9. EARLY DETECTION OF CHRONIC COMPLICATIONS: DIABETIC NEPHROPATHY

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Chronic complications resulting from diabetes mellitus are the third leading cause of death attributable to disease in Europe. Patients with juvenile-onset insulin-dependent diabetes mellitus (Type 1 diabetes) have, at the age of forty, twenty times higher mortality rate and on average at least fifteen years shorter life span when compared with healthy controls of the same age. The principal chronic complications of diabetes mellitus are retinopathy, neuropathy, angiopathy and nephropathy. Among them diabetic nephropathy (DN) with concomitant coronary artery disease (CAD) is the complication with the worst outcome.

DN refers to damage of the glomerulus (filtering apparatus of the nephron) and capillaries associated with the glomerulus, leading to a reduction in the filtering capability of the kidneys and clinical picture of marked proteinuria (>3.5 g/day), red blood cell casts, oedema and hypertension. DN develops in 30 to 40% of patients with type 1 diabetes and in around 10 to 20% of patients with maturity-onset diabetes mellitus (Type 2 diabetes). Nowadays DN is the single most common cause of end-stage renal disease (ESRD) in the Western world. Approximately 25 to 30% of individuals treated for end-stage renal failure are diabetics. In 1997, 44% of all new cases of ESRD in U.S. were diagnosed as diabetic patients, >80% of them had type 2 diabetes.

DN is characterised by specific morphometric changes mainly of glomerular structures. Glomerular basement membrane shows marked hyaline thickening as a result of a deposition of proteins or glycoproteins from plasma. However, the major changes are found in the mesangial area where there is a proliferation of mesangial cells with increased formation of mesangial matrix, which resembles hyaline deposition in the basement membrane. At the beginning these changes are diffuse and less specific. However, with the progression of the disease glomerular basement membrane becomes thicker and mesangial fractional volume increases. Finally, nodular glomerular lesions highly specific for DN (Kimmelstiel-Wilson nodules) develop. There is a correlation between diffuse diabetic glomerulosclerosis and deterioration of renal function determined as increasing diastolic blood pressure, proteinuria, blood urea and creatinine, and decreasing serum albumin, urea and creatinine clearances. Nodular lesions are not functionally important but are important diagnostically. Beside glomerular features DN shows marked hyaline thickening of afferent and also efferent arterioles, which may lead to severe ischaemia and is responsible for further tubular atrophy, thickening of the tubular basement membrane and intestinal fibrosis.

In general the longer the duration of the diabetes the more advanced are the glomerular changes. The correlation between severity of DN and duration of diabetes is, however, not very close. There is also a wide variation in the rate of development of the lesions. It seems that all patients with type 2 diabetes appear to develop glomerular structural changes of diabetes, albeit some at very slow rates. Others develop lesions so fast that they result in overt DN in as little as ten years. Early detection of risk leading to the possibility of interven-

tion before advanced renal damage has occurred is an obviously important goal. This goal is made difficulty by the fact that much of the important diabetic renal structural injury can occur in absolute clinical silence. Furthermore, it may not be practical to treat all diabetic patients with all potentially useful therapies. It would be far better to focus the available health care resources on those most likely to benefit.

1.1. Microalbuminuria

Based on studies in patients with type 2 diabetes, it has been generally considered that once overt DN, manifesting as persistent proteinuria, is present, it is possible only to slow, but not halt, the progression toward ESRD. This led investigators during the early 1980s to search for early predictors of DN through the measurement of low concentrations of albumin in the urine. Some diabetic patients were found to have increased urinary albumin excretion rate (UAER) not detectable by standard laboratory methods, and this condition was termed microalbuminuria (MA). These early studies further led to a consensus conference in which a general agreement was reached on the definition of MA, which is UAER between 20 to 200 mg/min or between 30 to 300 mg/day.

Initial retrospective studies observed an approximate 80% rate of progression from MA to proteinuria over the subsequent 6-14 years in patients with type 1 diabetes and led to the broad acceptance of MA as a useful clinical predictor of increased DN risk. However, more recent studies have observed, in patients with type 2 diabetes, only about a 30-45% risk of progression of MA to proteinuria over 10 years, while about 30% of patients with MA become normoalbuminuric and the rest remained microalbuminuric. Furthermore, around 40% of all patients designated to progress to proteinuria are normoalbuminuric at initial screening, despite many years of diabetes. The finding, that some MA patients have only mild diabetic renal lesions is consistent with the lower than originally estimated risk of progression from MA patients to proteinuria and with the notion that some MA patients revert to normoalbuminuria. To increase the complexity of the scenario, some normoalbuminuric long-standing patients with type 1 diabetes have well-established DN lesions. In these patients MA is a marker rather than a predictor of advanced renal structural changes and treatment for these patients could be less effective than at earlier stages of the disease. A similar picture is emerging in patients with type 2 diabetes though fewer studies have been conducted. Despite that, UAER remains the strongest broadly available predictor or marker of DN risk and should be regularly measured according to established guidelines. However, we should be aware that UAER may be unable to define patients who are safe from or at risk of DN with an accuracy that is adequate for optimal clinical decision making or for the design of certain clinical trials, so we need improved predictors and markers of DN risk.

There are two general approaches. The first is to improve the usage of existing methods such as using repeated measures of UAER over time, different set points for the definition of MA, or both. In addition, the combination of measures of UAER with multiple clinical and renal structural parameters, such as increased baseline blood pressure or HbA1c, may lead to the development of more precise risk estimates for DN risk. The second approach is to look for new predictors and markers of DN risk, such as renal functional reserve.

1.2. Renal functional reserve

The kidney has the ability to modify the plasma flow subject to different stimuli, which interfere with body homeostasis. Therefore glomerular filtration rate (GFR) fluctuates during the day in order to maintain stable environment optimal for different physiological processes. Autoregulation of the plasma flow is the result of the changes in afferent and efferent arteriolar resistance, and the endothelium has a major role in regulation of vascular tone.

Endothelium modulates vascular tone by releasing numerous vasoactive substances including the endothelium-derived relaxing factor (EDRF), which has been identified as nitric oxide (NO). NO is formed from amino acid L-arginine by the enzyme NO synthase. Animal studies suggest that diabetes or even hyperglycaemia, per se, can result in impaired endothelium-dependent vasodilatation by reduced bioavailability of endothelium-derived NO. However, loss of NO results not only in enhanced contractility, but also in proliferation of vascular smooth muscle cells, increased platelet aggregation, coagulation and generation of endothelin, and increased leukocyte or monocyte adhesion to the endothelium. Endothelial dysfunction, which is nowadays a generally accepted term for the decreased bioavailability of NO, disturbs the physiological protective regulatory balance and ultimately contributes to disease progression such as atherosclerosis and ischemic coronary events. Most recent evidence leads us to the conclusion that endothelial dysfunction may also have a substantial role in the development of DN.

The ability of endothelium to modify renal haemodynamic and glomerular function can be tested in vivo by measuring changes in GFR after amino acid ingestion. Oral protein load or amino acid infusion increases L-arginine concentration and bioavailability of NO, which leads to vasodilatation of afferent and efferent arterioles, and increases renal plasma flow and GFR. The difference between basal or “unstimulated” GFR and “stimulated” GFR is defined as renal functional reserve (RFR). There is a general agreement that RFR reflects the capacity of the healthy kidney to achieve a higher degree of function by vasodilatation of glomerular arterioles. During kidney disease, RFR demonstrates the ability of available nephrons to increase its GFR, which compensates the reduced number of functioning nephrons resulting from deterioration of the kidney function.

In patients with type 2 diabetes an altered renal haemodynamic response to protein load or amino acid infusion has been consistently reported. It is generally believed that these patients have decreased ability to vasodilate afferent arterioles due to endothelial dysfunction and reduced bioavailability of NO, which results in decreased or blunted RFR. Several clinical data suggest that in DN renal haemodynamic regulation as assessed by RFR may be lost prior to a decline of the resting, unstimulated GFR. For instance, RFR was found to be reduced in non-albuminuric-normotensive patients with recently diagnosed diabetes and was considered to identify a unique group of patients with significantly elevated mean basal GFR, which is a frequent hallmark of early DN. Thus, RFR appear to provide a much more sensitive assessment of the functional renal damage than usual baseline evaluation, and decreased or blunted RFR may be an early marker of DN.

A relevant question one can ask is whether decreased or blunted RFR may be also a predictor of DN. Patients with long-term type 1 diabetes without DN have normal RFR and therefore their glomerular function and renal haemodynamic can be considered as normal. On

the other hand, RFR is suppressed in macroalbuminuric hypertensive patients with overt DN in spite of the similar long-term course of the disease and remarkably maintained basal GFR. These results justify the interpretation that the decreased or blunted RFR may have a relevant role in the pathophysiology and the progression of DN. The reduction or the absence of functional reserve in renal disease could imply that the residual nephrons are already in a state of permanent glomerular hyperfiltration. Such a glomerular hyperfiltration associated with a reduction of RFR has been found as causal in the initial stages of the pathogenesis of diabetic microangiopathy leading finally to further vasodilatation and destruction of originally less damaged capillaries in organs with endarterial circulation such as kidney and retina, and the progression toward renal failure regardless of the primary disease. In one 8-year prospective study, glomerular hyperfiltration was found to be the only significant independent predictor of DN in initially normoalbuminuric patients with diabetes. Whatever the inner pathological mechanism, the glomerular filtration process appears to be impressively well-protected in overt DN at every time of the disease, while irreversible impairment of glomerular function is evidenced by the increased resistance and by the suppressed ability to vasodilate and to demonstrate normal RFR. The assumption that the decreased or blunted RFR may be the predictor of DN could be additionally strengthened by the evidence that RFR is normalised by strict metabolic control or dietary protein restriction, which are known to ameliorate the clinical outcome of DN in patients with type 1 diabetes.

1.3. Von Willebrand factor

Endothelial injury is probably a key feature of diabetic nephropathy, even during the subclinical phase. Evidence for this includes the observation that the transcapillary escape rate of albumin is increased in diabetic subjects with albuminuria. As a healthy glomerulus will retain albumin, it follows that a raised UAER implies damage to the endothelium of the kidney. A further corollary may be that those factors that damage the glomerular endothelium also damage endothelial beds elsewhere, and hence, possibly systemically. Indeed, microalbuminuria was found to be a marker of widespread vascular damage, which may underline the propensity of microalbuminuric patients to develop severe extrarenal vascular disease such as retinopathy, neuropathy, hypertension and macrovascular disease, and was also proven to be a predictor of atherosclerotic cardiovascular disease in patients with diabetes and in non-diabetic subjects.

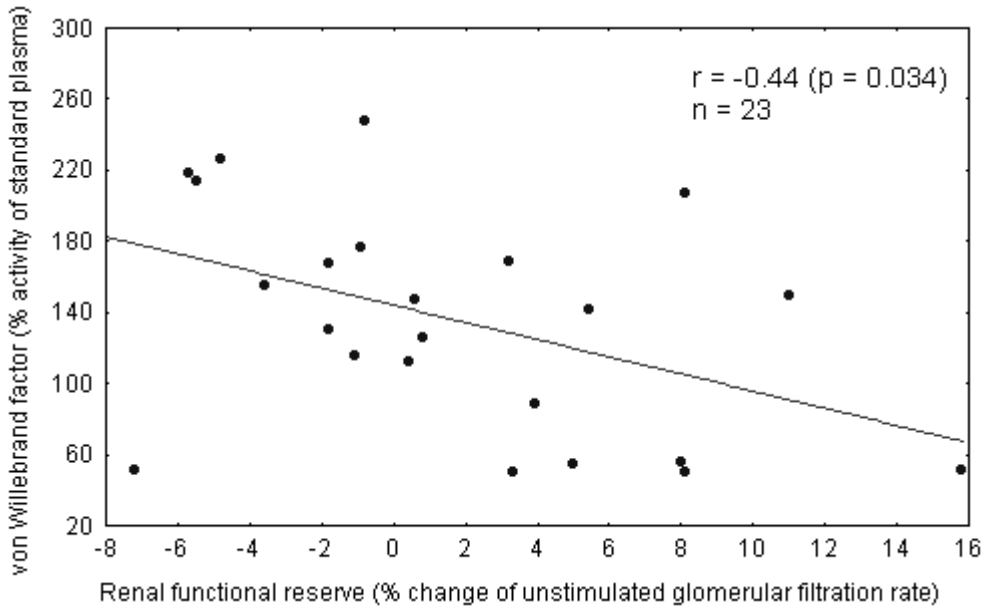
An alternative method of assessing endothelial disintegrity is to look for changes in the levels of various secreted products such as von Willebrand factor (vWf), soluble thrombomodulin, soluble E-selectin or tissue plasminogen activator, which are markers of generalized endothelial cell injury or endothelial dysfunction. VWf binds factor VIII and mediates platelet adhesion and spreading on endothelium. Following vascular injury, adhesion of platelets mediated by vWf is a pivotal element of haemostasis. Vascular endothelial cells are the major source of vWf and release vWf constitutively or from the Weibel-Palade bodies by a regulated pathway. Stimulation of the constitutive pathway of vWf secretion via endotoxin increases plasma levels of vWf after several hours. In contrast, stimulation of the regulated pathway of vWf-secretion through various mediators like thrombin, fibrin or vasopressin causes rapid release of vWf from intracellular endothelial stores, which is complete at 30 min. It might be important also to know that NO has dampening effect on vWf release from endothelial cells.

Several groups have measured plasma vWf activity in patients with diabetes. In cross-sectional analysis vWf was found to be higher in patients with type 1 diabetes manifesting microalbuminuria or overt DN. Furthermore, in patients with type 1 diabetes or type 2 diabetes with or without hypertension, increased plasma vWf activity correlated positively with UAER, in particularly in patients manifesting retinopathy. In longitudinal studies in patients with type 2 diabetes the development of microalbuminuria was associated with the baseline level of vWf. Elevated plasma vWf even preceded the development of microalbuminuria in patients with type 1 diabetes by approximately 3 years. Most recently, high levels of plasma vWf were found to be associated with progression in UAER also in clinically healthy subjects during a follow-up period of 4 years. To conclude, endothelial damage as estimated by plasma vWf activity preceded and may therefore predict the development of microalbuminuria in patients with diabetes and in health.

1.3.1. Negative (decreased, blunted) renal functional reserve associates increased von Willebrand factor plasma activity in patients with diabetes

Since RFR, as the indicator of renal haemodynamic impairment due to renal endothelial perturbation, is decreased or blunted and vWf, as the indicator of generalized endothelial vascular injury, is increased in diabetic patients with incipient DN, we hypothesized that RFR is negatively associated with vWf activity in plasma. To test the hypothesis, we measured RFR and vWf activity in 23 normoalbuminuric patients with type 1 diabetes manifesting retinopathy. Results are graphically presented in Figure 1.

Figure 1. Association between renal functional reserve and von Willebrand factor activity in plasma in normoalbuminuric patients with insulin-dependent diabetes mellitus manifesting retinopathy.



RFR was determined by the method of Estelberger et al. (1). Briefly, GFR determination relies essentially on system identification of the two-compartment kinetics of sinistrin, an inulin-like polyfructosan injected as an i.v. bolus. The temporal concentration profile was sampled over 3 hours. After ingestion of a protein-rich meal a second i.v. bolus of sinistrin was

applied and the ensuing sinistrin concentration contour observed during next 3 hours. RFR was finally calculated as a relative difference in GFRs after and before stimulation of renal function with the protein-rich meal. As tested previously, the method is sensitive enough to measure even acute short-term changes in renal haemodynamic after amino acid stimulation offering the precise determination of RFR within the required error bounds for the system parameter estimates in individual patients.

As demonstrated in Figure 1, we found great variety in RFR in our very homogeneous group of normoalbuminuric diabetic patients, with retinopathy showing positive (normal) or negative (decreased, blunted or bluntly increased) values, thus possibly identifying a subgroup of high-risk patients more likely to develop DN. The subgroup of patients with negative RFR did not differ from the subgroup of patients with positive RFR regarding any other parameter observed which may interfere GFR, such as age, duration of diabetes, blood pressure, HbA1c, lipids and apolipoproteins.

The most striking feature was that these RFRs were negatively associated with vWf activity in plasma. As high vWf activities precede and predict the development of microalbuminuria in patients with diabetes and in health, the above mentioned association suggests, that negative RFR may be a predictor of microalbuminuria and subsequent progression to DN too. However, it is still not clear whether the prognostic value of vWf is related to its specific function, i.e., enhancement of platelet adhesion and factor VIII availability, or whether it is simply a marker of endothelial injury and dysfunction arising from the problems of the specificity of the marker. Furthermore, increase in vWf activity in plasma is non-specific with respect to the type of injury and can be induced by hypertension, smoking, hypercholesterolaemia, hyperglycaemia, activation of coagulation, and cytokines arising to the problems of sensitivity of the marker. Therefore, RFR, as a sensitive and stage-specific indicator of the changed renal haemodynamic due to renal endothelial perturbation possibly predicting development of microalbuminuria and subsequent progression to DN, deserves further attention.

Recommended literature:

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