The morphology of immune mediated glomerulonephritis – a literature review

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1. Membranous glomerulonephritis

Membranous glomerulonephritis have as a characteristic the precipitation of immune complexes [Ig G, Ig M, C3, membranary atack complexes (C5b-C9)] in all segments of the glomerulus (Trautwein and Hewicker-Trautwein, 2000).

The name of this type of lesion is borrowed from the anglo-saxone literature and illustrates the particular aspect of the basal membranes of the glomerular capillaries. Still, in some situations it has been possible to identify the antigen within the structure of the immune complex retained within the glomerulus. Following this ideea, membranous glomerulonephritis have been observed following infection with adenoviruses in dogs, infestation with *Dirofilaria immitis* in dogs (Grauer and colab., 1989) and infection with feline leukosis virus in cats (FeLV). Still, in 70% of cases these lesions are idiopathic (Lees and col., 1997).

Immune complexes, no matter of their origin, cause glomerular lesions following a similar mechanism. Through elecron-microscopy studies performed on dogs suffering from idiopathic membranous glomerulonephritis the evolutionary steps of this lesion could be described.

In the early phases, very discrete, electron-dense immune deposits could be observed on the epithelial surface of the glomerular basal membranes. Later on, a slight thickening of the basal membrane can be seen as well as a fusion of the podocytic processes on top of the membranary immune deposits with a diffuse or sometimes focalized aspect. Podocytes look swollen and the podocytic processes contain a granular material.

Subsequently, the thickening of the basal membranes becomes evident through the newly synthetized membranary material, visible between the membranary deposits and the basal membrane. This material may be visualized as small dots and spikes on the epithelial side of the glomerular basal membrane imprinting a pectinated aspect, easily recognizable through silver impregnation (Moreau G., 1989, Vilafranca and col., 1994).

Studies regarding membranous glomerulonephritis in humans have pointed out that the spikes are actually extensions of the basal membrane formed of $\alpha 3$ and $\alpha 5$ chains of type IV collagen with laminin and fibronectin (Nevins T.E., 1985, Verlander J.W., 1998).

In the late evolutive stages the membranary spikes fuse with the membranary immune deposits, thus determining the thickening of the capillary wall. Also, we may notice a slight proliferation of the mesangial cells and an increase in the quantity of the mesangial matrix.

Finally, the glomerular basal membrane becomes distorted, folded and loses its polyanionic charge, thus becoming very permissive, especially for seric proteins, leading to proteinuria and nephrotic syndrome.

Histologically, the glomerular basal membrane appears thickened (5-6 times), especially at the level of the peripheral glomerular capillaries (solitary), being distorted and folded (*wire loop* aspect). The lumen of the damaged capillaries appears normal or even enlarged and empty. Also, a slight proliferation of the mesangial cells could be noticed and an increase of the mesangial

matrix. Steatosis and tubular granular dystrophy were also common, as signs of alteration of the glomerular membranes (fig. 1, fig. 2).

In 15% of cases the evolution in favorable, the lesions being reversible in most part, but there are also cases where a slow, progressive evolution towards glomerular sclerosis was noticed, as a consequence of the obliteration of the capillaries. (Moreau G., 1989).

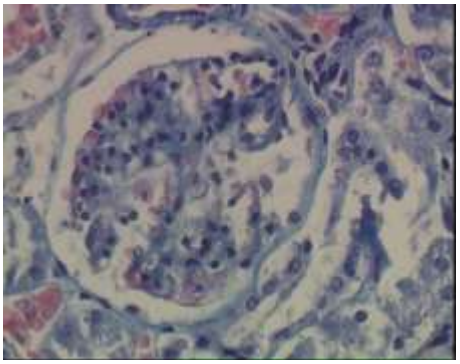

Fig. 1. Membranous glomerulonephritis. Masson trichromic stain, x1000

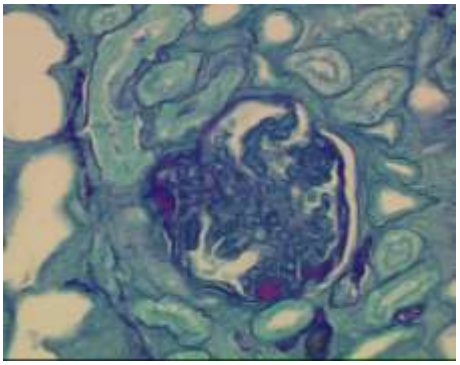

Fig. 2. Membranous glomerulonephritis. PAS stain – light green, x1000

From a clinical point of view, membranous glomerulonephritis are frequently found associated with nephrotic syndrome. Most membranous glomerulonephritis have a generalized and global evolution, are idiopathic (the structure of the circulating immune complexes could not be determined) or the expression of various pathological processes (infections, neoplasia, poisonings or autoimmune diseases) (Carpenter and col., 2002; Scott and col., 2001).

Membranous glomerulonephritis may also be an expression of old age (continuous synthesis of membranous material), heavy metal poisoning (gold, mercury), chronic septic diseases (pyometra in bitches), parasitic infestations, interstitial nephritis (in dogs), systemic metabolic disorders (diabetes, thyroiditis) or idiopathic (nonidentified immune complexes).

2. Mesangial glomerulonephritis (mesangio-proliferative)

Histologically, mesangio-proliferative glomerulonephritis is dominated by cellular proliferations which lead to an appearence of pluricellularity or polynucleosis of the Malpighi corpuscle (Oprean O.Z., 2002; Paul I., 1991) (fig. 3).

This type of immune glomerulonephritis is the most frequently encountered, being dominated by the cellular and matriceal changes of the glomerular mesangium, translated morphologically through a more or less evident increase in the number of cells inside its structure, due to the proliferation of the mesangial and endothelial cells associated with inflammatory type cells (polymorphonuclear cells, mononuclear cells), and an increase of the mesangial matrix and the accumulation of mesangial deposits. The capillaries have thin basal membranes, their lumen is collapsed, they are emptied of blood and sometimes non-existent (Pașca and col., 2006) (fig. 4).

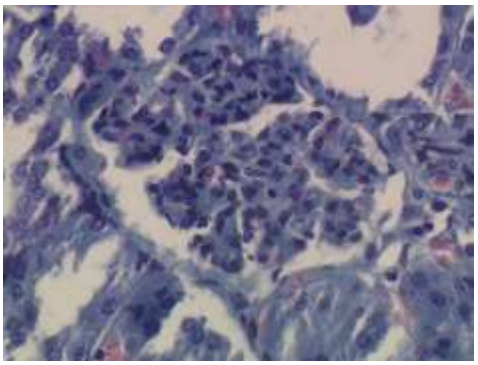

Fig. 3. Mesangio-proliferative glomerulonephritis. Mesangial proliferations lead to the tendency of separation between the glomerular lobules
Masson trichrome stain, x900

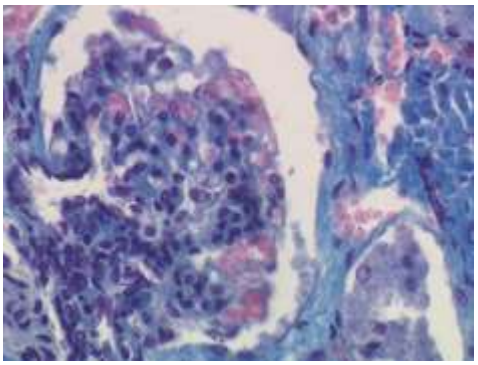

Fig. 4. Mesangio-proliferative glomerulonephritis.
Masson trichrome stain, x900

Proliferative glomerulonephritis may be generalized, affecting the whole glomerulus or segmentary, causing damage to only a few glomerular lobules.
In the case of pure mesangio-proliferative glomerulonephritis, the mesangial and matriceal changes are exclusively limited to the axial region of the glomerulus, without altering the lumen of the glomerular capillaries (Moreau G., 1989).
In segmentary glomerulonephritis, the hyperplasia that occurs within the glomerulus (glomerular polynucleosis) may cause a severe reduction in the caliber of the glomerular capillaries through external compression, leading to complete stenosis, thus determinig a reduction of the glomerular blood perfusion up to sclerosis (Jergens A.E., 1987).
Using electron microscopy we can observe the fusion of the podocytic pedicles. The extra-membranary deposits may be seen with extreme clarity using electron microscopy and using immunohistochemistry we may also highlight regulated, homogenous or granular deposits located in the subendothelial space, along the glomerular basal membrane, deposits that contain IgG and IgM, without the C3 fraction of the complement (Trautwein and Hewicker-Trautwein, 2000).

Most part of cellular proliferations are mediated by factors derived from the complement and platelets. It has been pointed out that the increase in numbers of the cells and in quantity of the mesangial matrix is accompanied by an increase in the level of expression of PDGF (platelet derived growth factor) produced by the endothelial cells and that of the receptor proteins for PDGF, demonstrating the fact that the cellular proliferation is based on an autocrine mechanism.
These proliferative phenomena have as a consequence segmentary glomerular sclerosis, or sometimes even a generalized such transformation, along with the collapse of the glomerular capillaries and the formation of adhesions between the vascular bundle and the Bowman capsule.

The quantitative and qualitative outcomes of these lesions are in indispensable in formulating a histologically based prognosis.

The appearence of segmentary or generalized proliferative glomerulonephritis was observed in infections with *Leishmania sp*. in dogs, mink plasmacytosis, horses and chikens infected with *Streptococcus zooepidermicus*, etc.

3. Membrano-proliferative glomerulonephritis (mesangio-capillary)

Membrano-proliferative glomerulonephritis (MPGN), also called mesangio-capillary, represent a morphoclinical entity characterized through the proliferation of the mesangial cells and at the same

time the thickening of the glomerular basal membrane,thus reuniting both aspects of membranous and mesangio-proliferative glomerulonephritis (fig. 5, fig. 6).

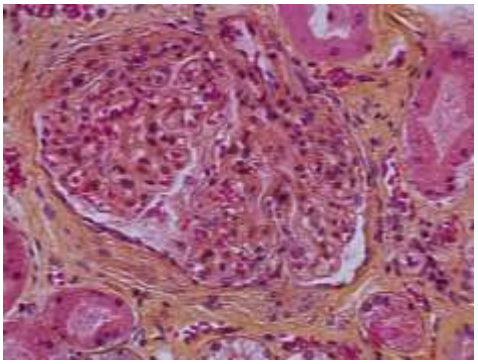

Fig. 5. Membrano-proliferative glomerulonephritis; Adherences glomerulus – capsule. Masson trichrome stain, x200

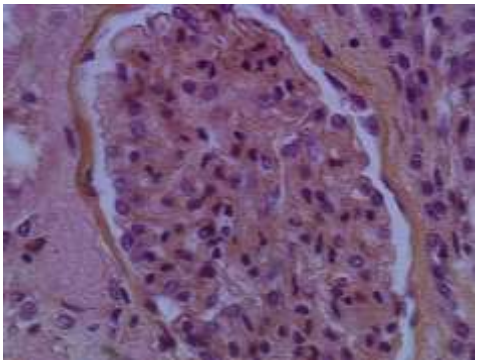

Fig. 6. Membrano-proliferative glomerulonephritis. Masson trichrome stain, x400

These morphological aspects are considered intermediary lesions that become final through generalized chronic glomerulonephritis, leading to chronic renal insuficiency.
Structural changes within this type of lesion explains why these animals manifest with hematuria and nephritis concomitant with proteinuria and nephrotic syndrome.

Membrano-proliferative glomerulonephritis may be associated with systemic illnesses or with a well determined etiology (secondary GN.) or, like in most cases, may be idiopathic (primary).
Membrano-proliferative glomerulonephritis, as a dominant-recessive type autosomatic disease, was described in 20 Bernese Mountain dogs, with ages between 2 and 5 years who showed a renal insufficiency syndrome with severe proteinuria. It appears that the infection with *Borrelia burgdorferi* represents a triggering factor for this pathological process (Gough and Thomas, 2004).
Based on the clinical and pathological aspects in humans and animals we can recognize 3 types of membrano-proliferative glomerulonephritis: type I, II and III, the last being the least known and controversed.

a. MPGN type I

Type I membrano-proliferative glomerulonephritis are characterized, from a histological point of view, by an accentuated lobulation of the glomerulus caused by the proliferation of the mesangial cells and an increased amount of mesangial matrix.

The process of epithelial and membranary proliferation may be extended to the whole glomerulus, from the vascular pole to the urinary one, or may be localized solely to a few capillary branches (glomerular lobules).
In the first situation the glomerulus appears shrunken, more or less atrophic, partially thrombotic or sclerotic.
In the second situation, the affected glomerular branches appear compacted, the rest of the capillaries remaining permeable.

As a whole, this category of glomerulonephritis is characterized by severe lesions that do not ensure healing without sequelae.

The walls of the glomerular capillaries appear thickened due to the subendothelial deposists composed of immune complexes containing immunoglobulins (Ig G, Ig M) and the C3 fraction of the complement (C3 nephrotic factor).

Highlighting within the structure of the immune complex deposits of C1q and C4 complement

fractions indicates that the classical path for activating the complement plays an important role in the pathogenesis of type I MPGN.

Another characteristic aspect of this type of glomerulonephritis is the so called "mesangial interpositioning" that regards the interpositioning of the cells and the mesangial matrix between the endothelium and the basal membrane. This strucure confers a double contour to the glomerular basal membrane also known as a "tram track" aspect, easily observable through silver impregnation and PAS stain.

In dogs, type I membrano-proliferative glomerulonephritis evolve mainly as an idiopathic disease (Trautwein and Hewicker-Trautwein 2000). Still, some data suggest that sometimes it is possible to associate this lesion with systemic diseases, bacterial (leishmaniasis and boreliosis in dogs) or viral infections (feline infectious peritonitis, feline leukosis, equine infectious anemia, african swine fever) and neoplasia (Brostoff and col., 1991, Dambach and col., 1997; Osborne and Finco, 1995).

b. MPGN type II (*Dense Deposit Disease*)

The characteristic histological aspects are represented by the dense thickening of the PAS-positive glomerular basal membrane. The mesangium will appear distended due to the proliferation of the mesangial cells or the matrix.

The definitive diagnosis may be established after highlighting through electron-microscopy the deposits formed by a dense, osmophilic material in the lamina densa of the glomerular basal membrane and the glomerular mesangium.

The same characteristic aspect of "mesangial interpositioning" may also be used to describe the type II membrano-proliferative glomerulonephritis (Cotran and col, 1999).

Also, through immunohistochemistry it has been observed that these deposits are mainly made out of the C3 fraction of the complement and properdine.

Within the pathology of glomerulonephritis in humans it has been noted the intense participation of Ig G auto-antibodies against C3-convertasis, also called C3 nephritic factors (C3NeF). The basal memebrane stabilizes the C3-convertasis, the C3bBb enzyme of the alternative path for the activation of the complement, thus continuing the activation of the C3 fraction with the fomation of C3b and C3a.

The continous activation of the complement leads to its comsumption (hypocomplementemia). The seric level of the C3 fraction is very low, but at the same time the C1q and C4 fractions remain within normal limits.

c. Type III MPGN are characterized by the presence of immune deposits on both sides of the basal membrane at the same time (sub-endothelial and sub-epithelial). The lesion seems to be a variant of the type one (Cheville N., 1994).

Ig A Nephropathy (Berger's Disease)

IgA nephropathy is the most common type of glomerulonephritis in humans and a frequent cause of asymptomatic hematuria. It can also be seen in dogs and experimentaly in mice (Osborne C.A., 1995)

From a morphological point of view it is characterized by IgA mesangial deposits, the proliferation of mesangial cells and the expansion of the mesangial matrix (Brostoff and col., 1991).

Histologically, this glomerular lesion falls into the category of membrano-proliferative glomerulonephritis.

The reseaches of the Béné collective (1984) have established that the disease occurs due to an abnormal synthesis of IgA or the synthesis of IgA with an abnormal structure (Béné and col, 1984).

In the first case, the increased synthesis of IgA is due to a prolongued exposure of the mucosas to an antigen represented by the pathogen during respiratory and digestive infections, which overcomes

the elimination capacity of the monocytic-macrophage system, thus leading to the precipitation of IgA deposits in the renal glomerulus, in the glomerular mesangium (Day and Penhale, 1988).

The amount of circulating Ig A immune complexes is directly proportional with the severity of the infection in the patient due to this abnormal synthesis.

In the second situation, the immune deposits located in the mesangium are composed of IgA1-glycosylate with an abnormal structure. Due to this abnormal structure of IgA1, syaloglyco-proteins (receptors form macrophages) cannot couple with the macrophages of the monocytic-macrophage system to initiate phagocytosis, thus leading to mesangial precipitation (Brostoff and col., 1991).

Glomerular lesions owed to the depositing of immune complexes containing IgA have been observed with an increased frequency in patients with liver cirrhosis. Studies on mice with experimentally induced cirrhosis through poisoning with carbon tetrachloride helped highlight the presence of IgA with an abnormal structure circulating in the blood stream but also located on the glomerular basal membranes.

The presence within the mesangial deposits of the C3 fraction and the final components of the complement, except for C1 and C4, suggests that the activation of the complement through the alternative path mediated by IgA plays an important role in the pathogenesis of this type of mesangio-proliferative glomerulonephritis **(**Brostoff and col., 1991; Slauson and Cooper, 2002).

The diagnostic of IgA nephropathy is based on highlighting through immunofluorescence the Ig A deposits present in the glomerular mesangium (Mc Cluskey R., 1983).

The highest prevalence of glomerular IgA deposits was seen in dogs with enteritis and hepatitis.

In the examined patients deposits containing IgM and IgG could also be observed.

4. Anti basal membrane glomerulonephritis

The name of this category of glomerulonephritis is reserved for the glomerular lesions caused by antibodies targeting intrinsical anti-antigens of the basal membrane which, through interaction lead to the accumulation of membranary immune deposits.

The experimental prototype is called the *Masugi nephritis* or *toxic nephritis* and has been achieved on mice through the administration of mouse anti-kidney antibodies, prepared on rabbit, after immunization with mouse renal tissue.

The glomerulonephritis caused by the anti-basal membrane antibodies is an autoimmune disease with severe and rapid evolution due to the formation of anti-basal membrane antibodies and their depositing inside the renal parenchyma or other extra-renal tissues, more frequently in the glomerulus and sometimes, but not always, in the walls of the pulmonary alveoli. The cause leading to the formation of these antibodies is still unknowkn.

This disease was experimentally induced in sheeps and dogs in order to understand the evolution of immune based glomerular lesions.

Following this lead, the circulating antibodies produced through the experimental immunization using heterologus anti-basal membrane antigens also react with their endogenous antigens.

The diagnostic of this type of glomerulonephritis is based on highlighting the lineary deposits located on the external side of the glomerular capillaries, in the subepithelial space, constituted by anti-basal membrane antibodies and IgG. Much more rarely IgM and IgA could also be observed.

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