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Microalbuminuria and Risk for Cardiovascular Disease: Analysis of Potential Mechanisms

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Microalbuminuria is a strong and independent indicator of increased cardiovascular risk among individuals with and without diabetes. Therefore, microalbuminuria can be used for stratification of risk for cardiovascular disease. Once microalbuminuria is present, cardiovascular risk factor reduction should be more "aggressive." The nature of the link between microalbuminuria and cardiovascular risk, however, remains poorly understood. There is no strong evidence that microalbuminuria causes atherothrombosis or that atherothrombosis causes microalbuminuria. Many studies have tested the hypothesis that a common risk factor underlies the association between microalbuminuria and cardiovascular disease but, again, have found no strong evidence in favor of this contention. At present, the most likely possibility is that a common pathophysiologic process, such as endothelial dysfunction, chronic low-grade inflammation, or increased transvascular leakage of macromolecules, underlies the association between microalbuminuria and cardiovascular disease, but more and prospective studies of these hypotheses are needed.

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Microalbuminuria is strongly associated with risk for cardiovascular disease, but the nature of this link remains controversial and poorly understood. This is reflected by expressions such as "microalbuminuria is a risk factor for cardiovascular disease," "microalbuminuria is a risk indicator for cardiovascular disease," and "microalbuminuria is a marker of endothelial dysfunction."

We define risk *factors* as variables that are (believed to be) causally related to cardiovascular disease, whereas risk *indicators* (or risk markers) are indirectly associated with cardiovascular disease, for example because they reflect a pathophysiologic mechanism that causes atherothrombosis or because they are strongly associated with an unknown (and unmeasured) risk factor. With regard to risk stratification, both risk indicators and risk factors are useful because the issue of causality is irrelevant with regard to risk prediction. Nevertheless, it is important to try to understand the nature of the association between (presumed) risk indicators and cardiovascular disease, because this may provide insight into the potential usefulness of adding the risk indicator to existing risk scores and of targeting the risk indicator to decrease risk. In addition, the exact nature of the association between a risk indicator and subsequent atherothrombotic disease may provide new insights into the pathobiology of vascular disease. This brief review discusses the nature of the association between microalbuminuria and cardiovascular disease in light of the above considerations.

Microalbuminuria as a Cardiovascular Risk Indicator

Microalbuminuria is a widely recognized, strong and independent risk marker of cardiovascular disease among individuals with diabetes. For example, Dinneen and Gerstein (1), in a systematic review, showed microalbuminuria among individuals with type 2 diabetes to be associated with a 2.4-fold (95% confidence interval [CI] 1.8 to 3.1) increased risk for cardiovascular death as compared with normoalbuminuria. In addition, similar associations exist in hypertensive individuals (without diabetes) and in the general population (2–6). Importantly, recent studies have added three novel findings to the well-established association between microalbuminuria and cardiovascular disease. First, the association between urinary albumin excretion and risk for cardiovascular disease does not begin at the traditional thresholds for defining microalbuminuria (*i.e.*, a urinary albumin-to-creatinine ratio of 2.5 mg/mmol in men and of 3.5 mg/mmol in women or equivalent urinary albumin excretion rates) but instead has a much lower threshold, starting at 1 mg/mmol creatinine or even below (7,8). Second, in individuals with diabetes, *progression* of microalbuminuria has been shown to be associated with a further increase in the risk for cardiovascular disease in a way that is independent of the

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initial urinary albumin excretion (9,10). Third, during 4.8 yr of antihypertensive treatment in 8206 patients with hypertension and left ventricular hypertrophy in the Losartan Intervention For Endpoint reduction in hypertension (LIFE) study, any decrease of urinary albumin excretion during treatment was associated with a proportional reduction in the risk for the primary composite end point (cardiovascular mortality, stroke, and myocardial infarction), which was not explained by the on-treatment level of BP (11,12).

Such associations between urinary albumin excretion and cardiovascular disease extend beyond microalbuminuria. For example, in a 10-yr follow-up study, Samuelsson *et al.* (13) showed that macroalbuminuria (*i.e.*, albumin excretion above the microalbuminuria threshold) was associated with an approximately three-fold increased cardiovascular risk among hypertensive men. In the Reduction of Endpoints in NIDDM with the Angiotensin II Antagonist Losartan (RENAAL) study, any decrease in albuminuria was strongly related to decreased risk for cardiovascular as well as renal outcomes (14,15). Understanding these relationships is important, because, although the prevalence of microalbuminuria is low among young people, it rises with age and is strongly associated with the presence of hypertension and diabetes, resulting in a prevalence of approximately 8 to 10% in the general elderly population, approximately 20% among hypertensive individuals, and approximately 30% among individuals with type 2 diabetes (5,6,16,17).

A final issue is whether microalbuminuria is associated with a particular phase of the atherothrombotic process, as suggested by some studies that analyzed the temporal association between microalbuminuria and cardiovascular events. One study, for example, found that microalbuminuria predicted cardiovascular mortality only in the relatively short term of 5 yr and not beyond, suggesting that microalbuminuria is associated with some adverse process that occurs relatively late in the atherothrombotic disease course (18). A compatible recent finding in the elderly in the Cardiovascular Health Study was that microalbuminuria was associated with cardiovascular events but not with subclinical atherosclerosis (as measured by carotid intima-media thickness), leading the authors to conclude that microalbuminuria may be associated with plaque destabilization rather than with atherosclerosis itself (19). However, these issues are not settled and deserve further study before firm conclusions can be drawn.

Nature of the Link Between Microalbuminuria and Cardiovascular Disease

A systematic analysis of the association between microalbuminuria and cardiovascular disease needs to take into consideration the following possibilities: (*1*) microalbuminuria causes cardiovascular disease; (*2*) cardiovascular disease causes microalbuminuria; and (*3*) both are caused by a common denominator, *e.g.*, a common risk factor or pathophysiologic process.

*Can Microalbuminuria Cause Cardiovascular Disease (*i.e., *Atherothrombosis)?*

There is no direct evidence for this contention, but the possibility that, for example, increased renal albumin (or protein) trafficking elicits a renal response that enhances the atherothrombotic process needs to be examined.

Can Atherothrombosis Cause Microalbuminuria?

It has been suggested that microalbuminuria is simply a marker of generalized atherosclerosis and that this explains its association with clinical cardiovascular disease. To test this hypothesis, we compared the association between microalbuminuria and cardiovascular disease with that between peripheral arterial disease (an accepted marker of generalized atherosclerosis) and cardiovascular disease in an age-, gender-, and glucose tolerance–stratified sample ($n = 631$) of a population-based cohort of individuals who were aged 50 to 75 yr and followed prospectively for 5 yr (2). This study showed that both microalbuminuria and peripheral arterial disease were strongly associated with 5-yr risk for cardiovascular death. However, only approximately 25% of individuals with microalbuminuria also had peripheral arterial disease and *vice versa*. In addition, mutual adjustment did not markedly affect the relative risk estimates, which argues against the idea that microalbuminuria is a marker of generalized atherosclerosis, because, if microalbuminuria affected risk through generalized atherosclerosis, then including the presence of peripheral arterial disease in the multivariate regression model would have been expected to lower the increased risk for mortality that is conferred by the presence of microalbuminuria.

Is There a Common Risk Factor Underlying the Association between Microalbuminuria and Cardiovascular Disease?

Many cross-sectional and somewhat more limited prospective data indicate that microalbuminuria is associated with many cardiovascular risk factors or risk indicators, such as advanced age, male gender, hypertension, diabetes, smoking, obesity, dyslipidemia (low HDL cholesterol and high triglycerides more clearly so than high LDL cholesterol), and hyperhomocysteinemia (20). At first glance, therefore, the association between microalbuminuria might simply be due to confounding by such risk factors, which might cause both microalbuminuria and atherothrombosis and thus explain their association. However, many studies have investigated this and have concluded that common risk factors explain at most a small part of the association between microalbuminuria and atherothrombosis. Nevertheless, it should be emphasized that confounding by other risk factors remains a distinct possibility. First, more precise measurements of potential confounders (*e.g.*, 24-h BP rather than office measurements) may show more extensive confounding than has hitherto been supposed. Such studies certainly need to be done. Second, some potential confounders, such as autonomic dysfunction (21), have not yet been examined in prospective studies. Third, subtle impairments of GFR, which themselves are associated with increased risk for cardiovascular disease (22), may link microalbuminuria to cardiovascular disease. However, microalbuminuria often is associated with a high rather than a low GFR (hyperfiltration), both in individuals with and in individuals without diabetes, so this mechanism seems less likely. Nevertheless, the associations among microalbuminuria, GFR, and cardiovascular disease in-

cidence need to be examined in prospective, population-based studies. Finally, it has been suggested that microalbuminuria is part of the metabolic or insulin resistance syndrome, although this is controversial (16). Again, this hypothesis needs to be tested in a prospective study by investigating whether the association between microalbuminuria and cardiovascular disease disappears when adjusted for insulin resistance and (or) the other variables included in the metabolic syndrome.

Clearly, the spectrum of cardiovascular risk factors remains to be discovered fully. Hence, there remains a possibility that microalbuminuria reflects the presence of a hitherto undiscovered factor that is causally related to atherothrombosis. However, because adjustment for known risk factors only marginally explains the cardiovascular risk that is associated with microalbuminuria, it would seem unlikely that other, yet undiscovered, risk factors would turn out to explain this association fully.

Is There a Common Pathophysiologic Process Underlying the Association between Microalbuminuria and Cardiovascular Disease?

Microalbuminuria and cardiovascular disease may be linked not by a common risk factor but rather by a common pathophysiologic process. Again, several hypotheses have been put forward. One is that dysfunction of the vascular endothelium causes both microalbuminuria and cardiovascular disease (see references 20,23 for reviews). Endothelial dysfunction can be defined as any change in endothelial properties that is inappropriate with regard to the preservation of organ function. Therefore, many types of endothelial dysfunction exist depending on which function is affected (*e.g.*, the regulation of hemostasis and fibrinolysis, vasomotor activity, permeability to macromolecules, leukocyte adhesion, vascular smooth muscle cell proliferation). Nitric oxide (NO) is a particularly important endothelium-derived mediator because of its vasodilator, antiplatelet, antiproliferative, antiadhesive, permeability-decreasing, and anti-inflammatory properties. Generalized endothelial dysfunction (*i.e.*, affecting many endothelial functions) now is considered a transducer of atherogenic risk factors and is thought to play an important role in both the initiation and the progression of atherosclerosis. Therefore, an association of microalbuminuria with generalized endothelial dysfunction, if it exists, could explain why microalbuminuria strongly predicts cardiovascular disease. Indeed, microalbuminuria in type 1 and type 2 diabetes usually is accompanied by endothelial dysfunction with regard to the regulation of hemostasis, fibrinolysis, leukocyte adhesion, and NO synthesis and/or availability, as estimated by plasma levels of endothelial function markers such as von Willebrand factor, tissue-type plasminogen activator, soluble vascular cell adhesion molecule-1, and soluble E-selectin and by endothelium-dependent vasodilation in response to increases in flow or to agonists such as cholinergic agents (20,23). Therefore, endothelial dysfunction in individuals with diabetes and microalbuminuria, at the very least, is extensive. Whether this occurs in all vascular beds is extremely difficult to test in humans but obviously is an important question. One small study did find a disturbed endothelial vasomotor response associated with microalbuminuria in not only the brachial artery but also the renal interlobar arteries (24), but we are not aware of any studies that have been done, for example, in the coronary circulation.

There are fewer data on the extent of endothelial dysfunction in nondiabetic individuals with microalbuminuria, but such endothelial dysfunction, as in diabetes, has been suggested to involve the regulation of hemostasis, fibrinolysis, leukocyte adhesion, and NO synthesis and/or availability. For example, a recent, large, population-based study of 645 individuals (mean age 68 yr; 248 with normal glucose metabolism, 137 with impaired glucose metabolism, and 260 with type 2 diabetes) showed that endothelial NO synthesis, as estimated from ultrasonically measured brachial artery endothelium-dependent, flow-mediated dilation, was impaired in individuals with diabetes as compared with those without and also was impaired in individuals with (micro)albuminuria as compared with those without, regardless of whether they had diabetes (25,26). Specifically, flow-mediated dilation of the brachial artery was 0.12 mm in the presence of (micro)albuminuria (defined as urinary albumin-creatinine ratio $\geq$2 mg/mmol; $n = 93$; 49 with diabetes) and 0.18 mm in its absence ($P = 0.002$). After adjustment for age, gender, baseline arterial diameter, and other potential confounders, flow-mediated dilation was 0.038 mm (95% CI 0.001 to 0.075) lower in the presence of (micro)albuminuria ($P = 0.04$) and decreased linearly across (micro)albuminuria categories (by 0.027 mm [95% CI 0.007 to 0.046] per category [$<$2, $\geq$2 to 5, $\geq$5 to 10, and $\geq$10 mg/mmol] increase of (micro)albuminuria; $P = 0.007$). Endothelium-independent, nitroglycerin-induced vasodilation was similar in individuals with and without (micro)albuminuria. All results were similar in individuals without and with diabetes.

These findings support the concept that impaired endothelial NO synthesis plays a role in the association of microalbuminuria with cardiovascular disease risk regardless of whether diabetes is present. Indeed, several studies have shown that endothelial dysfunction *precedes* and *predicts* the onset of microalbuminuria in individuals without and with diabetes (20,23). It therefore is tempting to postulate that endothelial dysfunction in microalbuminuria explains why microalbuminuria is a consistent marker of increased risk for atherothrombosis. This in turn raises the question of how endothelial dysfunction could cause microalbuminuria. Albumin is a relatively large, negatively charged protein (molecular weight 69 kD; size 36 Å). The filter through which albumin must pass before entering the urine, the glomerular capillary wall, is size and charge selective. Microalbuminuria is thought to be a consequence of an increased albumin leakage through the glomerular capillary wall as a result of increased permeability of the wall, an increased intraglomerular pressure, or both. For example, hyperglycemia and high BP are generally accepted risk factors for development of microalbuminuria. Both can increase intraglomerular pressure. In addition, hyperglycemia can alter the charge selectivity of the glomerular capillary wall, thereby increasing its permeability. In a healthy kidney, $>$99% of filtered albumin is reabsorbed in the proximal tubules. Some data suggest that microalbuminuria, at least in patients with type 2

diabetes, is associated not only with increased glomerular protein passage but also with an absence of a compensatory increase in tubular reabsorption of albumin (27). A pronounced increase in albumin filtered by the glomerulus will lead to excessive supply of albumin to the renal tubule, eventually exceeding tubular reabsorptive capacity, and thus to increased albumin excretion in the urine.

Theoretically, endothelial dysfunction could cause albuminuria by increasing glomerular pressure and glomerular barrier permeability. Previously, glomerular barrier permeability was thought to depend mainly on glomerular basement membrane composition and slit diaphragm structure. Recent evidence, however, has pointed toward a more important, direct role of the endothelium in determining permeability to albumin. In particular, the glycocalix that fills the endothelial fenestrae seems to be important for glomerular size and charge selectivity (28,29). Abnormalities in the endothelial glycocalix may contribute to (micro)albuminuria but also have been implicated in the pathogenesis of atherosclerosis, thus providing a potential direct link between albuminuria and cardiovascular disease (30). In particular, this recent knowledge of a possible common endothelial mechanism for increased glomerular albumin leakage and generalized vascular disease sheds new light on the concept that microalbuminuria reflects a systemic transvascular leakage of albumin, which might predispose to greater penetration of atherogenic lipoprotein particles into the arterial wall—the Steno Hypothesis (31). This hypothesis has been tested by examination of the transcapillary escape rate of labeled albumin, as a marker of transvascular leakage, in individuals without and with microalbuminuria. However, the association between increased transcapillary escape rate of labeled albumin and microalbuminuria does not seem to hold under all circumstances (31–33). A recent animal study, conversely, did suggest that endothelial glycocalix loss is associated with an increased permeability to macromolecules in the coronary circulation (34).

Ideally, to test the hypothesis that endothelial hyperpermeability to macromolecules plays a central role in the association between microalbuminuria and cardiovascular disease, one would need to investigate whether this association disappears when adjusted for macromolecular permeability (*i.e.*, transcapillary escape rate of labeled albumin).

Atherothrombosis currently is understood as a process in which endothelial dysfunction and chronic, low-grade inflammation are important early events. Indeed, chronic, low-grade inflammation can be both cause and consequence of endothelial dysfunction, and the two are tightly linked. Chronic, low-grade inflammation can be assessed by measurement of plasma levels of C-reactive protein and cytokines such as IL-6 and TNF-α. Studies using such markers have shown that, regardless of the presence of diabetes, chronic, low-grade inflammation is associated with the occurrence and the progression of microalbuminuria and with risk for atherothrombotic disease (35–37).

In view of the above considerations, endothelial dysfunction and chronic, low-grade inflammation are important candidates to explain the association between microalbuminuria and cardiovascular disease. Nevertheless, studies that have examined this hypothesis have (unexpectedly) observed that microalbuminuria, endothelial dysfunction, and low-grade inflammation, although tightly linked, were *independently* associated with risk for cardiovascular death (32,33). This may mean that, in these studies, endothelial dysfunction and low-grade inflammation were not measured with sufficient precision. Alternatively, the types of endothelial dysfunction (particularly measures of NO availability and biochemical markers of endothelial function) and low-grade inflammation (particularly C-reactive protein) tested may have been irrelevant with regard to cardiovascular risk in microalbuminuria. A final possibility to consider is that endothelial dysfunction and low-grade inflammation, although associated with microalbuminuria, do not explain the link between microalbuminuria and cardiovascular disease.

Conclusion

Both in nondiabetic and diabetic subjects, microalbuminuria is associated with an increased cardiovascular risk, independent of known risk determinants. As such, microalbuminuria is potentially useful for improved cardiovascular risk stratification. Certainly, in the presence of microalbuminuria, tight control of atherothrombotic risk factors is warranted. How exactly microalbuminuria is linked to cardiovascular risk is unclear. It is doubtful whether microalbuminuria causes atherothrombosis or *vice versa*. A common risk factor may underlie the association between microalbuminuria and cardiovascular disease, but clear evidence for this is lacking. The association between microalbuminuria and cardiovascular disease is probably explained by a common pathophysiologic process, such as endothelial dysfunction or chronic, low-grade inflammation. Further studies are clearly required to expand our understanding in this field.

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