STAGE REPORT- 2010

The research material was represented by the kidneys from 28 body of dogs, deceased or euthanized, from stray dogs, with various organopathies, out of which 19 presented morphopathological changes with different intensities, at kidney level.

The cytological examinations of the kidneys in live dogs were performed through echo guided biopsy with thin needle. The dogs were anesthetised, the lumbar region was shaved and disinfected for the biopsy. The puncture was performed through aspiration with a 5 ml syringe with G22 needle. The smears were stained May-Grunwald-Giemsa and examined at optical microscope.

The organs collected after the necropsies were photographed and data sheets were filled in for each of them. Subsequently, 3 to 6 samples were collected from each case, from kidneys and other anatomically and physiologically connected organs (heart, liver, lung, intestine, spleen) for histological examination.

The collected samples were fixed in 10% formaldehyde aqueous substance and/or fixing Bouin liquid, trimming, embedded in paraffin and sectioned at 5 m and stained with Haematoxylin-Eosin method (bichrome colour, HE) Haematoxylin-Eosin – Methyl blue (Masson trichrome colour, HEA), Haematoxylin-Eosin-Safran (HES), Periodic Acid - Schiff Fuxin (PAS), Periodic Acid - Schiff fuxin – light green (PAS-light green), Congo red.

HYSTOLOGICAL ASPECTS IN IMMUNE GLOMERULONEPHRITIS

1. Membranous glomerulonephritis

Membranous glomerulonephritis is an unspecific lesion determined by circulatory immune deposits, very discrete, on the epithelial surface of the glomerular basal membrane and without any inflammatory signs, at first.

Afterwards, a slight thickness of the basal membrane and diffuse or focalised fusion of the podocytes processes takes place over the immune deposits present at membrane level. The podocytes are tumefied and podocytes processes contain a granular material.

In advanced progressive stages, the membrane spiculi are fusing with the immune membrane deposits, the capillary wall being considerable thickened. The thickness of the basal membrane becomes obvious due to the new synthesized membrane material.

For the examined cases, the lesions had no correspondent macroscopically.

From the extension of the process point of view, the lesion is emphasized by a segmented focal character.

Histological, the glomerular basal membrane appeared thicker (of approximately 5-6 time more than normal), especially at the level of the peripheral glomerular capillaries (solitary), being distorted and having a *wire loop* aspect. The lumen of the capillaries was normal, or even distained and empty. A slight proliferation of the mesangial cells was also noticed and the increase of the mesangial matrix. Moreover, it was noticed steatonephrosis and tubular granular dystrophy, resulted from the permeability alteration of the glomerular membranes **(Fig. 1-4).**

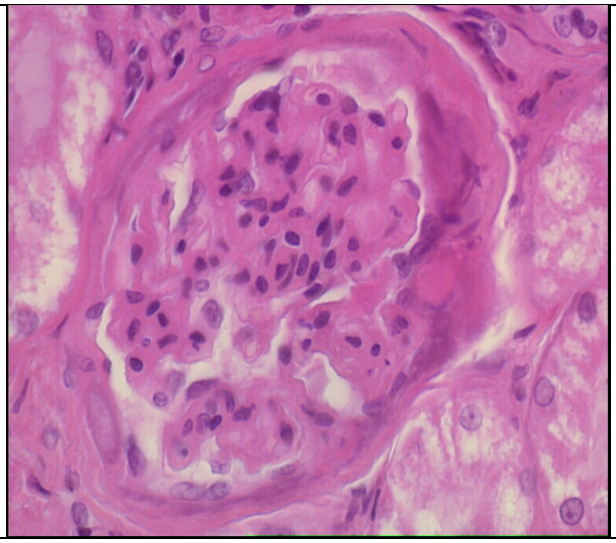

Fig.1 Membranous glomerulonephritis, Col. HE, x 400

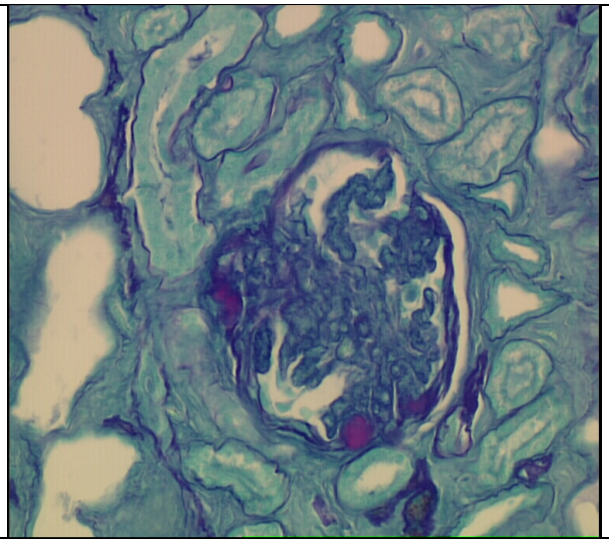

Fig.2. Glomerular basal membranes thickened and doubled.
Col. PAS-bright green, x200

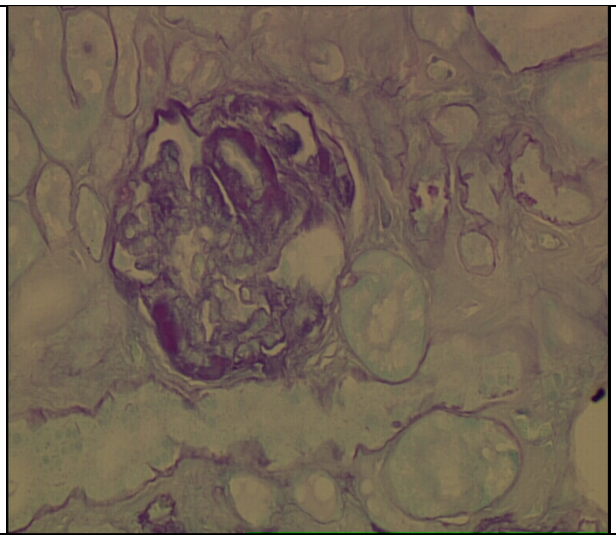

Fig.3 Glomerular basal membranes thickened and doubled.
Col. PAS-bright green, x200

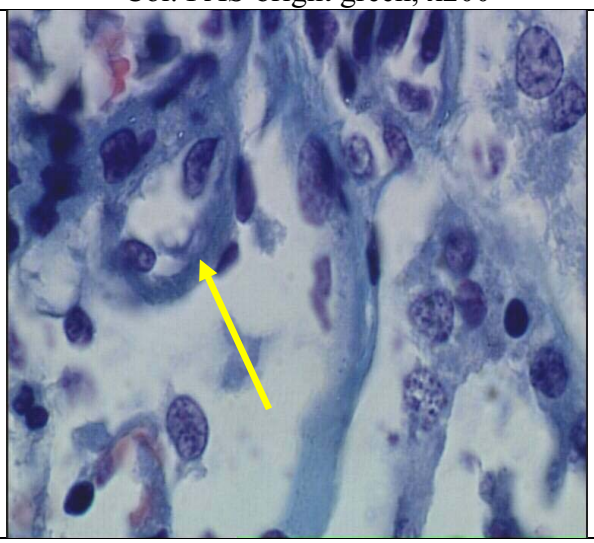

Fig.4 Membranous glomerulonephritis. Col. HEA, x1600

Finally, the glomerular basal membrane is losing its polyanionic charge and becomes very permissive, especially for the serous proteins. Thus, appears proteinuria and nephritic syndrome.

Some of the cases, the evolution is favourable, mostly, the lesions being reversible, in other cases is observed a lent progressive evolution to glomerular sclerosis, as a result of obliteration of the capillary lumen.

From clinically point of view, membranous glomerulonephritis are integrated part of the nephritic syndrome (15). Most of membranous glomerulonephritis evolves generalised or globally, being idiopathic (the composition of the circulatory immune complexes could not be identified), or are the expression of various pathological processes (infections, neoplasic processes, intoxications or autoimmune diseases).

The membranous glomerulonephritis may be the expression of geriatric age (continuous synthesis of membranous material), intoxication with heavy metals (gold, mercury), chronically septic disease (pyometra in bitches), parasitic infestation, association with chronically interstitial nephritis (in dog), systemic metabolic disorders (diabetes, thyroiditis) or idiopathic (unidentified immune complexes).

Mesangio-proliferative glomerulonephritis

Under histological aspect, the mesangio-proliferative glomerulonephritis is a predominantly cellular proliferation that leads in the end to a pluricellular or polynucleosis aspect of the Malpighian corpuscle.

This type of immune glomerulonephritis is frequently encountered, being dominated by the cellular and matrix modifications of the glomerular mesangium, morphologically expressed through an increase more or less notable of the cell number from its structure, as a result of the proliferation of mesangial and endothelial cells associated with inflammatory cells (polynuclear, mononuclear cells), increase of the mesangial matrix and of the mesangial deposits.

In histological examination, the glomeruli were increased in volume, occupying most of the glomerular cavity. The capillaries had thin basal membranes. The lumens of the capillaries were pressed, without blood, sometimes lacking.

The mesangial proliferations led to separation tendency of the glomerular lobules.

The observed proliferative cells were mesangial cells, visceral epithelial cells, monocytes, endothelial cells and sometimes even the cells of the parietal epithelial cells. Were also underlined adhesions between glomerule and Bowman’s capsule **(Fig. 5-9).**

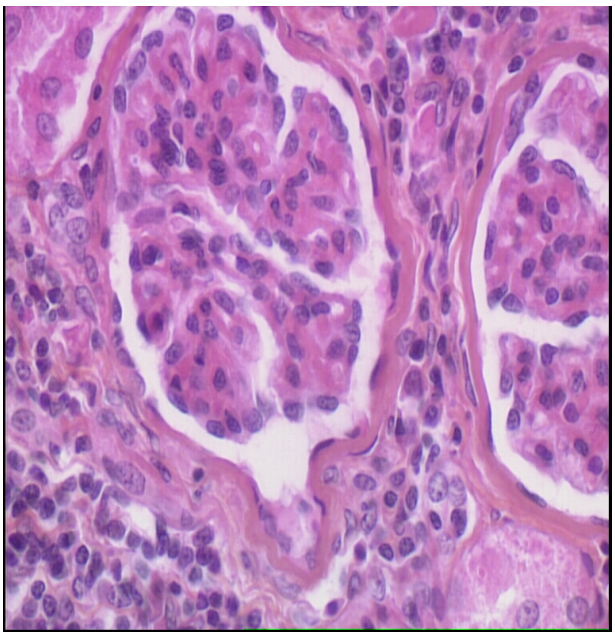

Fig.5 Mesangio-proliferative glomerulonephritis. Mesangial proliferations led to glomerular lobules separation tendency. Col.HES, x400

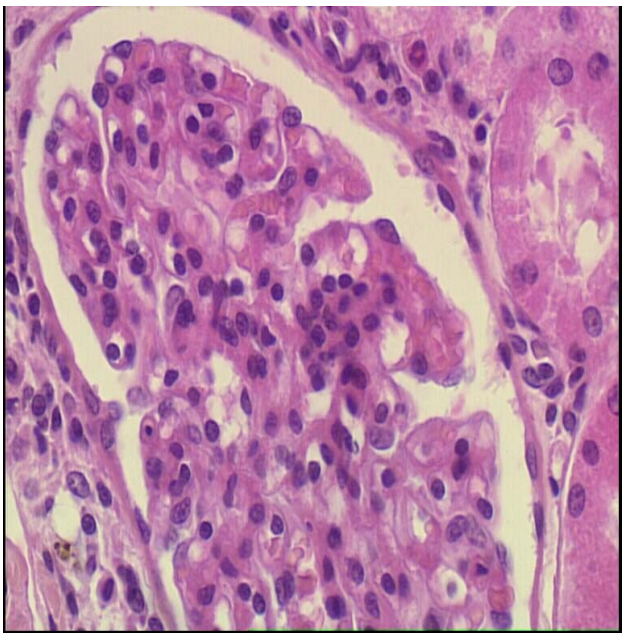

Fig. 6 Proliferations of the mesangial and endothelial cells associated with inflammatory cells (polynuclear, mononuclear cells), increase of the mesangial matrix and mesangial deposits,
Col. HE, x1000

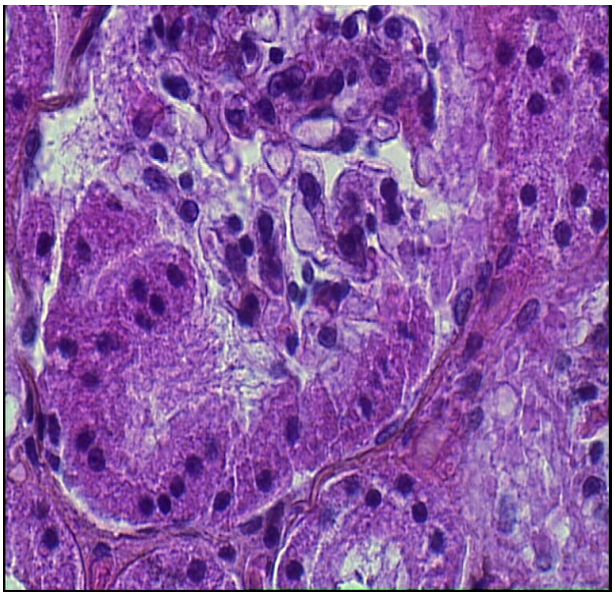

Fig.7 Parietal epithelial proliferation – Bowman's capsule. Col. HE, x1000

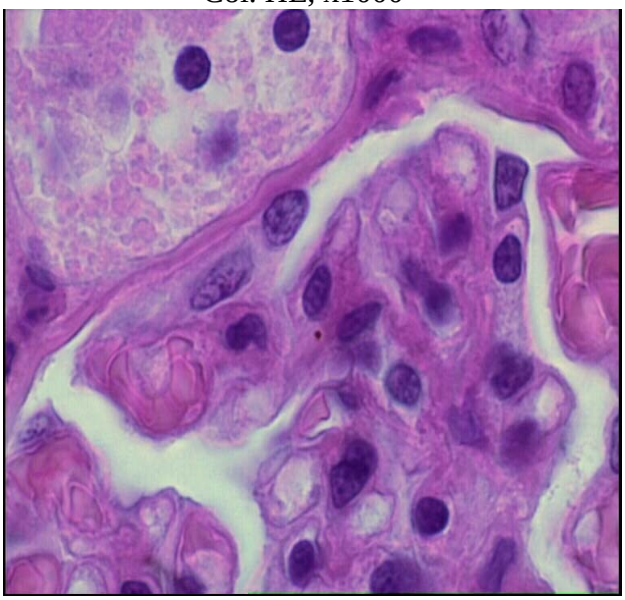

Fig.8 Adhesions between glomerule and Bowman's capsule Col. HE, x1600

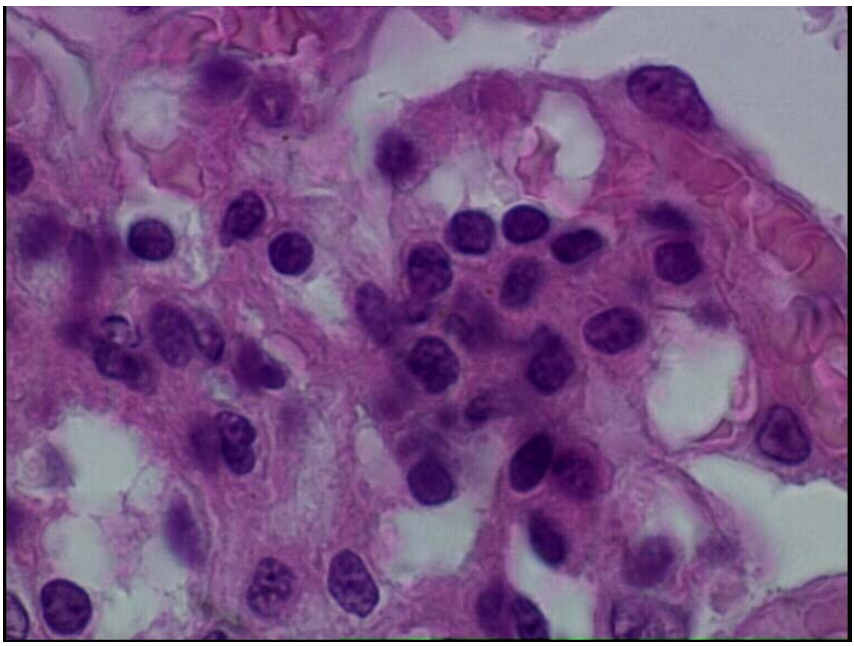

Fig. 9. Mesangio-proliferative glomerulonephritis.
Col. HE, x 1600

The mesangio-proliferative glomerulonephritis may evolve either globally, involving the entire glomerule, or segmental, affecting only the singular glomerular lobules.

The hyperplasic phenomena from the glomerular structure (glomerular polynucleosis) produced a remarkable reduction to the lumen of the glomerular capillaries, by external compression, reaching to complete stenosis, determining the decrease of the glomerular sanguine perfusion and finally, the sclerosis.

In some cases, the mesangio-proliferative glomerulonephritis are represented by mesangial and matrix changes exclusively limited to the axial region of the glomerule, without changing the lumen of the glomerular capillaries.

These proliferative phenomena have as consequence the segmental glomerular sclerosis and even general, collapse of the glomerular capillaries and adhesion between rounded tuft of blood capillaries and Bowman's capsule.

In electron microscopy it may be observed the fusion of the pedicels of podocytes. The extra-membrane deposits are extremely rare observed in electron microscopy but with immunohistochemistry are emphasized regular, homogenous or granular deposits, placed subendothelial along the glomerular basal membrane that contain Ig G and Ig M, without C3 fraction of the complement.

Mostly from the cell proliferation are mediated by derivative factors from complement and platelets. Was underlined the fact that the increase of the number of cells and of mesangial matrix is followed by the increased expression of PDGF (platelet derivative growth factor)

secreted by the endothelial cells and PDGF-receptor proteins, proving that the cellular proliferation is based on an autocrine mechanism.

The quantitative and qualitative features of these lesions is indispensable for histological prognostic.

In scientific literature, the description of the segmental or global proliferative glomerulonephritis was observed in Leishmania infections in dog, plasmocytosis in mink, in horse and poultry in infections with *Streptococcus zooepidermicus.*

Membrane-proliferative glomerulonephritis

Membrane proliferative glomerulonephritis (MPGN), also called mesangio-capillary glomerulonephritis represent a morpho-clinical entity characterised by proliferation of the resident cells (endothelial and mesangial) and at the same time by thickness and duplication of the glomerular basal membrane, hence fulfilling both aspects of the membranous and mesangio-proliferative glomerulonephritis.

Moreover, were emphasized other changes represented by leucocytes afflux, micro thromboses and proliferations of the parietal epithelium of the Bowman's capsule. The interstitial lesions depend on duration and gravity of the glomerulonephritis (alteration of the permeability of the glomerular capillaries) **(Fig.10).**

In chronically evolution were observed periglomerular and peritubular conjunctive proliferations and sequent atrophy of the nephron.

These morphological aspects are considered intermediary lesions finalised through generalised chronically glomerulonephritis and that lead to chronic renal failure.

The structural changes in this type of lesion explain why the patients present hematuria and nephritis concomitantly with proteinuria and nephritic syndrome.

The membrane-proliferative glomerulonephritis may be idiopathic (primary GN) as in most of the cases or associated with systemic diseases or to a well defined aetiology (secondary GN). In dogs, the membrane-proliferative glomerulonephritis evolves predominantly as idiopathic disease (Trautwein, 2000). Yet, recent data suggest that in some situation is possible the association of this type of lesion with systemic diseases or bacterial infections (leishmaniosis and borreliosis in dog) and neoplasm.

In conclusion, this category of glomerulonephritis is represented by severe lesions which are not allowing the healing without leaving marks.

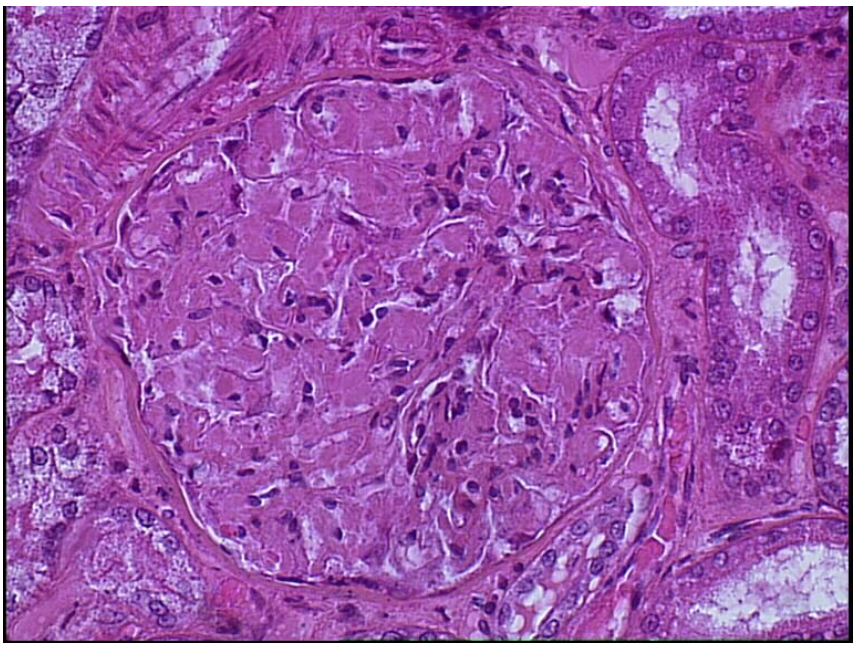

Fig.10 Membrane-proliferative glomerulonephritis, Col. HE, x1000

Proliferative glomerulonephritis

Sclerosing glomerulonephritis

The lesions may be determined either by glomerular causes (compression from inside the capsule, circulatory disturbances, types of glomerulopathies previously described), or by extra glomerular causes (interstitial inflammations, exterior compressions on glomerule, interruption of the sanguine glomerular circulation).

From morphological point of view, there were more aspects in the evolution of the glomerular sclerosis. In this study the lesions were identified in 5 dogs, with segmental or diffuse evolution.

For some cases, it was noticed the centrifuge debut of the sclerosis from the level of the mesangium (sometimes from the basal pole) and of the glomerular capillaries towards the Bowman's capsule by activating the mesangial cells and increase of the synthesis of mesangial matrix with different collagen degrees, proliferation of the parietal epithelium of the Bowman's capsule and appearance of inter-epithelial glomerular synechias/ adhesions (visceral epithelium-parietal epithelium).

Other aspect frequently emphasized was represented by eosinophil deposits, homogenous on the structures of the Malpighian corpuscle, thickening of the glomerular basal membranes, thickening of the basal membrane of the Bowman's capsule and even its dublation, degeneration of the visceral epithelium, proliferation of the cells and conjuctive cells in glomerular mesangium and periglomerular.

For other cases there was emphasized a centripetal sclerosis, proliferation of the conjunctive fibers from the level of the glomerular capsule and reaching to entire glomerule.

The sclerosis is finalized by transformation of the glomerule or only of the rounded tuft of blood capillaries in a mass of cells and concentrically stratified conjunctive fibers or it may be localized only to constitution of fibrous scar where it may be scarcely distinguished vestigial from the structure of the rounded tuft of blood capillaries. In time, a high number of glomerulus disappears together with dependent tubules **(Fig.10-13).**

Lymphocytic infiltration is extended to interstice replacing the uriniferous tubules with conjunctive scars. The connected tubules to functional glomerule are dilating and are presenting regenerative signs.

The sclerosing glomerulonephritis is that type of inflammation towards which may evolve all forms of acute and subacute glomerulonephritis.

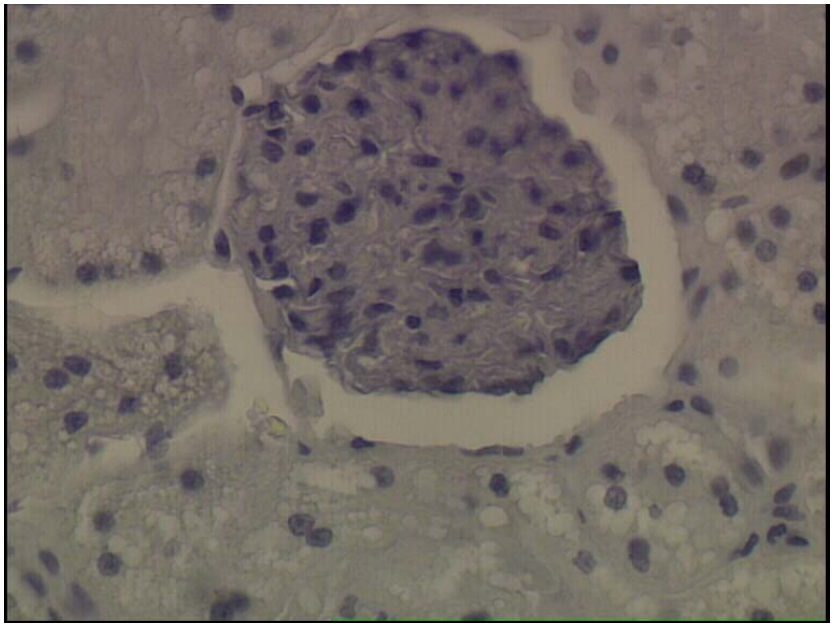

Fig.11 The proliferation of the cells and conjunctive fibres in glomerular mesangium periglomerular,
Col. Haematoxylin Mayer, x400

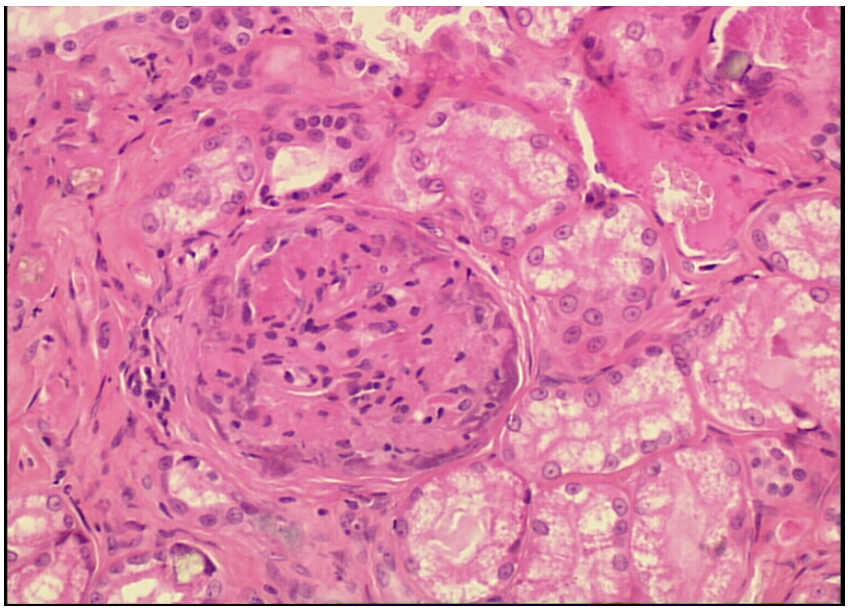

Fig.12 The proliferation of the cells and conjunctive fibres in glomerular mesangium periglomerular,
Col. HE, x200

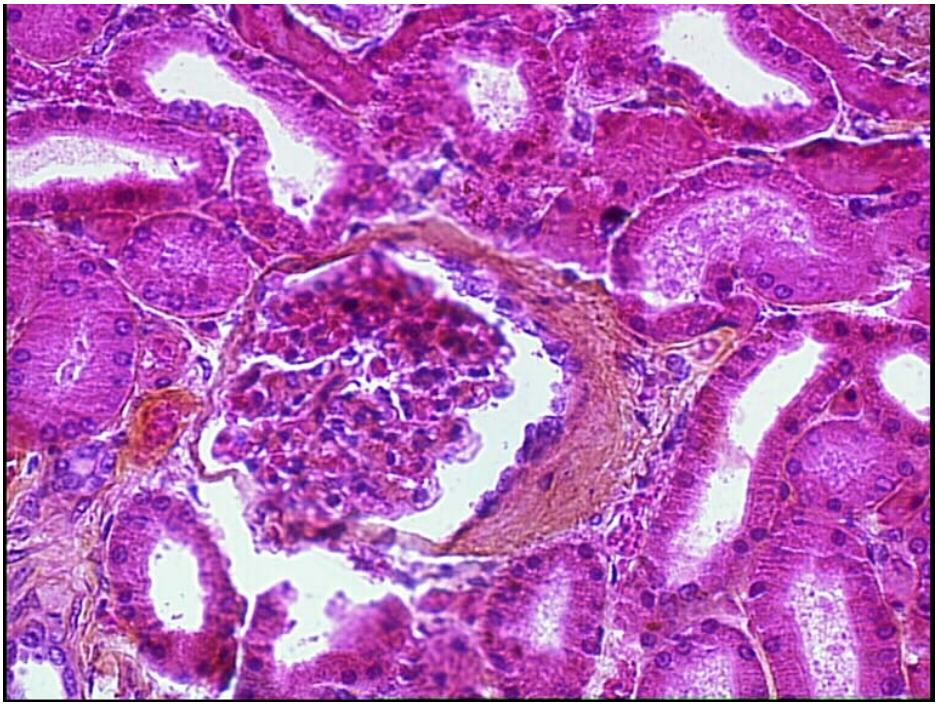

Fig. 13. Glomerular sclerosis, Col. HES, x400

CYTOLOGICAL ASPECTS IN IMMUNE GLOMERULONEPHRITIS

Cytological examination may represent an extremely useful diagnose item for some renal lesions, especially of the neoplasm.

Although the cytological examination of the collected samples by aspiration with thin needle does not allow the characterisation of numerous lesions due to its impossibility to

generally visualise cell architecture, yet, it offers sufficient information for a diagnose meant to guide clinical process and is considerably less invasive than biopsy.

The complications consequently to aspiration with thin needle of the renal tissue samples, are extremely rare, if the technique is properly applied. The first indication for aspiration with thin needle technique is identification of the size anomalies or shapes of the kidneys. The samples collected from patients with renomegaly uni- or bilateral often offers the information necessary for diagnose; on the contrary, in the case of kidneys with decreased volume, rarely it may be identified the cause. The lesions from where the samples are collected may be liquid or solid and the cytological examination may characterise the process as being cystic, inflammatory or neoplasm. It must be taken into account the fact that the impossibility to identify a lesion by cytological examination is not a certitude indication of its absence as it may exists the probability that the collected sample is not representative. The use of echographic examination for sample collection may be extremely useful for detecting the optimal areas with lesions. Taking into account the rich vascularisation of the kidneys, sanguine contamination of the samples is a frequent issue. The use of a non-aspiratory technique facilitates the collection of not very contaminated samples. It should also take into account that the solid lesions, rich in cells, offer samples with an increased number of cells than the inflammatory or degenerative lesions. Te cytological examination of the kidney has poor contraindications, the main being related to establishing the bleeding time for each patient.

A good contention and immobilization of the patient is very important together with manual fixation of the kidney closely to abdominal wall. For diffuse lesions, puncture may be blindly performed; it is preferred the echo-guided punctures on sedated or anesthetized patients.

The skin is cleaned and disinfected as it is prepared for a surgical procedure; the patient is immobilized in lateral decumbency and the kidney is manually immobilized towards the abdominal wall. It is preferred the use of a technique without aspiration: the needle is attached to a syringe of 10-12 cc previously filled with air; concerning the nature of the lesion, the needle is pointed either in the center of lesion (focal lesion) or tangentially (diffuse lesions). The renal hilum must be avoided to prevent serious hemorrhage. The needle must enter equally into the tissue to approximately 2/3 from the thickness of the lesion; the action is repeated for 5-7 times. The puncture must be performed in a single area, to avoid increased vascular lesions. The sample may be collected from 2-3 different areas of the lesion, repeating each time the described technique.

The collection of minimally 5 slides from each area of the lesion increases the chances to obtain a sample proper for diagnose. If inside the syringe or needle appears blood, the collection

must be stopped. After collection, the specimen from the needle must be dispersed on a side of a glass slide and then slowly placed with another glass slide.

If the collected sample is liquid and clear, which suggests a reduced number of cells, it is recommended its sedimentation.

Fig.14 Preparing the patient

Fig. 15 Echo-guided renal biopsy with thin needle

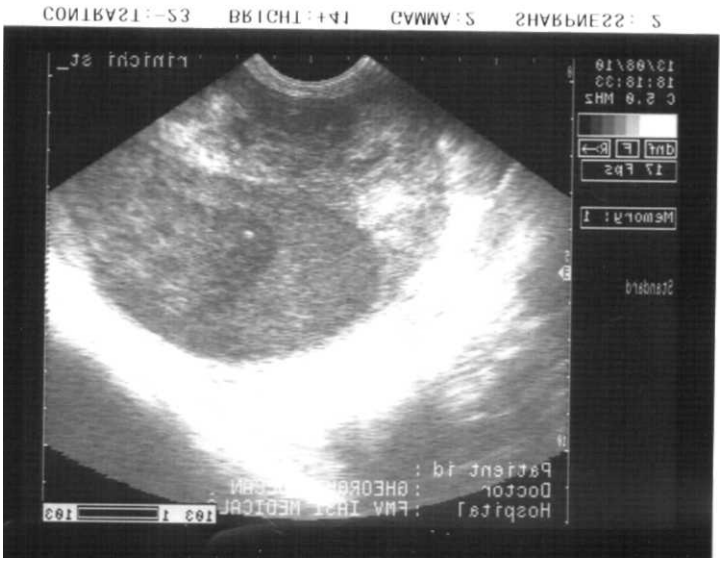

Fig.16 Echographic image of the kidney when puncture is performed

In 9 cases of renal failure, clinically diagnosed by urine and blood biochemical examinations, it was performed echo-guided renal biposy with thin needle. The examination of the MGG stained smears emphasized purulent pielonephritis by the presence of renal tubules epithelial cells, vacuolated with round central nucleus, with abundant cytoplasm, intensely basophile, visible nucleoli, of different dimensions and hyperhydrated hypersegmented neutrophiles granulocytes **(Fig.17, 18).**

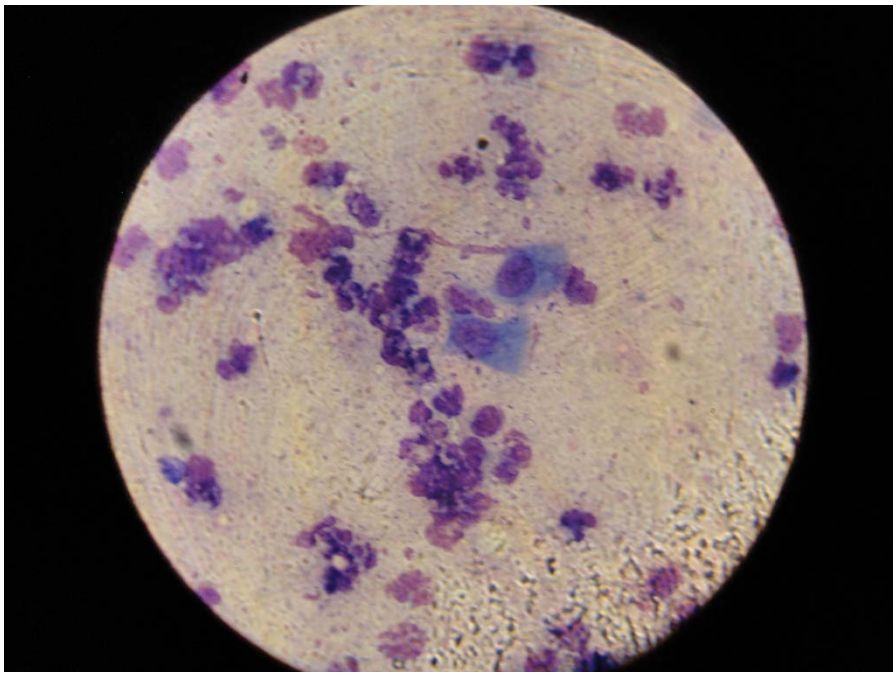

Fig.17 Degenerated neutrophiles, rare tubular epithelial cells. MGG, x 1000

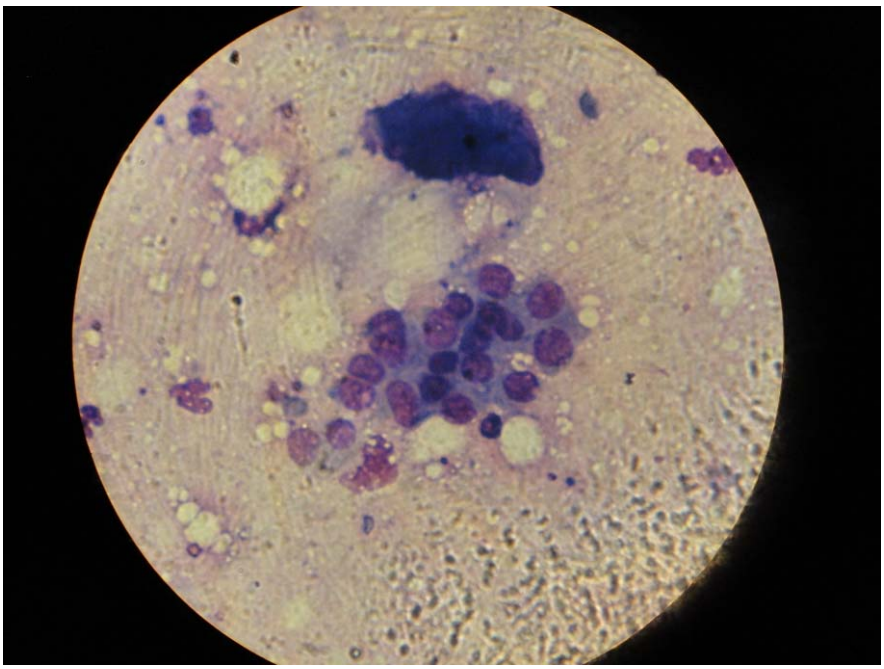

Fig.18 Deposits of tubular epithelial cells. MGG, x 1000