Multiple marker approach to risk stratification in patients with stable coronary artery disease

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Aims

Multimarker approaches for risk prediction in coronary artery disease have remained inconsistent. We assessed multiple biomarkers representing distinct pathophysiological pathways in relation to cardiovascular events in stable angina.

Methods and results

We investigated 12 biomarkers reflecting inflammation [C-reactive protein, growth-differentiation factor (GDF)-15, neopterin], lipid metabolism (apolipoproteins Al, B100), renal function (cystatin C, serum creatinine), and cardiovascular function and remodelling [copeptin, C-terminal-pro-endothelin-1, mid-regional-pro-adrenomedullin (MR-proADM), mid-regional-pro-atrial natriuretic peptide (MR-proANP), N-terminal-pro-B-type natriuretic peptide (Nt-proBNP)] in 1781 stable angina patients in relation to non-fatal myocardial infarction and cardiovascular death (n = 137) over 3.6 years. Using Cox proportional hazards models and C-indices, the strongest association with outcome for log-transformed biomarkers in multivariable-adjusted analyses was observed for Nt-proBNP [hazard ratio (HR) for one standard deviation increase 1.65, 95% confidence interval (CI) 1.28–2.13, C-index 0.686], GDF-15 (HR 1.59, 95% CI 1.25–2.02, C-index 0.681), MR-proANP (HR 1.46, 95% CI 1.14–1.87, C-index 0.673), cystatin C (HR 1.39, 95% CI 1.10–1.75, C-index 0.671), and MR-proADM (HR 1.63, 95% CI 1.21–2.20, C-index 0.668). Each of these top single markers and their combination (C-index 0.690) added predictive information beyond the baseline model consisting of the classical risk factors assessed by C-index and led to substantial reclassification (P-integrated discrimination improvement <0.05).

Conclusion

Comparative analysis of 12 biomarkers revealed Nt-proBNP, GDF-15, MR-proANP, cystatin C, and MR-proADM as the strongest predictors of cardiovascular outcome in stable angina. All five biomarkers taken separately offered incremental predictive ability over established risk factors. Combination of the single markers slightly improved model fit but did not enhance risk prediction from a clinical perspective.

Keywords

Multiple biomarkers • Cohort study • Risk stratification • Reclassification • Coronary artery disease

Introduction

Increasingly, detailed knowledge of pathways contributing to myocardial infarction and heart failure, as well as the availability of new technologies, has led to the recognition of a growing number of novel biomarkers detectable in peripheral blood. In patients with angiographically confirmed stable angina, the risk of non-fatal myocardial infarction and cardiovascular death is considerable. From a
clinical perspective, there is a need to improve risk stratification with biomarkers that provide long-term discriminative information beyond established cardiovascular risk factors. So far, these efforts have been disappointing, and we and others have concluded that most contemporary biomarkers only add moderate incremental prognostic information in the setting of primary prevention and in those with manifest disease.2–5 Further exploration of novel biomarkers and the use of newer analytical methods have been encouraged.6

We selected a broad panel of biomarkers to represent multiple pathways that have been invoked in coronary artery disease (CAD). Natriuretic peptides, in our study represented by N-terminal pro B-type natriuretic peptide (NT-proBNP) and mid-regional pro atrial natriuretic peptide (MR-proANP), provide valuable information about cardiovascular stress.3,7 The availability of novel immunoassays for markers of vascular function with potent autocrine and paracrine actions such as C-terminal(Ct)-pro-endothelin-1, copeptin (a precursor of vasopressin), and mid-regional pro adrenomedullin (MR-proADM) permits a large-scale comparative study of these biomarkers with existing risk factors such as natriuretic peptides and the inflammatory marker C-reactive protein. Growth-differentiation factor (GDF)-15, a distant member of the transforming growth factor beta family, has recently been shown to be related to mortality and cardiovascular events in patients with CAD.8,9 Its prognostic ability in stable CAD in direct comparison with other biomarkers has not been established. Biomarkers that sensitively mirror impaired renal function such as cystatin C also have been noted to improve risk stratification in various settings.10

Finally, it remains unclear, whether a strategy that uses simultaneous assessment of several novel biomarkers, which have little interdependence, is superior to strategies that use single-biomarker assessments or established risk factors only for cardiovascular risk prediction.2,3

Methods

Study sample

AtheroGene is a prospective cohort study of consecutive patients with manifest CAD and at least one stenosis of 30% or more present in a major coronary artery.11 The combined outcome for this study was non-fatal myocardial infarction and cardiovascular mortality. Information on events was collected by regular follow-up questionnaires mailed to the participants and telephone interviews, and verified by death certificates and hospital or general practitioner charts. As the purpose of the analyses was to establish the incremental value of biomarkers in stable patients with proven CAD, patients with acute coronary syndrome (n = 282) or missing covariate data (n = 231) were excluded, leaving n = 1781 participants with complete data sets for the analysis. Further details of the study are provided in the Supplementary material.

The study was approved by the local Institutional Review Board, all participants gave written informed consent. The authors had full access to the data and take responsibility for its integrity.

Laboratory methods

Twelve biomarkers reflecting lipids [apolipoproteins (Apo) AI, Apo B100], renal function (serum creatinine, cystatin C), inflammation (C-reactive protein, GDF-15, neopterin12), vascular function (copeptin, Ct-pro-endothelin-1, MR-proADM), and haemodynamics and remodelling (MR-proANP, NT-proBNP) were measured by commercially available assay systems or antibodies. Serum creatinine was measured by the modified Jaffe routine method. Further details on the tests used are provided in see Supplementary material online, Table S1.

Statistical methods

Biomarker concentrations were logarithmically transformed if the distribution strongly deviated from a normal distribution if it enhanced model fit. Associations of the biomarkers were assessed by Spearman correlation coefficients.

The prognostic abilities of all biomarkers were investigated through multivariable proportional hazards regression adjusting for age, sex and in a second model additionally for body mass index (BMI), LDL/HDL ratio, smoking, diabetes, hypertension, and number of diseased vessels. Hazard ratios (HRs) per one unit of the biomarker and for one standard deviation (SD) increase in (log-transformed) biomarker concentration along with the 95% confidence intervals (CIs) are provided.

We further examined net reclassification improvement (NRI) for the risk categories 0–6, >6–20, and >20% for 3-year event-free survival and integrated discrimination improvement (IDI) by adapting recent approaches in order to make them suitable for survival data.6 For NRI we computed the predicted probability of an event over 3 years of follow-up for each individual using a Cox proportional hazards model based on the classical risk factors and categorized individuals according to their predicted risk. For reclassification analyses, the information of the novel biomarker was then added to the model. In individuals with a future event upward reclassification was indicated by adding +1, downward classification into a lower risk category was assigned −1 and missing reclassification was indicated by zero. These scores are summed and divided by the number of individuals in the event group. Individuals in the non-event group are reclassified the same way but assigned +1 for classification into a lower risk category, correct reclassification and −1 for reclassification into a higher risk rank and the overall net reclassification is derived as the NRI. The IDI is less intuitive. The advantage is that it does not depend on pre-specified risk categories. Integrated discrimination improvement evaluates reclassification as a continuous outcome across the spectrum of risk; a value of zero indicates no movement in predicted risk.

To derive the primary risk categories, we used the combination of the classical cardiovascular risk factors (age, sex, BMI, LDL/HDL ratio, smoking, diabetes, hypertension, and number of diseased vessels). We also present data on the additive value of the investigated biomarkers when used on top of the ESC (European Society of Cardiology) Score.13

For biomarkers with a significant IDI (P < 0.05), as an additional measure for assessing their discriminative ability C-indices were calculated and tested in bootstrap analysis.14 We evaluated models for the top biomarkers separately and for the combination of these biomarkers in linear regression models.

For the best biomarkers and their combination, we defined cut-off values for event-free survival using survival trees, generated by the rpart package, classification and regression trees (CART) in R.15 A binary tree is calculated from the whole group of predictors. The best predictor and its optimal cut value are chosen at each step. A log-rank statistic is used as a criterion for goodness of split for each subsequent node. In order to obtain a binary classification, only the first split was used. P-values for the likelihood-ratio-tests using the dichotomized variables are provided. We also present Kaplan–Meier curves for tertiles of the biomarker score (Figure 1).
*P*-values and CIs have been corrected for multiple testing (Bonferroni correction).

All analyses were carried out with R 2.10.1.\(^{16}\)

### Results

#### Baseline characteristics

Cardiovascular events were observed in 137 patients over a median follow-up of 3.6 (maximum 6.9) years. The baseline characteristics of the study participants are provided in Table 1. The median age was 63 years, 22.5% of study participants were female. Risk factor distribution was more unfavourable in individuals with cardiovascular event during follow-up. About half of the patients in the event group (51.8%) had a history of myocardial infarction. Approximately 80% of the sample had hypertension, one-fifth was diabetics, and more than half of the sample was treated with cardiovascular medication at baseline. Multivessel disease was present in ~70% of the total sample and 80% of the event group. Most biomarker concentrations were higher in patients with cardiovascular events except for Apo Al, which tended to be lower in the event group.

#### Correlations

Overall, partial correlations (age- and sex-adjusted) between biomarkers were weak to moderate. The strongest association between renal function, reflected by cystatin C, and biomarkers was observed for MR-proADM (\(r = 0.60\)) and GDF-15 (\(r = 0.50\)) (see Supplementary material online, Table S2). Between the biomarkers, correlations with \(r > 0.5\) are observed within the cluster of neurohumoral and vasoactive biomarkers MR-proADM, Ct-pro-endothelin-1, and the inflammatory marker GDF-15. In addition, a positive association is observed between the biomarkers MR-proANP and Nt-proBNP (\(r = 0.58\)). The absolute value of correlations between apolipoproteins and other biomarkers was <0.2 except for C-reactive protein and Apo AI (\(r = -0.25\)).

#### Single biomarkers and cardiovascular outcome

The Cox proportional hazards assumption was not violated. Analyses of biomarker concentrations revealed a positive association with cardiovascular events for most of the markers investigated (Table 2). Overall, the strongest association with cardiovascular outcome was observed for Nt-proBNP, GDF-15, MR-proADM, MR-proANP, Ct-pro-endothelin, and cystatin C. Results remained similar after adjustment for classical risk factors and after additional adjustment for cardiac medication (ACE-inhibitors, beta-blockers, and statin intake). In a subgroup of 1214 participants with information on left ventricular ejection fraction, the additional adjustment for this variable did not markedly change the results (data not shown).

#### Risk information beyond classical risk factors

To further explore whether any of the biomarkers added to the predictive value of traditional risk factor screening, we assessed reclassification (Table 3). The strongest reclassification for single biomarkers with a NRI of over 10% was observed for GDF-15 (0.16), Nt-proBNP (0.15), MR-proANP (0.13), and cystatin C (0.12). All other biomarkers yielded a NRI of <0.1. An IDI with a significance of \(P < 0.05\) was observed for MR-proADM (0.022), Nt-proBNP (0.022), GDF-15 (0.021), cystatin C (0.015), and MR-proANP (0.014). The strongest biomarkers, MR-proADM, GDF-15, Nt-proBNP, cystatin C, and MR-proANP entered further analyses.

#### Comparison of single biomarkers and a multimarker panel

The combination of the top biomarkers (Nt-proBNP, GDF-15, MR-proANP, cystatin C, MR-proADM) reclassified 30 individuals with events into a higher risk category, 15 into a lower risk group. Of the event-free subgroup 169 patients were upwardly classified, 341 were reclassified in the correct direction. The NRI was 0.24, \(P = 0.0001\) and the IDI 0.036, \(P < 0.0001\). Correct reclassification was thus achieved in the majority of individuals with events as well as for down-classification of participants without event during follow-up.

We computed the C-index for risk prediction models based on estimated 3-year event-free survival. The information obtained through the individual biomarkers was assessed on top of the basic model (C-index basic model 0.656, 95% CI 0.608–0.705) (Figure 2). The C-index improved for all biomarkers and the combination of the top five biomarkers. Adding the information on all the top five biomarkers to the baseline model increased the C-statistic from 0.656 to 0.690 (95% CI 0.640–0.739). The effect estimate is
comparable with the best single biomarkers on top of the classical risk factors, e.g. the addition of Nt-proBNP to the baseline model increased the C-index to 0.686 (95% CI 0.637–0.735). The C-index for the combination of all biomarkers was not significantly different from the C-index resulting from single biomarkers. Cut-off analyses for risk stratification

To give an idea of the strength of the single biomarkers when used as dichotomized variables, we calculated cut-offs. Cut-off values for discrimination of high- and low-risk individuals were defined using survival trees. Results confirmed the single biomarkers Nt-proBNP, GDF-15, MR-proANP, cystatin C, and MR-proADM and the combination of the five biomarkers as strong risk indicators (Figure 2). Results were comparably strong when the top biomarkers were added to risk estimates derived from the ESC Score (see Supplementary material online, Table S3). Since HRs and cut-offs may vary by end-point we also present the final analyses separately for cardiovascular death (see Supplementary material online, Table S4). As the cut-offs are optimal for the used data sets, the P-values were expected to be small.

Discussion

We evaluated the prognostic value of 12 contemporary biomarkers reflecting atherosclerosis and heart failure associated pathways in a prospective cohort of stable angina patients. Our data indicate that the most informative biomarkers that offer incremental predictive ability over traditional risk factors were concentrations of circulating Nt-proBNP, GDF-15, MR-proANP, MR-proADM, and—to a slightly lesser extent in this data set—

### Table I  Baseline characteristics of the study sample

<table>
<thead>
<tr>
<th>Variable</th>
<th>All, n = 1781</th>
<th>No event, n = 1644</th>
<th>Event, n = 137</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>63 (56/69)</td>
<td>63 (55/69)</td>
<td>64 (60/70)</td>
</tr>
<tr>
<td>Male sex, n (%)</td>
<td>1381 (77.5)</td>
<td>1283 (78.0)</td>
<td>98 (71.5)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>27.4 (25.0/30.1)</td>
<td>27.4 (25.0/30.1)</td>
<td>27.6 (25.0/29.8)</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>1402 (78.7)</td>
<td>1293 (78.6)</td>
<td>109 (79.6)</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diet</td>
<td>74 (4.2)</td>
<td>70 (4.3)</td>
<td>4 (2.9)</td>
</tr>
<tr>
<td>Oral medication</td>
<td>176 (9.9)</td>
<td>158 (9.6)</td>
<td>18 (13.1)</td>
</tr>
<tr>
<td>Insulin dependent</td>
<td>155 (8.7)</td>
<td>135 (8.2)</td>
<td>20 (14.6)</td>
</tr>
<tr>
<td>Smoking, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>647 (36.3)</td>
<td>599 (36.4)</td>
<td>48 (35.0)</td>
</tr>
<tr>
<td>Former smoking</td>
<td>829 (46.5)</td>
<td>765 (46.5)</td>
<td>64 (46.7)</td>
</tr>
<tr>
<td>Current smoking</td>
<td>305 (17.1)</td>
<td>280 (17.0)</td>
<td>25 (18.2)</td>
</tr>
<tr>
<td>Positive family history, n (%)</td>
<td>685 (38.5)</td>
<td>638 (38.8)</td>
<td>47 (34.3)</td>
</tr>
<tr>
<td>History of myocardial infarction, n (%)</td>
<td>713 (40.0)</td>
<td>642 (39.1)</td>
<td>71 (51.8)</td>
</tr>
<tr>
<td>Beta-blocker, n (%)</td>
<td>1181 (66.3)</td>
<td>1094 (66.5)</td>
<td>87 (63.5)</td>
</tr>
<tr>
<td>ACE-inhibitors, n (%)</td>
<td>949 (53.3)</td>
<td>864 (52.6)</td>
<td>85 (62.0)</td>
</tr>
<tr>
<td>Statins, n (%)</td>
<td>970 (54.5)</td>
<td>896 (54.5)</td>
<td>74 (54.0)</td>
</tr>
<tr>
<td>LDL/HDL ratio</td>
<td>2.50 (1.86/3.19)</td>
<td>2.48 (1.85/3.18)</td>
<td>2.69 (2.19/3.31)</td>
</tr>
<tr>
<td>Multivessel disease, n (%)</td>
<td>1280 (71.8)</td>
<td>1165 (70.9)</td>
<td>115 (83.9)</td>
</tr>
</tbody>
</table>

Continuous variables are presented as median (25th/75th percentile). For discrete variables absolute (relative) frequencies of patients are given.
cystatin C. A multimarker approach combining these biomarkers was most strongly related to outcome, added incremental risk information, and showed the highest model stability and precision. However, the combined measurement did not improve risk stratification or reclassification compared with the strongest single biomarkers from a clinical perspective. Based on event-free survival probabilities, Nt-proBNP and GDF-15 in direct comparison proved to be the strongest single markers with substantial reclassification. In contrast to data derived in initially healthy individuals,5 the top biomarkers achieved correct reclassification in both, patients with future events in whom the majority was upwardly reclassified and in individuals without cardiovascular events during follow-up who were predominantly reclassified in lower risk categories. Results were similar when the ESC Score was used as the basic model for primary risk classification.

Our results on natriuretic peptides confirm findings for Nt-proBNP3,17,18 and demonstrate a comparable strength of association for MR-proANP. Similarly to the natriuretic peptides, GDF-15 is associated with outcome in heart failure and in patients with CAD.8,9 GDF-15 has been detected in high concentrations in autopsy samples after acute myocardial infarction.19 Along these lines, GDF-15 concentrations have been found to closely correlate with CAD severity and impaired systolic function in patients with acute coronary syndrome, suggesting that the circulating concentrations of this stress-inducible cytokine may integrate information on vascular and cardiac pathologies.20 Consistent with this hypothesis, GDF-15 concentrations were related to inflammatory markers and Nt-proBNP in AtheroGene. Notably, GDF-15 added prognostic information to these markers in patients with acute coronary syndrome and heart failure, as well as in the present patient cohort, indicating that this marker may provide insights into additional disease pathways.

Adrenomedullin is widely expressed, suggesting a crucial role in the control of central body functions such as endothelial regulation of vascular tone and blood pressure,21 modulation of cardiac output,22 protection against organ damage in hypoxia or sepsis,
and fluid and electrolyte homoeostasis. Plasma concentrations of adrenomedullin are elevated in a number of cardiovascular pathologies including hypertension, diabetes, heart failure, or acute coronary syndrome. Whereas adrenomedullin concentrations are difficult to assess \textit{in vivo}, the identification of the more stable MR-proADM with favourable analytic characteristics allows

### Table 3  Net reclassification improvement and integrated discrimination improvement for all biomarkers

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>NRI</th>
<th>P-value</th>
<th>Individuals with event</th>
<th>Individuals without event</th>
<th>IDI</th>
<th>P-value</th>
<th>R² difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal function</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.0084</td>
<td>1.0</td>
<td>1</td>
<td>2</td>
<td>78</td>
<td>49</td>
<td>0.0030</td>
</tr>
<tr>
<td>Cystatin C</td>
<td>0.12</td>
<td>0.066</td>
<td>16</td>
<td>6</td>
<td>179</td>
<td>128</td>
<td>0.015</td>
</tr>
<tr>
<td>Inflammation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-reactive protein</td>
<td>0.076</td>
<td>0.36</td>
<td>9</td>
<td>5</td>
<td>180</td>
<td>113</td>
<td>0.0063</td>
</tr>
<tr>
<td>GDF-15</td>
<td>0.16</td>
<td>0.015</td>
<td>19</td>
<td>10</td>
<td>253</td>
<td>120</td>
<td>0.021</td>
</tr>
<tr>
<td>Neopterin</td>
<td>0.041</td>
<td>1.0</td>
<td>8</td>
<td>6</td>
<td>138</td>
<td>100</td>
<td>0.0087</td>
</tr>
<tr>
<td>Cardiac remodelling and vascular function</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Copeptin</td>
<td>0.038</td>
<td>1.0</td>
<td>6</td>
<td>3</td>
<td>122</td>
<td>103</td>
<td>0.0052</td>
</tr>
<tr>
<td>Ct-pro-endothelin</td>
<td>0.028</td>
<td>1.0</td>
<td>11</td>
<td>12</td>
<td>154</td>
<td>93</td>
<td>0.016</td>
</tr>
<tr>
<td>MR-proANP</td>
<td>0.13</td>
<td>0.043</td>
<td>17</td>
<td>8</td>
<td>228</td>
<td>138</td>
<td>0.014</td>
</tr>
<tr>
<td>MR-proADM</td>
<td>0.087</td>
<td>0.78</td>
<td>15</td>
<td>11</td>
<td>223</td>
<td>138</td>
<td>0.022</td>
</tr>
<tr>
<td>Nt-proBNP</td>
<td>0.15</td>
<td>0.056</td>
<td>22</td>
<td>13</td>
<td>303</td>
<td>180</td>
<td>0.022</td>
</tr>
<tr>
<td>Lipid metabolism</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apo AI</td>
<td>0.048</td>
<td>0.064</td>
<td>3</td>
<td>0</td>
<td>105</td>
<td>69</td>
<td>0.0025</td>
</tr>
<tr>
<td>Apo B100</td>
<td>−0.0006</td>
<td>1.0</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>7</td>
<td>−0.00001</td>
</tr>
</tbody>
</table>

NRI stands for net reclassification improvement, IDI for integrated discrimination improvement. The basic model comprised age, sex, BMI, LDL/HDL ratio, smoking, diabetes, hypertension, and number of diseased vessels. P-values are Bonferroni corrected.

**Figure 2** Hazard ratios and 95% confidence intervals for cut-off values for event-free survival determined from survival trees for single markers and for the strongest biomarkers combined. P-values derived from Cox regression analyses were all < 0.0001. The C-index for continuous biomarker concentrations was calculated on top of the basic model comprising the classical risk factors (age, sex body mass index, LDL/HDL ratio, smoking, diabetes, hypertension, and number of diseased vessels), C-index 0.656. The coefficients used to derive the combined cut-off were log-transformed N-terminal-pro-B-type natriuretic peptide 0.2702, log-transformed growth-differentiation factor-15 0.4595, log-transformed mid-regional-pro-atrial natriuretic peptide 0.0119, log-transformed cystatin C 0.2527, and log-transformed mid-regional-pro-adrenomedullin 0.2310.
the application in epidemiological studies. Recent reports highlight the prognostic power of MR-proADM after acute myocardial infarction, and its relation to cardiovascular events in high-risk patients. The present data on MR-proADM have two major implications. First, we were able to demonstrate that MR-proADM is strongly associated with cardiovascular outcome in stable angina; and second, the inclusion of MR-proADM improves the model accuracy comparable with Nt-proBNP, one of the strongest predictors of outcome in secondary prevention. A single MR-proADM determination provides information for cardiovascular risk stratification incremental to that obtained from risk models simultaneously incorporating the classical risk factors, and similar accuracy of risk information obtained from the combination of several biomarkers of different pathophysiological backgrounds.

Our biomarker evaluation accounts for renal function by incorporation of cystatin C, which is well correlated with a glomerular filtration rate even in patients with only minor renal impairment. Kidney disease may affect both biomarker concentrations and cardiovascular outcome. All top biomarkers revealed relevant bivariate correlations with cystatin C. However, single-marker analysis and the multimarker model suggest that the top biomarkers, natriuretic peptides, GDF-15, and MR-proADM, offer predictive information beyond cystatin C.

Clinical application and decision-making is based on cut-off values. Therefore, we dichotomized the single biomarkers and their combination by identifying an optimal cut-off for the prediction of cardiovascular events. Interestingly, the statistically optimal cut-offs correspond comparatively well with recently observed thresholds of concentrations for the longer known biomarkers such as Nt-proBNP and cystatin C (Figure 2). The confirmation and refinement of cut-off values might help to identify patients at high risk for cardiovascular events in the clinical setting.

Further establishment of the pathophysiological role of MR-proADM and GDF-15 is necessary to improve our understanding of the underlying mechanisms that lead to the additional risk information provided by these novel markers and to assess their potential for clinical application.

**Limitations**

The number of events in this study is relatively small, which may limit our ability to detect small effects and to compare individual biomarkers head-to-head. In particular, the strength of association may differ by end-point (non-fatal myocardial infarction, cardiovascular death). But the number of outcomes in the two subgroups was too small to derive stable risk estimates in separate analyses and results have to be interpreted with caution. In confirmatory analyses, we observed similar effect sizes and directions as reported in the literature, which supports the reliability of our current results. To enhance comparability of the biomarkers, we only used individuals with complete biomarker data for analyses. The exclusion of participants with missing values may have introduced a bias. The maximum values of the C-indices for single markers and the combination of the top markers did not exceed 0.70, the range when model discrimination is considered satisfactory. This finding may, in part, be due to the underlying expected event rate in a stable CAD sample and the overall predictability of events. Of note, the basic model comprising classical cardiovascular risk factors only achieved a C-index of 0.656. In contrast to initially healthy samples, validated risk scores for patients with manifest disease and stable angina do not exist. Our efforts can be viewed as an attempt at stratifying patients by combining some of the known variables strongly related to outcome to provide the basis for the assessment of additional, novel biomarkers. We used risk categories derived from primary prevention settings. These may not be the most appropriate classifications in manifest CAD, and at this point, therapeutic implications cannot be directly derived from these categories, thus precluding cost-benefit analyses of the additional measurement of the novel biomarkers. Overall, our analyses are exploratory and need confirmation in independent samples.

Strengths of the study are clearly the homogeneous prospective cohort with almost complete long-term follow-up and the availability of a large panel of contemporary biomarkers some of which were, to our knowledge, evaluated in a stable angina cohort for the first time. We were able to demonstrate that among a panel of novel and established cardiovascular risk indicators, single biomarkers such as Nt-proBNP, GDF-15, MR-proANP, cystatin C, and MR-proADM provided information on cardiovascular outcome in stable angina with similar accuracy as the combination of the five strongest biomarkers combined on top of classical risk factors. In conclusion, we can state that the robust neurohormonal precursor Nt-proBNP remains one of the strongest and best examined biomarkers in cardiovascular risk prediction. None of the other promising novel markers proved to be clearly superior. From a clinical perspective, the measurement of one of the top biomarkers provided comparative information to the determination of all five markers and a multiple biomarkers approach combining the presented biomarkers does not seem to be favourable. Whether the additional measurement of any of the top biomarkers affects clinical decision-making and outcome has to be investigated in future studies.

**Supplementary material**

Supplementary material is available at European Heart Journal online.

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