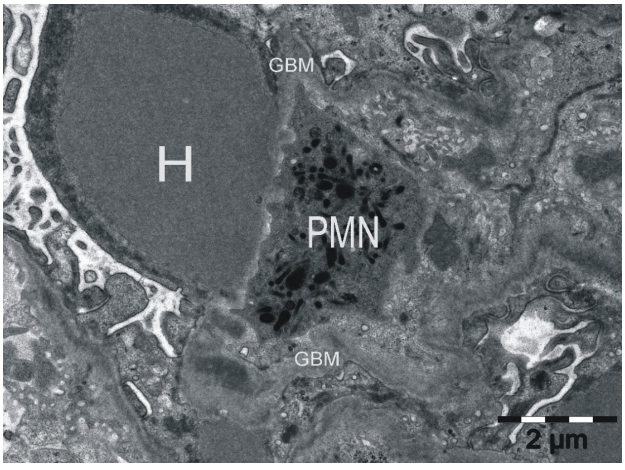

Figure 8 – ***A hump (H) in close relationship with a polymorphonuclear (PMN). A very thin GBM layer is still separating the two structures.***

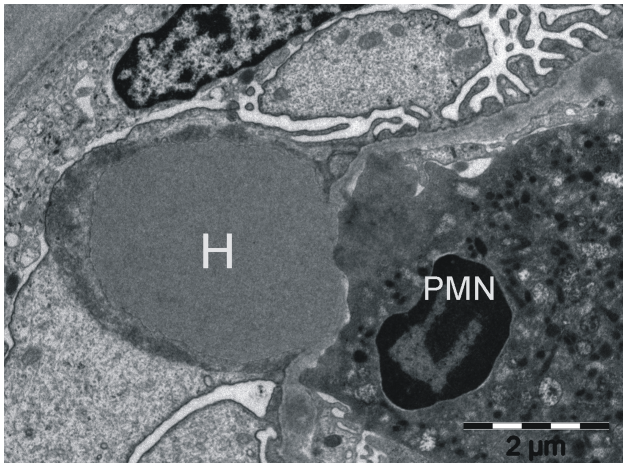

Figure 9 – ***A PMN in direct contact with a hump (H). The basement membrane is optically missing.***

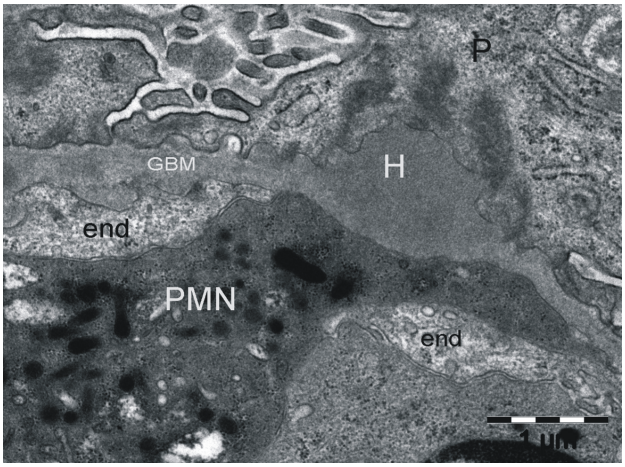

Figure 10 – ***A polymorphonuclear (PMN) process in contact with a hump (H). The hump lost its round regular shape, and became wrinkled, with a sinuous contour. Endothelium removed (end), and podocyte (P).***

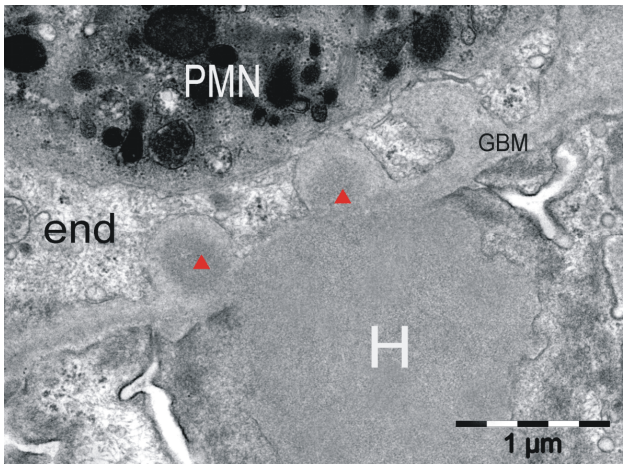

Figure 11 – ***Polymorphonuclear (PMN) and hump (H) in contact, through two endothelial (end) fenestrae (red marks). The GBM seems to be dissolute.***

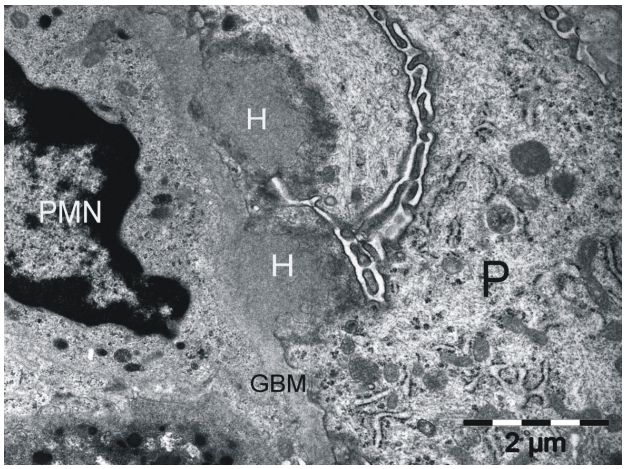

Figure 12 – ***Two small humps (H) with irregular contour and decreased density in contact with an intracapillary PMN. GBM is disorganized and a podocyte (P) surrounds the humps, while foot processes are missing.***

Discussion

The most attractive feature of our investigation was the interdependency and affinity of leukocytes with humps. This vicinity has been mentioned earlier [1, 4] and considered as a component of the inflammatory process.

Judging upon the humps electron density and the time elapsed between the onset of APSGN and the biopsy we are endowed to consider that the above mentioned humps as being deposits on their way to be cleaned out. Thus, the interrelationship leukocytes-humps concern a possible additional mechanism of deposit dissolution. A sustaining argument in this direction is also the fact that the last surviving humps are those located in the paramesangial areas were the leukocytes have no access [1].

It seems that the active element participating in this mutual relationship is the leukocyte. Infiltration of leukocytes may be promoted by chemotactic factors of the complement system [11]. Increased circulating levels of IL-6, IL-8, tumor necrosis factor-α, and protein-1 have been found in acute post-streptococcal glomerulonephritis [12, 13].

The leukocytes have the ability to generate cytoplasmic processes, in this case penetrating in the subendothelial space. Once in contact with the GBM, such a process gets bigger, covering the entire base of a hump or even larger. In fact, this is the location of the antigen nephritis-associated plasmin receptor (NAPlr) and its corresponding antibody [14]. NAPlr can activate the complement pathway, and thus inducing an income of polymorphonuclears and monocytes. Frequent features associated with the leukocyte-GBM contacts show disorganized basement membrane segments. The capacity of NAPlr, planted on the subendothelial side of GBM, to bind plasmin and consequently to cause tissue destruction by direct and indirect effects may be the impulse of GBM melting. Furthermore, the leukocyte plasma membranes develop in these areas short processes towards the hump, feature that signifies a local activity. Once disorganized, the GBM looses its limitation capacity for antigen-antibody complexes of

the hump deposits. Thus, the immune complexes may find the free way back from humps to circulation [10].

Another endorsing feature for the immune complex dissolution in the circulation was the wrinkled shape of some humps in contact with leukocytes. These shrunken humps seem to free their content in the circulation because of the leukocyte activity.

Conclusions

The polymorphonuclear-hump association can be taken into consideration as an alternative way of hump deposits dissolution by discharge of immune complexes in the circulation during the healing process of acute postinfectious glomerulonephritis.

References

[1] D'Agati VD, Jennette JC, Silva FG, *AFIP Nontumor Fascicle 4: Non-neoplastic kidney diseases*, American Registry of Pathology, Washington D.C., 2005, 269–296.

[2] Sorger K, Gessler M, Hübner FK, Köhler H, Olbing H, Schulz W, Thoenes GH, Thoenes W, *Follow-up studies of three subtypes of acute postinfectious glomerulonephritis ascertained by renal biopsy*, Clin Nephrol, 1987, 27(3):111–124.

[3] Sorger K, Gessler U, Hübner FK, Köhler H, Schulz W, Stühlinger W, Thoenes GH, Thoenes W, *Subtypes of acute postinfectious glomerulonephritis. Synopsis of clinical and pathological features*, Clin Nephrol, 1982, 17(3):114–128.

[4] Michael AF Jr, Drummond KN, Good RA, Vernier RL, *Acute poststreptococcal glomerulonephritis: immune deposit disease*, J Clin Invest, 1966, 45(2):237–248.

[5] Roy S 3rd, Pitcock JA, Etteldorf JN, *Prognosis of acute poststreptococcal glomerulonephritis in childhood: prospective study and review of the literature*, Adv Pediatr, 1976, 23:35–69.

[6] Herdson PB, Jennings RB, Earle DP, *Glomerular fine structure in poststreptococcal acute glomerulonephritis*, Arch Pathol, 1966, 81(2):117–128.

[7] Bhimma R, *Acute poststreptococcal glomerulonephritis*; Chief Editor: Langman CB, Medscape Reference: Drugs, Diseases & Procedures, http://emedicine.medscape.com/article/980685-overview, 2011.

[8] Yoshizawa N, Treser G, Sagel I, Ty A, Ahmed U, Lange K, *Demonstration of antigenic sites in glomeruli of patients with acute poststreptococcal glomerulonephritis by immunofluorescein and immunoferritin technics*, Am J Pathol, 1973, 70(1):131–150.

[9] Yoshizawa N, Yamakami K, Fujino M, Oda T, Tamura K, Matsumoto K, Sugisaki T, Boyle MD, *Nephritis-associated plasmin receptor and acute poststreptococcal glomerulinephritis: characterization of the antigen and associated immune response*, J Am Soc Nephrol, 2004, 15(7):1785–1793.

[10] Törnroth T, *The fate of subepithelial deposits in acute poststreptococcal glomerulonephritis*, Lab Invest, 1976, 35(5): 461–474.

[11] Rodríguez-Iturbe B, Batsford S, *Pathogenesis of poststreptococcal glomerulonephritis a century after Clemens von Pirquet*, Kidney Int, 2007, 71(11):1094–1104.

[12] Soto HM, Parra G, Rodríguez-Iturbe B, *Circulating levels of cytokines in poststreptococcal glomerulonephritis*, Clin Nephrol, 1997, 47(1):6–12.

[13] Besbas N, Ozaltin F, Catal F, Ozen S, Topaloglu R, Bakkaloglu A, *Monocyte chemoattractant protein-1 and interleukin-8 levels in children with acute poststreptococcal glomerulonephritis*, Pediatr Nephrol, 2004, 19(8):864–868.

[14] Rodríguez-Iturbe B, Musser JM, *The current state of poststreptococcal glomerulonephritis*, J Am Soc Nephrol, 2008, 19(10):1855–1864.

Corresponding author

Eugen Mandache, MD, PhD, "Carol Davila" Clinical Hospital for Nephrology, 4 Griviţei Avenue, Sector 1, 010731 Bucharest, Romania; Phone +40727–170 204, e-mail: mandache.eugen@yahoo.com

Received: March 8th, 2012

Accepted: August 20th, 2012