N-Terminal Pro-B–Type Natriuretic Peptide Is Related to Retinal Microvascular Damage
The Rotterdam Study

Unal Mutlu, M. Arfan Ikram, Albert Hofman, Paulus T.V.M. de Jong, Caroline C.W. Klaver, M. Kamran Ikram

Objective—N-terminal pro-B–type natriuretic peptide (NT-proBNP) is a marker of cardiac dysfunction and has been linked to various indices of large vessel disease. However, it remains unclear whether NT-proBNP also relates to microvascular damage. In a community-dwelling population, we studied the association between NT-proBNP and retinal microvascular damage.

Approach and Results—From the population-based Rotterdam Study, we included 8437 participants (mean age 64.1 years and 59% women) without a history of cardiovascular disease, with NT-proBNP data and gradable retinal images. NT-proBNP serum levels were measured using an immunoassay. Retinopathy signs, that is, exudates, microaneurysms, cotton wool spots, and dot/blot hemorrhages, present on fundus photographs were graded in the total study population; retinal vascular calibers, that is, arteriolar and venular calibers, were semiautomatically measured in a subsample (n=2763) of the study population. We conducted cross-sectional analyses on the association between NT-proBNP and retinal microvascular damage using logistic and linear regression models, adjusting for age, sex, and cardiovascular risk factors. We found that NT-proBNP was associated with the presence of retinopathy (adjusted odds ratio [95% confidence interval] per SD increase in natural log-transformed NT-proBNP: 1.14 [1.03–1.27]). We also found that higher NT-proBNP was associated with narrower arteriolar calibers (adjusted mean difference in arteriolar caliber per SD increase in natural log-transformed NT-proBNP: −0.89 µm [−1.54 to −0.24]). This association remained unchanged after excluding participants with retinopathy signs.

Conclusions—In participants free of clinical cardiovascular disease, higher levels of NT-proBNP are associated with retinal microvascular damage, suggesting a potential role for NT-proBNP as marker for small vessel disease.

Key Words: arterioles ■ biomarkers ■ etiology ■ heart diseases ■ N-terminal pro-BNP ■ venules

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such patient populations are being recognized as a distinct entity representing a wide spectrum of coronary microvascular dysfunction. Against this background, it is conceivable that NT-proBNP—as marker of subclinical cardiac dysfunction—may also be related to indices of small vessel disease. Thus, the link between NT-proBNP and renal function may reflect cardiac stress and signify the presence of microvascular pathology. However, the link of NT-proBNP with direct visualization of microvascular damage has not been studied, primarily because of a lack of noninvasive markers of small vessel disease. The retina provides an opportunity to visualize the microvasculature in vivo. Microvascular damage in the retina often manifests itself as exudates, microaneurysms, cotton wool spots, dot/blot hemorrhages, and narrower arterioles, which can be quantified on retinal imaging. These measures of microvascular damage have been widely used in large population-based studies, and findings from these studies have often shown that retinal microvascular damage is related to subclinical and clinical cardiovascular diseases. In view of these observations, we hypothesize that, besides large vessel disease, cardiac stress due to microvascular dysfunction may also lead to higher levels of NT-proBNP. Therefore, in a community-dwelling population free of clinical cardiovascular disease, we investigated the association of NT-proBNP with the presence of retinopathy and retinal vascular calibers (Figure).

Materials and Methods
This study is embedded within the Rotterdam Study, an ongoing population-based cohort study in The Netherlands. Materials and Methods are available in the online-only Data Supplement.

Results
Table 1 shows the characteristics of the study population. Of the total 8437 participants, the average age was 64.1 years (SD: 9.4) and 59% were women. Participants with retinopathy had a mean NT-proBNP level of 10.7 pmol/L (interquartile range: 5.5–21.1), whereas participants without retinopathy had a mean of 7.3 pmol/L (interquartile range: 4.0–13.6). Table 2 shows the association between NT-proBNP and the presence of retinopathy. We found that higher levels of NT-proBNP were significantly associated with the presence of retinopathy: odds ratio per SD increase of natural log-transformed NT-proBNP: 1.22 (95% confidence interval, 1.10–1.35). The association attenuated after adjusting for cardiovascular risk factors, but it remained statistically significant. Furthermore, when categorizing NT-proBNP levels into quartiles, we found a graded increase in the likelihood of having retinopathy, although these effects attenuated slightly in model 2. Table 3 shows the association between NT-proBNP and retinal vascular calibers. Higher levels of NT-proBNP (both continuously and in quartiles) were significantly associated with narrower arteriolar calibers: mean difference in arteriolar caliber per SD increase of natural log-transformed NT-proBNP: −1.36 (95% confidence interval, −2.03 to −0.70). Adjusting for cardiovascular risk factors attenuated the association, but it remained statistically significant. In contrast, NT-proBNP levels were not associated with venular calibers.

Stratification on sex did not reveal any interaction with NT-proBNP (Pinteraction>0.05). After excluding participants with retinopathy (n=71) we found, if anything, stronger associations with retinal vascular calibers: mean difference in arteriolar caliber per SD increase of natural log-transformed NT-proBNP: −1.51 (−2.19 to −0.83) in model 1 and −1.01 (−1.64 to −0.35) in model 2.

Discussion
We found that in participants without cardiovascular disease, higher levels of NT-proBNP were associated with microvascular damage as reflected by the presence of retinopathy and narrower retinal arterioles. These associations were independent of cardiovascular risk factors. Thus far, evidence of a role of NT-proBNP in cardiovascular disease comes primarily from research focused on coronary heart disease and heart failure. In addition, it is increasingly being suggested that NT-proBNP might be a general marker of vascular disease beyond specific heart disease. For instance, previous studies have shown that higher NT-proBNP levels were associated with indices of large vessel disease, stroke, and mortality. In this sense, the strong link of NT-proBNP with various

Figure. Fundus photographs showing (A) signs of retinopathy and (B) measurement of retinal vascular calibers. A, White arrow: small hemorrhages; black arrow: hard exudates. B, Red lines: arteriolar calibers; blue lines: venular calibers.
leads to vasodilatation.25 It may also stimulate the production of nitric oxide, which also exerts its effect also directly. In vitro studies have demonstrated that BNP activates the guanylate cyclase receptors, on both endothelial and vascular smooth muscle cells, which subsequently activate potassium and calcium channels promoting arterial vasodilatation.24 Another important feature of BNP is that it stimulates the production of nitric oxide, which also leads to vasodilatation.25

Cardiovascular diseases may be explained by the strong link of cardiac dysfunction with vascular damage (ie, atherosclerosis, arteriosclerosis, and endothelial dysfunction), which is a substantial component of cardiovascular diseases. Although most studies investigating the association of NT-proBNP with vascular damage revolved around large vessel disease, there are now indications that NT-proBNP also relates to small vessel disease. Indeed, recent studies have shown NT-proBNP to be associated with kidney disease26 and cerebral small vessel disease,21 such as white matter lesions and silent brain infarcts. However, no study has investigated the association of NT-proBNP with direct visualization of microvascular damage. Our findings showed an independent link between higher NT-proBNP levels and markers of retinal microvascular damage. Several methodological issues need to be discussed. A limitation of our study is the cross-sectional design that prevents us inferring causality of the outcomes. Another limitation might be that we used a static measure of the microcirculation instead of dynamic functional measures synchronized on the cardiac cycle. A variation of 2% to 17% in retinal vascular caliber has been reported.30 This may have caused random misclassification, leading to an underestimated of our associations.

Furthermore, in our study, besides retinopathy signs, we did not have data on arterovenous nicking and focal arteriolar narrowing. Despite presumed differences in pathogenesis...
of specific retinal signs (eg, arteriogenous nicking and focal arteriolar narrowing are driven by hypertensive damage), both retinopathy signs and arteriogenous nicking and focal arteriolar narrowing are considered markers of microvascular pathology. As such, previous studies have shown the most consistent and strongest association between retinopathy signs and subclinical and clinical cardiovascular diseases.31 Although a positive association of NT-proBNP with arteriogenous nicking and focal arteriolar narrowing would have further supported our findings, we think that our current findings with retinopathy signs/NT-proBNP are in themselves in line with previous findings on retinopathy signs and cerebrovascular diseases. Strengths of our study are the population-based setting, large study size and extensive available data on cardiovascular risk factors, and clinical outcomes. In conclusion, we found that higher NT-proBNP levels were associated with retinal microvascular damage, independent of cardiovascular risk factors. Our findings add important corroboration to a growing body of evidence implicating NT-proBNP as a general marker of vascular disease, representing damage to not only large vessels but also small vessels.

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Disclosures
None.

References
15. Bhibis-Domingo K, Ansari M, Schiller NB, Massie B, Whooley MA. B-type natriuretic peptide and ischemia in patients with stable coronary

Table 3. The Association of NT-proBNP With Retinal Vascular Calibers

<table>
<thead>
<tr>
<th>Natural Log-Transformed NT-proBNP</th>
<th>Arteriolar Caliber, Mean Difference (95% CI)</th>
<th>Venular Caliber, Mean Difference (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Per SD increase</td>
<td>Model 1*</td>
<td>Model 2†</td>
</tr>
<tr>
<td>Quartiles (range)</td>
<td>Model 1*</td>
<td>Model 2†</td>
</tr>
<tr>
<td>1st quartile (0.59–3.33)</td>
<td>0 (reference)</td>
<td>0 (reference)</td>
</tr>
<tr>
<td>2nd quartile (3.34–6.02)</td>
<td>−1.18 (−2.55 to 0.19)</td>
<td>−1.38 (−2.69 to −0.08)</td>
</tr>
<tr>
<td>3rd quartile (6.03–11.24)</td>
<td>−1.94 (−3.36 to −0.51)</td>
<td>−1.72 (−3.09 to −0.36)</td>
</tr>
<tr>
<td>4th quartile (11.25–178.50)</td>
<td>−2.87 (−4.34 to −1.40)</td>
<td>−2.03 (−3.46 to −0.59)</td>
</tr>
<tr>
<td>P value for trend</td>
<td>&lt;0.001</td>
<td>0.01</td>
</tr>
</tbody>
</table>

CI indicates confidence interval; and NT-proBNP, N-terminal pro-B–type natriuretic peptide.

*Model 1: adjusted for age, sex, and the other vascular caliber.
†Model 2: as model 1, additionally adjusted for systolic blood pressure, diastolic blood pressure, use of antihypertensive medication, body mass index, total cholesterol, high-density lipoprotein cholesterol, diabetes mellitus, C-reactive protein, carotid plaque, and smoking.

Cl indicates confidence interval; and NT-proBNP, N-terminal pro-B–type natriuretic peptide.
These findings suggest that N-terminal pro-B–type natriuretic peptide reflects not only damage to large vessels but also damage to small vessels. These findings add important corroboration to a growing body of evidence implicating N-terminal pro-B–type natriuretic peptide as a general marker of vascular disease.
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