

## REVIEW

# The value of kidney biopsy in diabetes mellitus

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### Abstract

The purpose of this work is to emphasize the value of kidney biopsy in patients with diabetes mellitus and clinical renal impairment. Diabetes is the leading cause of end stage renal disease because diabetic nephropathy develops in 30 to 40% of patients. Multiple genetic predisposing conditions are involved in the development or not of a diabetic nephropathy, therefore supporting the existence of several factors in the pathogenesis of this disease. These predisposing conditions may also favor different other types of glomerulonephritis which can occur independently or in parallel with a diabetic nephropathy. All these renal diseases have different treatments, and therefore they must be correctly identified and managed accordingly. The processing of the kidney biopsy samples requires a very careful and highly qualified management to differentiate precisely the nature of each condition. In addition to the mesangial classical lesions, recent biopsy studies provided evidence that podocytes are injured very early in the diabetic nephropathy. On the other hand, transgenic mice models provide a unique opportunity to investigate the natural course of the disease. The paper underlines the main laboratory techniques required for this activity, and the main structural arguments to perform a satisfactory differential diagnosis.

**Keywords:** diabetes mellitus, nephropathy, differential diagnosis, immunofluorescence, electron microscopy.

### □ Introduction

Between one quarter and a half of the type II diabetic patients who are submitted to renal biopsy may have different nondiabetic nephropathies. These diseases can be either independent or associated (superimposed) with the diabetic nephropathy [1]. This situation occurs more often in type II diabetes. Higher reported frequencies are seen in biopsy studies because unusual clinical features, such as a proteinuria detected months or years after clinical onset of diabetes, or a rapid progression to renal failure, are suggestive of a nondiabetic renal disease and often prompt a biopsy [2]. Studies with proteinuria as the only criterion for biopsy show a frequency of 9% for nondiabetic renal disease [3].

Many other glomerulonephritis (GN) lesions have been described in diabetic patients [4, 5]. These include, in decreasing order of frequency, membranous nephropathy, acute post-infectious glomerulonephritis, IgA nephropathy, crescentic glomerulonephritis, membranoproliferative glomerulonephritis (types I and II), lupus nephritis, nonspecific immune complex disease, and amyloid [3, 6, 7]. These lesions may be present alone, or superimposed on diabetic glomerulosclerosis. In addition, both minimal change disease and focal segmental glomerular sclerosis have been reported [6, 7], although the occurrence of these two entities can be definitely established only in the absence of diabetes mellitus. Whether the incidence of any type of glomerular disease is increased in the diabetic population, or whether the coexistence of diabetic glomerulosclerosis with other glomerular lesions is a chance occurrence is still not known [8].

Diabetic nephropathy is probably the most common renal disease to be complicated by another distinct identifiable kidney disease. Although virtually any other glomerular disease can associate the diabetic nephropathy, membranous nephropathy is by far the most common.

Diabetic patients are particularly predisposed to severe urinary tract infections, such as papillary necrosis, acute pyelonephritis, and arteriolar nephrosclerosis. All these conditions are common and severe complications of advancing diabetic renal disease.

### □ Microscopic lesions in diabetic nephropathy (DN)

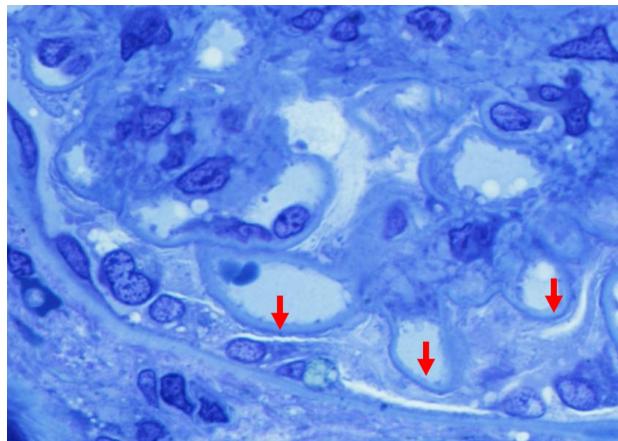
Resident and nonresident renal cells are stimulated by hyperglycemia in producing humoral mediators, cytokines, and growth factors that are responsible for structural alterations such as increased deposition of extracellular matrix (ECM) and functional alterations like enhanced permeability of glomerular basement membrane. All these alterations contribute to diabetic nephropathy [9].

Light microscopy (LM) characteristic features:

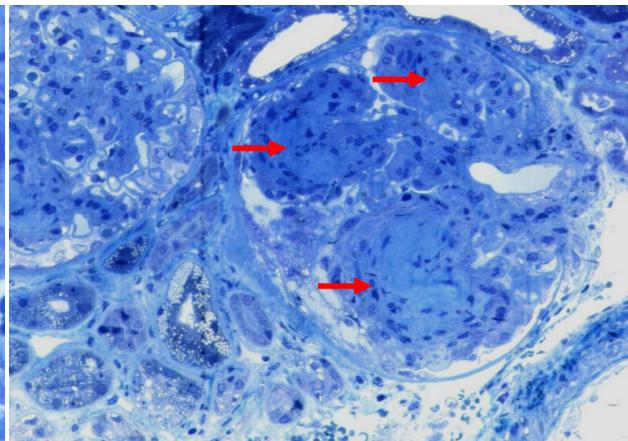
- Glomerular hypertrophy;
- Uniform thickening of glomerular basement membrane (GBM) (Figure 1);
  - Increase of mesangial matrix – diffuse;
  - Kimmelstiel–Wilson nodules [10] (Figure 2);
  - Podocyte hypertrophy and foot process broadening and effacement [11–15];
  - Hyalinosis of glomerular peripheral capillaries (plasmatic insudation);
  - Aneurismal peripheral glomerular capillaries;

- Capsular drops (Figure 3);
- Extracapillary proliferation (fibro-cellular crescents) in a small percentage (Figure 4);
- Hyalinosis of afferent and efferent arterioles;

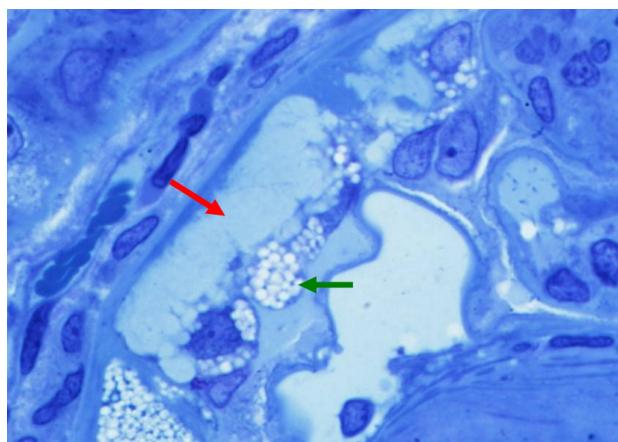
- Arteriosclerosis;
- Thickening of the tubular basement membrane (TBM) (Figure 5);
- Chronic interstitial inflammation and fibrosis.



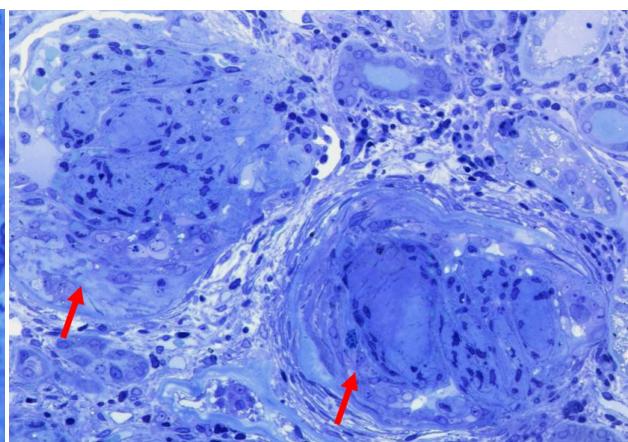
**Figure 1 – Cross-section through several glomerular capillaries having thickened glomerular basement membranes (GBM) (red arrows) (TB, ob. 100×).**



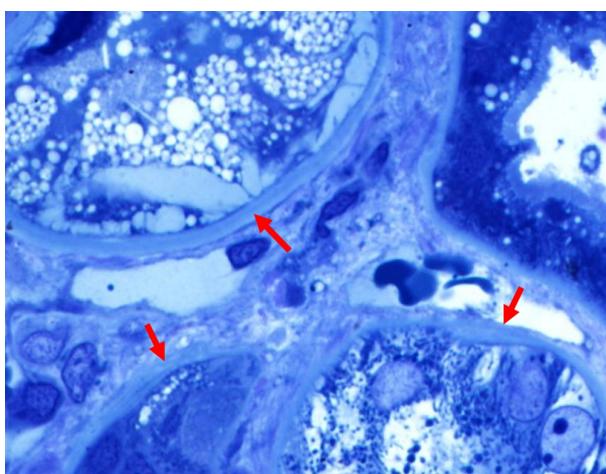
**Figure 2 – Glomerular hypertrophy and Kimmelstiel–Wilson nodules (red arrows) (TB, ob. 20×).**



**Figure 3 – Glomerular periphery and Bowman's capsule. Capsular drop (red arrow) and foamy parietal epithelial cells (green arrow) (TB, ob. 100×).**



**Figure 4 – Two glomeruli with nodular glomerulosclerosis and extracapillary proliferation (red arrows) (TB, ob. 20×).**



**Figure 5 – Cross section through three tubules having thickened basement membranes (TBM) (red arrows) (TB, ob. 100×).**

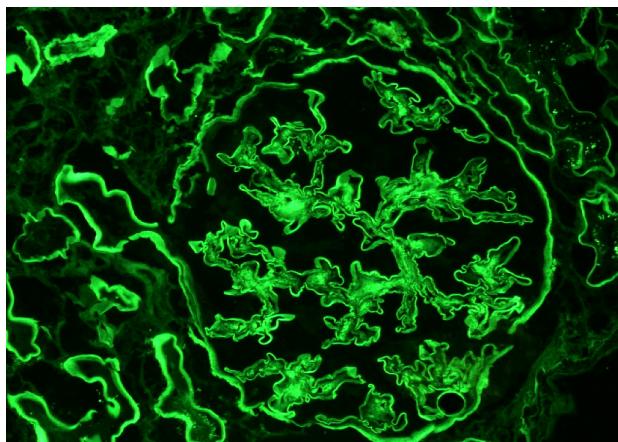
Immunofluorescent (IF) characteristic features:

- Linear labeling of GBM and TBM with anti-Albumin and anti-IgG antibodies (Figures 6 and 7).

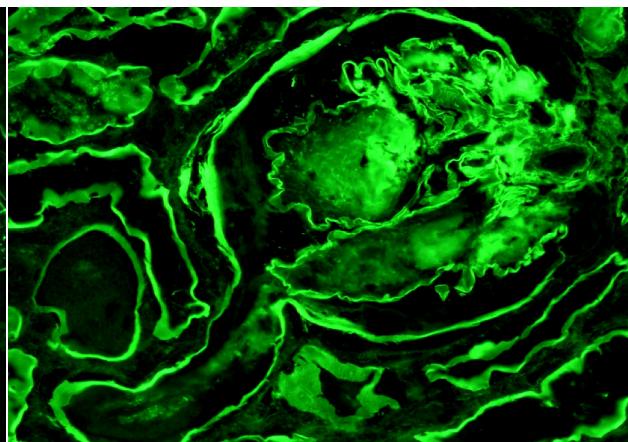
Ultrastructural characteristic details of diabetic nephropathy:

- Typical uniform thickening of GBM (Figure 8);
- Thickening of TBM (Figure 9);
- Capsular drops (Figure 10);
- Plasmatic insudation of glomerular capillaries (Figure 11).

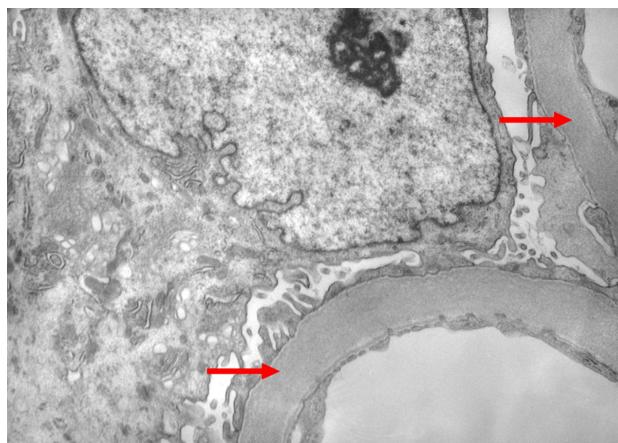
In summary, the well-formed nodular lesion with hyaline arteriolosclerosis is virtually pathognomonic of diabetes. In the absence of the nodular lesion, the presence of diffuse mesangiosclerosis, typical linear immunofluorescence staining of glomerular capillary walls and tubular basement membranes for IgG and albumin, and thickening of the GBM by electron microscopy provide a constellation of findings typical of diabetic glomerulosclerosis. Although often present in diabetic renal disease, hyalinosis lesions of glomerular capillaries and arterioles are nonspecific. All the above-mentioned lesions are helpful to differentiate the diabetic nephropathy from other glomerular nephropathies, either alone or associated.



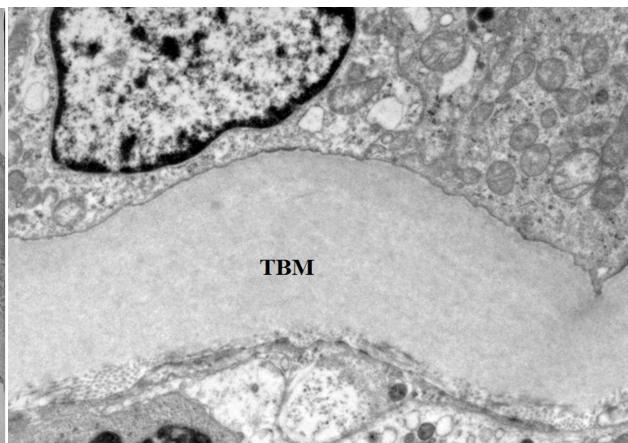
**Figure 6 – Linear labeling of GBM with FITC conjugated anti-albumin antibody (IF, ob. 20×).**



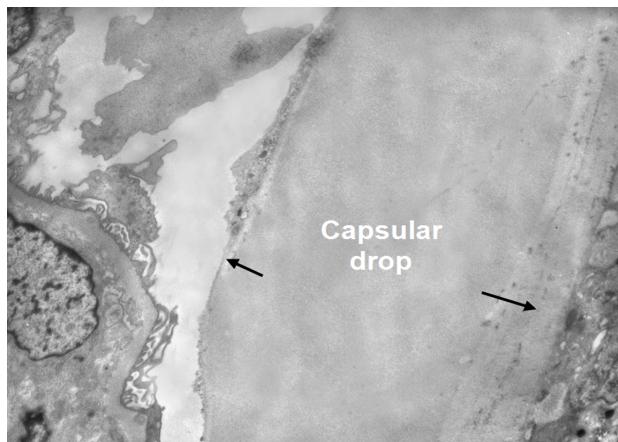
**Figure 7 – Linear labeling of GBM, Bowman's capsule and TBM with anti-IgG conjugated antibodies (IF, ob. 20×).**



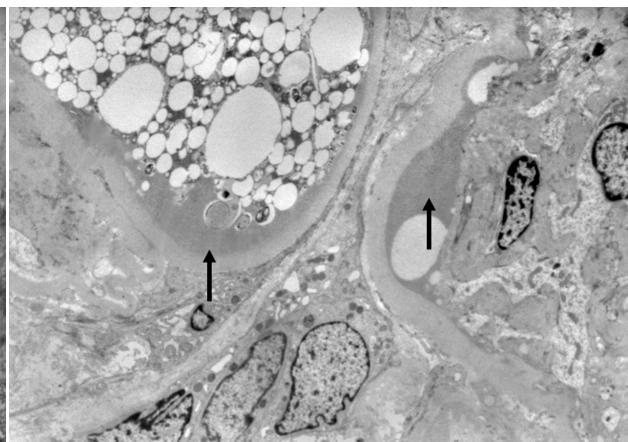
**Figure 8 – Intensely, uniform thickened GBM (red arrows), around 1000 nm (EM, 16 000×).**



**Figure 9 – Thickened tubular basement membrane (TBM). On the upper side it is in contact with a tubular epithelial cell (EM, 20 000×).**



**Figure 10 – Capsular drop. The insudative, amorphous material is placed between the Bowman capsule basement membrane, and the thin rim of parietal epithelial cell (black arrows) (EM, 18 000×).**



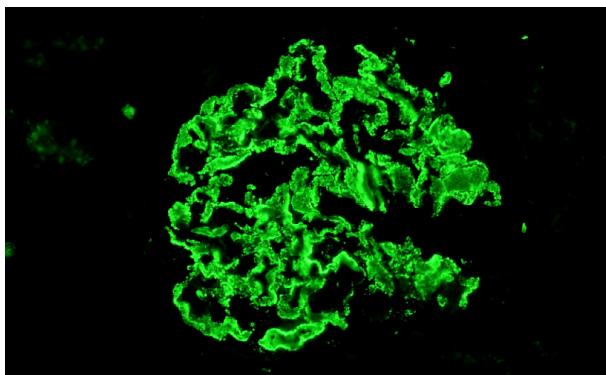
**Figure 11 – Small hyaline deposits (black arrows) and foamy cell in a glomerular capillary loop (EM, 12 000×).**

## ☒ Pathologic differential diagnosis

### Membranous nephropathy (MBN)

MBN is the most frequently associated GN with diabetes mellitus (DM) nephropathy.

Differentiation between these two conditions is easy due to the lack of glomerulosclerosis features either nodular or diffuse in LM, and the occurrence of typical granular deposits of IgG (Figure 12) in IF, exclusively in glomeruli, along the capillary walls in MBN, in contrast with the linear GBM pattern in DM.



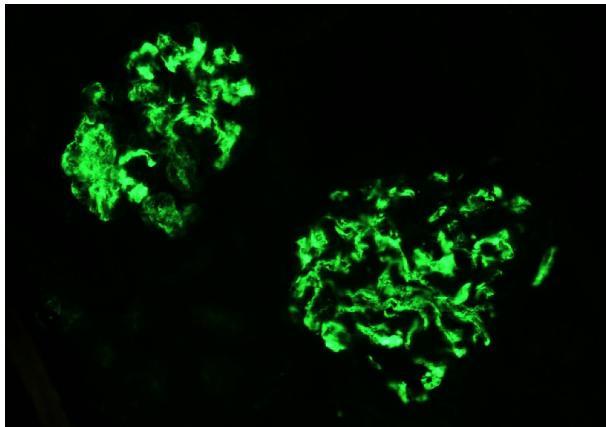
**Figure 12 – Characteristic granular pattern of labeling along the capillary walls in a membranous nephropathy. Anti-IgG FITC conjugated antibody (IF, ob. 20×).**

### Hypertensive renal disease

In both hypertension and diabetes mellitus, diffusely thickened GBM and a general increase of mesangial matrix are features frequently encountered. Other changes, such as the presence of Kimmelstiel–Wilson nodules, hyalinization of the efferent arterioles, and linear staining of GBM and TBM for IgG and Albumin by IF are indicative for DM nephropathy (Figures 6 and 7).

### IgA nephropathy (IgAN)

IgAN, either alone or associated with DMN, has its trademark label consisting of the specific staining of mesangial areas for IgA in IF (Figure 13). This granular, arborescent pattern of IF staining is unmistakable compared with the IgG linear labeling of GBM and TBM in DM.



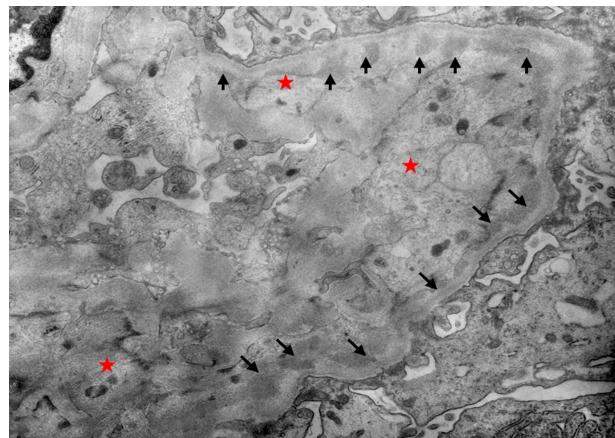
**Figure 13 – Typical mesangial, arborescent pattern of labeling with anti-IgA antibody in an IgA nephropathy (IF, ob. 20×).**

### Acute post-infectious glomerulonephritis

In post-infectious glomerulonephritis the most common finding is a coarsely granular capillary staining for complement C3c alone or associated with IgG. These patterns called either starry sky or garland are the equivalent of the dense deposits (humps) seen in electron microscopy, and they are quite different to the linear IgG and albumin pattern characteristic for diabetic nephropathy.

### Membranoproliferative glomerulonephritis type I

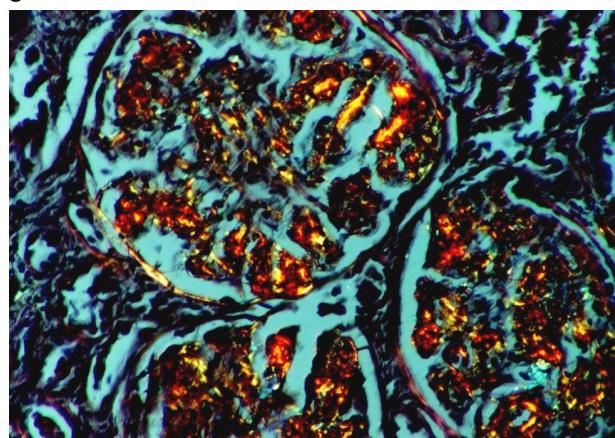
The membranoproliferative types of glomerulonephritis sometimes have a lobular accentuation with sclerotic intercapillary nodules that are similar to Kimmelstiel–Wilson nodules [1]. Increased intracapillary hypercellularity, circumferential mesangial cell interposition with diffuse “reduplication” or “tram-tracking” of the glomerular capillary wall, and the presence of discrete granular electron-dense immune deposits (detected by immunofluorescence or electron microscopy) are in favor of an immune-complex membranoproliferative GN (Figure 14).



**Figure 14 – Small, subendothelial dense deposits (black arrows), and mesangial interposition (red stars) in a membranoproliferative nephropathy (EM, 9000×).**

### Amyloidosis

Amyloidosis involving the mesangial areas may have a nodular appearance. The amyloid nodules are mostly acellular and negative to silver staining techniques. Instead, they stain with Congo red and give an apple-green birefringence under polarized light. Thioflavine T is also positive in amyloidosis. Electron microscopy shows long, unbranching filaments (8 to 12 nm in diameter) randomly arranged in cases of amyloidosis (Figure 15), while nonspecific collagen fibers are noted in the sclerotic areas of diabetic glomeruli.



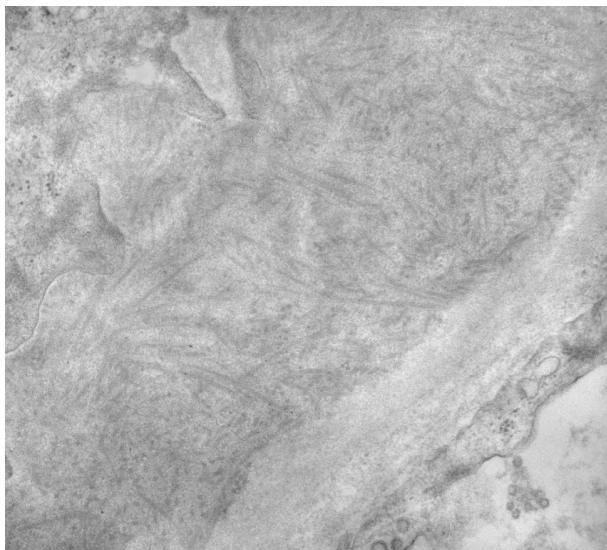
**Figure 15 – Renal amyloidosis. Three glomeruli stained with Congo red showing specific birefringence under polarized light (ob. 20×).**

## Lupus nephropathy

The differential diagnosis in this case must be done according to the class of lupus nephropathy. The case comes under attention for the class III and IV. Lupus nephropathy class III and IV may develop nodular features due to accentuation of the characteristic lobular appearance. In LM, there is a higher cellularity in lupus than in diabetic patients. In IF, there is a “full house” pattern of multiple glomerular immune deposits in lupus, compared with the linear pattern only for IgG and albumin in diabetes. In EM, the immune type dense deposits are polymorphic in lupus, compared with the lack of dense deposits in diabetes [1].

## Light chain deposition disease

Light chain deposition in plasma cell dyscrasias may lead to intraglomerular sclerotic lesions resembling Kimmelstiel–Wilson nodules. These nodules are often uniform in size. In contrast, the diabetic nodules vary in size and usually only two or three per glomerular tuft. The silver stain techniques reveal less argyrophilia in light chain disease glomerular nodules than in the diabetic ones. Discrete immune-type electron dense deposits are noted in patients with plasma cell dyscrasias (fibrillar or microtubular ultrastructural pattern) (Figure 16). In addition, the severe vascular disease and the diffuse marked thickening of GBM noted in diabetes are not present in light chain disease [8, 21].



**Figure 16 – Subendothelial organized dense deposit. Randomly placed microtubules in a plasma cell dyscrasia (EM, 25 000×).**

## Minimal change disease

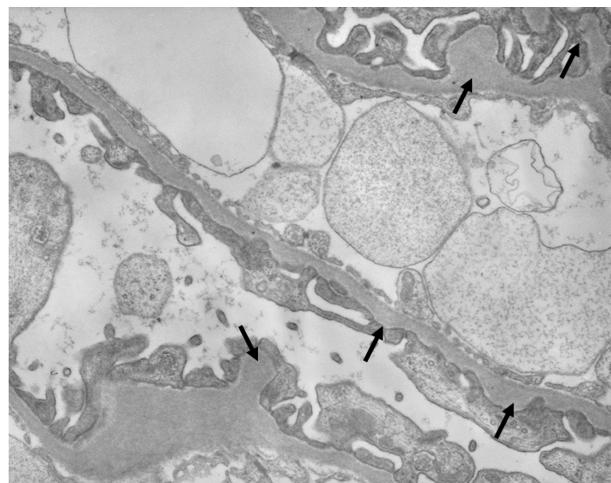
In this condition, the nodular sclerotic structures are completely missing. The only abnormal feature is the effacement of foot processes seen only in electron microscopy. The difference between minimal changes and diabetic patients can be easily done in IF; while in the first one, the immunofluorescence is negative or very weak, in the second one GBM and TBM are very clearly labeled with albumin and IgG in a linear pattern [8].

## Focal segmental glomerular sclerosis (FSGS)

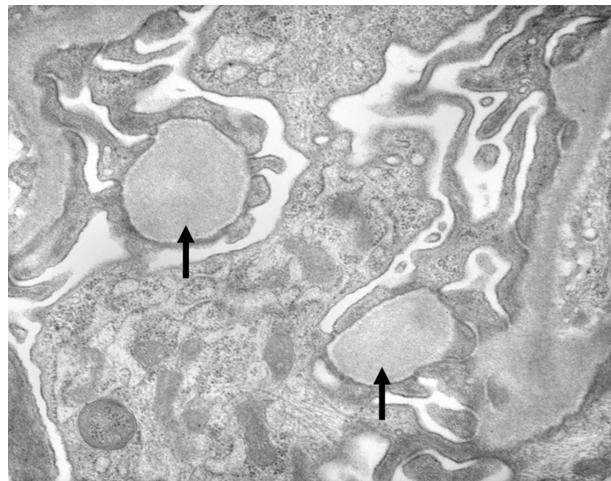
The glomerular capillary hyalinization (fibrin cap) seen in diabetic nephropathy is not specific and may be noted in focal segmental glomerulosclerosis too. The capsular drop lesion can also be rarely seen in advancing glomerular conditions. The lack of significant mesangial proliferation and the linear fluorescent pattern for IgG and albumin in diffuse diabetic glomerulosclerosis should aid in its distinction from an FSGS, or a mesangioproliferative form of glomerulosclerosis [1].

## Experimental diabetes mellitus

The characteristic lesional elements of the diabetic nephropathy involve the glomeruli, tubules and the interstitium. Concerning the glomeruli, the main remodelling transformations refer to podocyte hypertrophy and discontinuous thickening of GBM [11–13, 16]. These contiguous thickenings have a hump-like profile and are always placed on the subepithelial side (Figures 17 and 18) [17].



**Figure 17 – Experimental diabetes mellitus in transgenic mice. Discontinuous, irregularly thickened GBM (black arrows) and flattened foot processes (EM, 11 000×).**



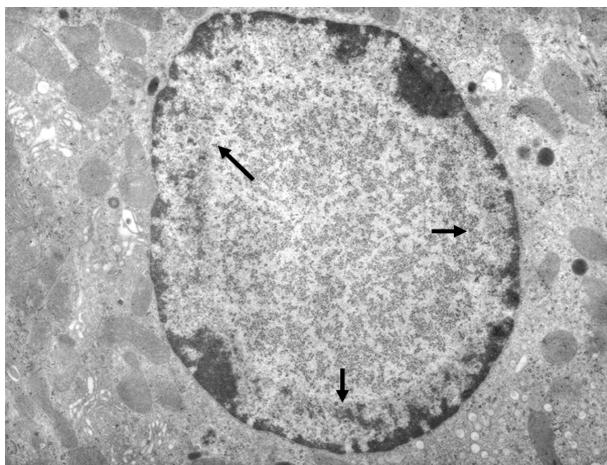
**Figure 18 – Experimental diabetes mellitus. Hump-like thickened GBM areas (black arrows) in cross section surrounded by foot processes and podocytic processes (EM, 18 000×).**

There is also a mesangial hypertrophy involving mainly the matrix, and having dense deposits, here and there (Figure 19). This mesangial expansion is caused by the excessive deposition of extracellular matrix (mostly collagen IV and fibronectin) and mesangial cell hypertrophy. Concomitant with the mesangial expansion, the thickening of GBM occurs due to the enhancement of extracellular protein synthesis. The result is not only a quantitative increase of GBM material, but also a qualitative transformation favoring a higher permeability to macromolecules. Concerning the collagen IV, there is an increase of  $\alpha_1$  and  $\alpha_2$  chains in mesangial area, and  $\alpha_3$  and  $\alpha_4$  in GBM [18].



**Figure 19 – Experimental diabetes mellitus. Vascular pole area of a glomerulus showing thickened GBM (black arrows), and dense deposits in the arteriolar wall and capsular basement membrane (EM, 10 000 $\times$ ).**

The renal tubules are involved in the pathologic process as a consequence of proteinuria which affects both epithelial cells and the tubular basement membrane. Some of the tubular epithelial cells may show intranuclear glycogen deposits (Figure 20).



**Figure 20 – Experimental diabetes mellitus. A tubular epithelial cell nucleus with a large inclusion of glycogen in the central area (EM, 11 000 $\times$ ).**

The arteriolar lesions consist of thickened vascular walls and hyaline deposits (Figure 19) [19, 20].

The kidney biopsy in diabetic patients is a valuable procedure to establish the stage of the renal disease, and

to differentiate this condition from other types of glomerulonephritis, either independent or in parallel with the diabetic nephropathy.

The processing of kidney samples requires a qualified and careful management involving light microscopy, immunofluorescence and electron microscopy.

Transgenic mice models provide a very useful scientific tool to investigate the natural course of the disease.

### Acknowledgements

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