SUMMARY - Structural changes underlying diabetic nephropathy in Type 1 diabetes are predominant in the glomerulus (thickening of glomerular basement membrane (GBM) and mesangial expansion), but also include arteriolar, tubular and interstitial lesions. The structural measure that correlates best with all renal functional parameters in Type 1 diabetes is mesangial fractional volume [Vv(mes/glom)], an estimate of mesangial expansion. Structural-functional relationships in Type 2 diabetes are much less known. These studies investigated renal structure in the early stages of nephropathy [microalbuminuria (MA)] in patients with Type 1 and Type 2 diabetes. Diabetic glomerulopathy was quite advanced in Type 1 diabetic patients with MA, and both Vv (mes/glom) and GBM width were increased as compared to normoalbuminuric (NA) patients when the albumin excretion rate (AER) was >30 µg/min. Serial renal biopsies were performed 5 years apart in 11 Type 1 diabetic patients to evaluate whether glomerular and interstitial lesions progress jointly. AER increased significantly in 5 years, while the glomerular filtration rate remained unchanged. All structural parameters were initially abnormal. Vv(mes/glom) and mean glomerular volume increased significantly, whereas GBM width and the interstitial volume fraction were unchanged. Moreover, the change in Vv (mes/glom) was correlated with the change in AER (r = 0.64, p < 0.05). Thus, at the disease stage during which some patients progress to MA or proteinuria, continuing mesangial expansion is the main variable, whereas further interstitial expansion does not occur. A large number of Type 2 patients were also studied. Early diabetic glomerulopathy was detected by electron microscopy in NA patients and found to be more advanced in those with MA and proteinuria. However, lesions were milder than in Type 1 diabetic patients, and there was considerable overlap between groups. Morphometric results by electron microscopy were similar to those by light microscopy, demonstrating the heterogeneity of renal structure in Type 2 diabetic patients. In fact, only 30% of MA patients had typical diabetic glomerulopathy, while 40% had more advanced tubulo-interstitial and/or vascular lesions and 30% had normal renal structure.

Key-words: renal structure, type 1 diabetes, type 2 diabetes, morphometric analysis, microalbuminuria, mesangial expansion.

RÉSUMÉ - Progression de l’atteinte anatomique dans la néphropathie du diabète de type 1 et de type 2.

Les anomalies structurales sous-tendant la néphropathie du diabète de type 1 impliquent surtout le glomérule (épaississement de la membrane basale (MBG) et expansion mésangiaire) mais aussi des lésions artério-laires, tubulaires, et interstitielles. Les relations structure/fonction sont moins bien connues pour le diabète de type2. Nous avons analysé la structure rénale aux stades précoces microalbuminuriques de la néphropathie chez des diabétiques de type 1 ou 2. La gloméropathie était assez évoluée chez les diabétiques de type 1 microalbuminuriques, et le Vv (mes/glom) et l’épaississement de la MBG étaient augmentés par rapport à des patients normalalbuminuriques avec albuminurie (ERA) >30 µg/min. Des biopsies rénales séquentielles à 5 ans d’écart ont été réalisées chez des diabétiques de type 1 pour savoir si les lésions glomériulaires et interstitielles évoluaient ensemble. L’ERA, mais pas la FGR, augmentait significativement en 5 ans. Tous les paramètres structuraux étaient anormaux lors de la première biopsie. Le Vv (mes/glom) et le volume gloméulaire moyen augmentaient significativement. En revanche, la MBG et le volume interstitiel ne changeaient pas en 5 ans. De plus, le changement du Vv (mes/glom) était corrélé à celui de l’ERA (r = 0.64, p < 0.05). Donc, au stade d’évolution vers une microalbuminurie ou une protéinurie, l’expansion mésangiale continue est la variable principale, alors qu’il ne se produit pas d’expansion interstitielle. Chez des diabétiques de type 2, une gloméropathie précoce était détectable en microscopie électronique (ME) chez les patients normalalbuminuriques, et était plus évoluée chez les patients microalbuminuriques ou protéinuriques (ces lésions étant plus faibles que dans le diabète de type 1). Ces études en ME, en cohérence avec les études en microscope optique, démontrent l’hétérogénéité de la structure rénale au cours du diabète de type 2; seuls 30% des patients microalbuminuriques ont une gloméropathie typique, 40% ayant des lésions tubulo-interstitielles et/ou vasculaires plus avancées et 30% présentant une structure normale.

Mots-clés : structure rénale, diabète, microscopie, membrane basale gloméulaire, volume fractionnel mésangial.

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Disabilities and mortality caused by end-stage renal disease (ESRD) are important health problems in Western countries. Diabetic nephropathy is currently the most common cause of ESRD in the United States, Japan and Europe, and there has been a dramatic increase in the proportion of ESRD patients affected by diabetes in recent decades [1-4]. Between 1982 and 1992, the number of patients with diabetic ESRD rose from 27% to 36% in the United States and from 11% to 17% in Europe. The rate of increase of ESRD in patients with diabetes has been much more rapid than that of ESRD from other causes. The majority of diabetic patients with ESRD have Type 2 diabetes. In the past, there was confusion regarding the classification of diabetes among ESRD patients because many on insulin treatment were also classified as Type 1 when they were affected by Type 2 diabetes, and 40% of Type 2 diabetic patients received insulin. Thus, the proportion of ESRD patients with Type 1 diabetes was overestimated. In Europe, Type 2 diabetes accounts for 43% of diabetic ESRD, and it has been estimated that 67% of Italian diabetic patients with ESRD have Type 2 diabetes [1, 4]. In the USA over 80% of diabetic patients with ESRD have Type 2 diabetes. Although Type 2 diabetes is the most common cause of ESRD, the majority of studies on renal morphology have been performed in patients with Type 1 diabetes. The clinical manifestations of diabetic nephropathy, proteinuria, decreased glomerular filtration rate and increasing blood pressure are similar in Type 1 and Type 2 diabetes [5-7], but the spectrum of renal lesions is not the same, and some differences in the clinical course of renal disease are apparent. For example, proteinuria or microalbuminuria is rarely present at the onset of Type 1 diabetes, whereas several patients have abnormal albumin excretion rates at the time of diagnosis of Type 2 diabetes [8, 9]. Although this may be explained by a long period of undiagnosed hyperglycaemia, it is also possible that the structural changes underlying abnormalities in albumin excretion rate differ in Type 1 and Type 2 diabetes. Some time ago, the risk of progression to proteinuria in microalbuminuric Type 1 diabetic patients was estimated to be about 80% [10-13]. More recently, some prospective studies have suggested that the risk of progression from microalbuminuria to proteinuria was overstimated and that it ranges from 18 to 45% [14-16]. Mogensen et al. found that only 20% of patients with Type 2 diabetes progressed to overt nephropathy over a decade of follow-up. This can be partly explained by the high mortality from cardiovascular disease among these patients, who do not have time to become proteinuric because of premature death. In fact, microalbuminuria in Type 2 diabetic patients is a strong predictor of cardiovascular disease. More recent prospective studies indicating lower mortality rates among microalbuminuric Type 2 diabetic patients have shown that the risk of progression to proteinuria was about 40% after 5 to 6 years of follow-up [17-22]. The risk of overt nephropathy over a longer period of follow-up is not known, but presumably greater.

### RENAL STRUCTURE IN TYPE 1 DIABETES

Glomerulopathy is the most important structural change in Type 1 diabetic patients, characterised by thickening of the glomerular basement membrane (GBM) and mesangial expansion, leading to progressive reduction in the filtration surface of the glomerulus [23-26]. Although the most important structural changes occur in the glomeruli [23-26], concomitantly and proportionally to the degree of glomerulopathy, the arterioles [27], tubules [28] and interstitium [29] also develop morphological lesions.

Although all the pathologic findings of diabetes can be observed in other renal diseases, a constellation of lesions such as thickening of glomerular (GBM) and tubular basement membrane (TBM), mesangial expansion, Kimmelstiel-Wilson nodules, and arteriolar hyalinosis is unique to this disease and separable from all other renal disorders [23-25]. The first change that can be quantified is thickening of the GBM [30]. There is also thickening of the TBM [22] and Bowman’s capsule. Within a few years after onset of diabetes, afferent and efferent arteriolar hyalinosis can be noted. This can progress to the replacement of the smooth muscle cells of these vessels by hyaline material. This lesion and other “exudative” ones (hyaline deposits) in the subendothelial space (hyaline caps) and along the parietal surface of Bowman’s capsule (capsular drops) contain a variety of plasma proteins, especially immunoglobulins, complement, fibrinogen, albumin, etc. [31, 32]. Increases in the volume of cellular and matrix components of the mesangium can be detected in some patients as early as 5 to 7 years after onset of diabetes [25, 30]. Diffuse and generalised mesangial expansion is termed “diffuse diabetic glomerulosclerosis”. Nodular glomerulosclerosis consists of areas of marked mesangial expansion forming large round fibrillar mesangial zones with palisading of mesangial nuclei around the periphery of the nodules. The first and extreme compression of the associated glomerular capillaries (Kimmelstiel-Wilson nodules). In most but not all instances, nodular glomerulosclerosis is seen with advanced diffuse mesangial changes. Many patients with clinical diabetic nephropathy (DN) have few or no nodular lesions. Additional abnormalities include global glomerular sclerosis (GS) [33] and interstitial expansion, especially in areas of tubular atrophy or GS [25]. Currently, our hypothesis is that the interstitial changes in DN follow from progressive glomerulosclerosis [26]. The critical lesion of DN that leads primarily to renal insufficiency in Type 1 diabetic patients is expansion of the glomerular...
mesangial [25]. The morphometric determination of mesangial expansion is mesangial fractional volume \([Vv\text{ (mes/glom)}]\) (the percentage of the glomerular tuft made up by mesangium); this is the structural parameter that best correlates with all functional parameters in Type 1 diabetes. A highly significant inverse correlation exists between glomerular filtration rate (GFR) and \(Vv\text{(mes/glom)}\) [25]. As the mesangium expands, it ultimately restricts glomerular capillary luminal volume and diminishes filtration surface, which is closely related to GFR [34]. Although GS and capillary closure can also influence this course of events, GFR can be reduced in patients with little or no glomerular scarring [33]. \(Vv\text{(mes/glom)}\) is also related to the presence of proteinuria and hypertension [25]. Thus, proteinuria, hypertension, and declining GFR (all manifestations of clinical DN) are related to mesangial expansion and necessarily to distortions in glomerular capillary architecture. Percent of global sclerosis [33] and interstitial expansion [29] are also correlated with the clinical manifestations of DN, and are somewhat independent variables of mesangial expansion in relation to proteinuria, hypertension, and declining GFR. However, the exact contribution of each of these lesions to the renal dysfunction of DN is difficult to determine. Current studies in Type 1 diabetes suggest that progression from normoalbuminuria (NA) to microalbuminuria (MA), and from MA to overt DN, is related to the development of glomerular rather than interstitial pathology. Arteriolar lesions also progress, but there is no significant relationship between this progression and the emergence of early clinical diabetic renal disease [26].

The thickness of GBM is not related to GFR or to the presence of hypertension, although there is a direct relationship between albumin excretion rate (AER) and GBM thickness. AER is related to all glomerular parameters. Normoalbuminuric patients (NA) may have diabetic glomerulopathy of variable severity: those with low levels of microalbuminuria (NA) to microalbuminuria (MA), and from MA to overt DN, is related to the development of glomerular rather than interstitial pathology. Arteriolar lesions also progress, but there is no significant relationship between this progression and the emergence of early clinical diabetic renal disease [26].

Changes in podocytes have also been considered important in determining altered glomerular permeability to proteins. Björn et al. found that the foot process width (FPW) of podocytes on peripheral basement membrane was increased in MA Type 1 diabetic patients as compared to NA patients, and that the width of filtration slits in NA- and MA diabetic patients was greater than in non-diabetic control subjects. Filtration slits were correlated with GFR in all diabetic patients. The authors concluded that changes in podocytes and filtration slits in Type 1 diabetic patients with nephropathy already occur at the microalbuminuria stage [35]. In contrast, Ellis et al. found that significant disturbances of epithelial cell structure occurred only in patients with Type 1 diabetes and overt proteinuria, and that there were no differences between patients with microalbuminuria and those with normal AER for foot process width and filtration slit length density. However, when all diabetic patients were considered, these parameters were correlated with AER [36].

Another possible mechanism accounting for albuminuria in diabetic glomerulopathy is the loss of the charge-selective barrier. In fact, the passage of plasma proteins larger than 70 kD across the GBM is believed to be normally restricted not only by the size-selective barrier but also by a charge-selective barrier. This is thought to be mainly due to polyanionic glycosaminoglycans in the GBM, which restrict the passage of small polyanionic plasma proteins (primarily albumin). For example, studies have revealed that the defect in minimal-change glomerulopathy results mainly from a loss of charge selectivity [37]. Vernier et al. found that heavy proteinuria in Type 1 diabetes was associated with a decrease in the number of anionic charge sites in the lamina rara externa due to a decrease of heparan sulphate proteoglycan. As the number of anionic charge sites was similar in NA and MA patients, there was no evidence that loss of heparan sulphate proteoglycans is a mechanism for early microalbuminuria [38].

### RENAL STRUCTURE IN TYPE 2 DIABETES

The pioneer paper by Kimmelstiel and Wilson in the field of renal pathology in diabetes clearly indicates that patients with Type 2 diabetes may develop glomerulopathy [39]. In Type 1 diabetes, non-diabetic renal diseases are rare, whereas the prevalence of non-diabetic renal lesions has been reported to be high.
(approximately 30%) in proteinuric Type 2 diabetic patients. Parving et al. reported that 23% of Type 2 diabetic patients with proteinuria had non-diabetic glomerulopathies [minimal lesion nephropathy, mesangio-proliferative glomerulonephritis (GN)] and sequelae of GN [40]. Heterogeneity in renal lesions has also been reported by Gambara et al., who found that 37% of proteinuric Type 2 diabetic patients had typical changes of DN, and that 33% showed glomerular disease superimposed on diabetic glomerulosclerosis [41]. In a more recent report from the same group, non-diabetic glomerulopathies occurred only in 18% of 65 Type 2 diabetic patients [42]. Other authors observed the presence of non-diabetic renal disease in 42% of 153 Type 2 patients with overt nephropathy [43]. However, the occurrence of non-diabetic renal disease was much lower (12%) in the series of 33 proteinuric patients studied by Olsen [44]. In all these studies, with the exception of that of Parving et al., patients were referred to the nephrologist, and kidney biopsies were performed for clinical indications. Therefore, these studies may not describe the usual Type 2 patients with diabetic nephropathy, but rather those with an unusual clinical course. Moreover, the varying results may reflect differences in the criteria for kidney biopsy. A large autopsy study of Type 2 diabetic patients did not confirm a high incidence of non-diabetic renal diseases [45]. Thus, available data on renal structure in Type 2 diabetic patients with proteinuria are contradictory.

Data on quantitative morphometric analysis are scarce. In Japanese Type 2 patients with a wide range of renal function, morphometric determinations of diabetic glomerulopathy showed correlations with renal functional parameters similar to those observed in Type 1 diabetes [46]. However, more recent studies suggest a high incidence of normal glomerular structure among microalbuminuric and proteinuric Japanese Type 2 diabetic patients [47]. In Caucasian Type 2 diabetic patients with proteinuria, Osterby et al. [48] found that all the morphometric glomerular parameters were on average normal, although some patients had glomerular structure within the normal range. On the other hand, in Type 1 diabetic patients with overt nephropathy, glomerular structure was always severely altered. Our recent study of a large group of Caucasian Type 2 diabetic patients [49] showed that several had normal glomerular structure despite persistent microalbuminuria or macroalbuminuria, even though diabetic glomerular structural parameters were more altered on average from normalalbuminuria to microalbuminuria and overt proteinuria. Moreover, diabetic glomerulopathy was less apparent in patients with Type 2 diabetes than in those with Type 1 diabetes and similar renal function. AER was directly related to both GBM width ($r = 0.47$, $p < 0.001$) and $V_v$(mes/glom) ($r = 0.44$, $p < 0.001$), whereas GFR was inversely related to $V_v$(mes/glom) ($r = 0.47$, $p < 0.001$) but not to GBM width. Although significant, these structural/functional relationships were less precise than in Type 1 diabetes.

These data are in agreement with those for Pima Indians which, in a much smaller group, showed no significant differences in glomerular ultrastructure between patients with long-term Type 2 diabetes with normoalbuminuria and those with microalbuminuria [50]. The parameters of diabetic glomerulopathy were more severely altered only in patients with proteinuria. These results were true for mesangial fractional volume and GBM width as well as for foot process width and the number of podocytes per glomerulus. A cross-sectional study in diabetic Pima Indians found that subjects with clinical nephropathy had fewer podocytes per glomerulus than those without nephropathy. The total surface area covered by the podocytes did not change since the remaining ones were obliged to grow and extend their foot processes to maintain the area covered. This suggests that podocyte loss and an increase in foot process width are important factors in the progression to overt nephropathy. In fact, changes in podocytes occur late in the course of diabetic renal disease and are probably more involved in mechanisms of progression than in those of genesis and early development [50].

In a recent longitudinal study, Meyer et al. considered the predictive value of glomerular structure in Pima Indians with Type 2 diabetes and microalbuminuria relative to the change in urinary albumin excretion during 4-year follow-up. They found that the number of podocytes per glomerulus was the strongest predictor of changes in albuminuria, and that fewer cells were predictive of more rapid progression during follow-up [51].

A number of mechanisms could account for podocyte loss in diabetes. Firstly, it has been suggested that sustained mechanical stress associated with glomerular hypertrophy and hypertension causes podocyte injury. Secondly, mesangial expansion beyond some critical point can cause closure of capillary loops and obliteration of podocytes.

In summary, regardless of whether the primary role concerns podocytes or mesangial expansion, diabetic glomerulopathy, on average, also develops in Type 2 diabetes, producing structural changes similar to those usually observed in Type 1 diabetes. However, renal structural lesions are much more heterogeneous in Type 2 diabetes.

In Type 1 diabetes, the most important structural changes involve the glomerulus predominantly, whereas light microscopy studies have shown that a substantial proportion of Type 2 diabetic patients have more advanced tubulo-interstitial and vascular than glomerular lesions [52, 53].

Fifty-three Type 2 diabetic patients with MA were studied in greater detail [53]. Many of these patients had either no glomerulopathy or mild mesangial ex-
pansion (as revealed by light microscopy), with or without tubulo-interstitial and arteriolar changes. On this basis, we proposed a classification system including 3 major groups:

**Category CI:** Normal or near-normal renal structure. These patients (41%) had biopsies which were normal or showed mild mesangial expansion, tubulo-interstitial changes, or arteriolar hyalinosis.

**Category CII:** Typical diabetic nephropathology. These patients (26%) had established diabetic lesions with an approximately balanced severity of glomerular, tubulo-interstitial and arteriolar changes. This picture was typical of that seen in Type 1 diabetic patients with obvious light microscopy DN changes.

**Category CIII:** Atypical patterns of renal injury. These patients (33%) had relatively mild glomerular diabetic changes despite disproportionately severe renal structural changes: (a) tubular atrophy, tubular basement membrane thickening and reduplication and interstitial fibrosis (tubulo-interstitial lesions); (b) advanced glomerular arteriolar hyalinosis commonly associated with atherosclerosis of larger vessels; and (c) global glomerular sclerosis. In the CIII group, these patterns were present in all possible combinations.

No cases of definable non-diabetic renal disease were found in this series of 53 patients.

Hyperglycaemia may cause different patterns of renal injury in older Type 2 as compared to younger Type 1 diabetic patients. Tubulo-interstitial and vascular changes could also be related to ageing, atherosclerosis, and systemic hypertension. However, hypertension, which was present in almost all patients in all three structural categories, cannot account per se for the different lesions observed in category III. Moreover, mean age was similar in patients in categories II and III (60 years), despite the different patterns of renal injury in both groups. Our observations in a large number of age-matched normal controls suggest that ageing is not a sufficient factor to account for most of the renal structural changes observed in CIII patients. Thus, it can be supposed that heterogeneity in renal structure may reflect the heterogeneous nature of Type 2 diabetes. However patients with “typical” DN lesions had longer known diabetes duration, worse metabolic control, and diabetic retinopathy in all cases (50% background, 50% proliferative). Conversely, none of the patients in categories CI and CIII had diabetic retinopathy, and background retinopathy was observed only in 50% of CI and 57% of CIII patients. In Type 1 diabetes, 28% of patients with MA had proliferative retinopathy, and 58% background retinopathy. This suggests that the different underlying pathophysiologic mechanisms responsible for Type 2 diabetes in these groups of patients may also underlie different renal pathophysiologic mechanisms or responses.

As MA is not associated with renal structural changes in a substantial subset of Type 2 diabetic patients, we considered the possibility that it might be due to endothelial dysfunction. Thus, plasma levels of von Willebrand factor (vWF), an endothelial-derived protein indicative of endothelial function, were measured in a group of MA patients undergoing kidney biopsy [54]. Contrary to our assumptions, vWF plasma levels in the 32 patients studied were significantly increased only in those with renal structural abnormalities (both allocated in category II-typical and category III-atypical patterns), but normal in those with normal renal structure (Category I) [54]. These studies suggest that there are two types of MA in Type 2 diabetes: one associated with increased vWF plasma levels, established renal structural lesions, and (frequently) diabetic retinopathy; and the other characterised by normal vWF plasma levels, normal renal structure, and no or mild diabetic retinopathy. Whether these two types of MA have a different prognostic impact in terms of end-stage renal disease and cardiovascular events deserves to be investigated in longitudinal studies.

Preliminary results on the course of renal function in these patients, involving repeated measurements of GFR over a follow-up of 4 years, suggest that glomerular structure has a strong impact on the course of kidney function. Those patients with more advanced diabetic glomerulopathy show a decline in GFR over time, whereas no significant changes in GFR occur in patients with no or mild glomerulopathy. Among 108 Type 2 diabetic patients studied (74 with MA and 34 with proteinuria), the number who showed a decline in GFR (“Progressor”) increased across quartiles of GBM width and Vv(mes/glom) [55].

This study also showed that AER and GFR at baseline, as well as mean blood pressure levels during follow-up, did not influence the change in GFR, whereas patients termed “Progressor” had the worst metabolic control and the odds ratio of being a “Progressor” increased across the quartiles of HbA1c [55].

Larger studies over a longer term are required to understand the clinical implications of these complex processes on renal structure and function in Type 2 diabetes.

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