Myocardial biomarkers for prediction of cardiovascular disease

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Abstract. The identification of those persons in the population who have the highest risk of future cardiovascular events is important for targeting intensive preventive efforts. This can be reliably done using a handful of long since established risk factors. The unmet need for new molecular biomarkers for prediction of cardiovascular events in the general population is therefore low. In order for a new biomarker to be used clinically for risk prediction, a statistically significant association of levels of the biomarker to adverse outcome is not enough, but the biomarker should also be demonstrated to add discriminative capacity beyond established risk factors. In contrast to the limited value of new biomarkers for risk prediction, their usefulness for unraveling the pathophysiology of cardiovascular disease is large. The myocardium is the source of a vast number of interesting biomarkers, of which a few may be useful for risk prediction in the general population. Two of these, troponin-I and the N-terminal fragment of brain natriuretic peptide, have passed tests of added discriminatory value. Numerous other biomarkers produced by cardiomyocytes or non-cardiomyocytes in the myocardium are promising, and if they are not proven useful for risk prediction, they will unquestionably enhance our understanding of cardiovascular disease.

Keywords: Risk, epidemiology, population, biomarker, peptide, myocardium

1. The need for prediction and prevention of cardiovascular events

Cardiovascular diseases kill half of the population in developed countries, and seriously disable a further large number. A high cardiovascular disease death rate in elderly persons is unavoidable, but prevention of cardiovascular deaths in younger individuals is highly prioritized, as recently outlined in the European Heart Health Charter [1].

Sudden cardiac death is sometimes the first manifestation of cardiovascular disease, and traditional risk factors can be identified beforehand in a minority of these persons [2]. Further, one out of four myocardial infarctions resulting in Q-waves are asymptomatic [3], and smaller myocardial infarctions may be asymptomatic

in as much as four out of five victims [4]. Hence, the high risk strategy will not be sufficient in these cases.

In order to prevent as many of premature cardiovascular deaths as possible, the Charter therefore calls for the use of both the population strategy (treating the whole population with measures such as smoking prevention or cessation, physical activity, and fruit and vegetable intake) and the high-risk strategy (identifying persons at high risk and treating risk factors aggressively). This article reviews some of the tools available for the latter strategy.

1.1. The role of biomarkers in the identification of high-risk persons

The term risk factor was coined fifty years ago [5], and soon thereafter it was established that the large majority of cardiovascular events can be predicted using a mere handful of risk factors. These include a few molecular biomarkers such as glucose and cholesterol, but the largest part of the equation is accounted for by other markers, such as age, sex, blood pres-

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sure, and smoking. Indeed, the non-modifiable markers are among the most powerful ones. Of the modifiable markers, the non-molecular markers blood pressure and smoking are the most important [6]. Among other non-molecular markers, electrocardiographic left ventricular hypertrophy is one of the most powerful risk factors. Other physiological measures of subclinical cardiovascular disease, such as those obtained by echocardiography, carotid ultrasound or cardiovascular magnetic resonance or computer tomography imaging, are also gaining ground as potential tools for identifying high risk persons.

The term “biomarker” used to include all of the factors mentioned above, but the meaning has recently shifted to merely indicate molecular markers. This is the meaning that will be used in this article. Biomarkers have a role in cardiovascular risk prediction, partly because of risk-predictive capacity of certain markers, but mainly because of accessibility and relatively low cost. Nearly three decades ago, more than 200 variables had been related to coronary disease, [7], and the number has been growing exponentially since then.

The unmet need for new risk factors is low [8]. In fact, a model without any laboratory testing could discriminate subsequent cardiovascular events as precisely as a model with limited laboratory testing (of the most important molecular biomarker, cholesterol) in a population-based sample [9]. One might thus argue that more time and money should be spent on implementing existing guidelines using already established risk factors instead of searching for new risk factors.

Today, new biomarkers have a tough case for being considered clinically useful [10]. Demonstrating a statistically significant relation between a new biomarker and a cardiovascular outcome simply isn't enough to take the marker from bench to bedside anymore. Nowadays, demonstration of added power to discriminate those who will suffer cardiovascular events from those who will not is warranted before a marker is considered for clinical use [11].

Although their immediate clinical usefulness may be limited, circulating biomarkers are important tools for increasing the understanding of biological pathways, and may thereby be of indirect clinical importance by e.g. propelling drug discovery. Further, if new biomarkers do not find use as risk prediction markers in the general population, they may help identify subgroups of patients, may be useful for risk prediction in certain clinical settings, and may find use in tailoring and monitoring treatment. Very few of the biomarkers reviewed in this article have passed reasonable tests of

usefulness [10] and even fewer the tests of discriminatory capacity [11], and their clinical utility in the general population is therefore presently low, at best. But quite a few of them are promising candidates for risk prediction in the future, if they survive such tests.

1.2. Sources of biomarkers

In this issue, biomarkers of various origins are reviewed. For risk prediction purposes, it does not matter much in which organ or cell type the biomarker is produced. But from the perspective of understanding the pathophysiology of cardiovascular disease, it may be purposeful to subdivide biomarkers according to their source. This article focuses on the role of biomarkers originating from the myocardium in risk prediction.

Myocardial biomarkers have been researched mainly within the scope of prediction of events in heart failure patients, in patients with acute coronary syndromes or those undergoing related procedures, and in small patient samples with less common diseases. Myocardial biomarkers may also be important for risk prediction in the community, mainly for prediction of incident heart failure, but also for prediction of ischemic heart disease, arrhythmias, or other adverse cardiac events. The general population setting will be the focus of this article.

In the heart, one third of all cells are cardiomyocytes, but these take up 75% of the volume. Consequently, two thirds of all cells are interstitial non-myocytes, mainly endothelial cells, vascular smooth muscle cells, fibroblasts and myofibroblasts, and macrophages and mast cells [12]. A selection of promising biomarkers from the cardiomyocyte and non-cardiomyocyte compartments are discussed separately in the following (Table).

2. Myocardial biomarkers reflecting the cardiomyocyte compartment

2.1. Troponins

Cardiac troponins are located on the thin filaments in the sarcomere which, activated by Ca^{2+} , generate the contractile force of the heart. Cardiac troponins circulating in peripheral blood have a widespread clinical use as a tool for diagnosing myocardial damage, mainly that from myocardial infarctions. The recent universal definition of myocardial infarction relies heavily on increased circulating cardiac troponin levels [13]. Circu-

Table 1

Cardiomyocyte compartment	Non-cardiomyocyte compartment
Troponin-I and -T	Interleukin-6 and -10
Natriuretic peptides ANP, BNP & NTproBNP	Myocyte chemoattractant protein-1
ST2	Transforming growth factor- β
Growth-differentiation factor-15	Galectin-3
Chromogranin-A	Connective tissue growth factor
Osteoprotegerin	Tumour necrosis factor α
Heart-type fatty-acid-binding protein	Matrix metalloproteinase-9
Myosin light chain-I	Tissue inhibitor of metalloproteinases-1
Creatine kinase type MB	Collagen propeptides PIINP, PICP & ICTP
Myoglobin	

lating troponin levels have been demonstrated to predict subsequent left ventricular systolic dysfunction [14] and mortality [15] in patients with acute coronary syndromes.

Myocardial damage identified by circulating cardiac troponins may be of prognostic importance also in the absence of an acute myocardial infarction. Circulating troponin levels may predict mortality in a variety of other clinical settings, such as acute decompensated heart failure [16–18] and chronic stable heart failure [19–22] but also in patients with end-stage renal disease without overt heart disease [23], as well as in apparently healthy people in the community [24].

Troponin-I is one of very few myocardial biomarkers (NTproBNP is another) that improves risk discrimination of subsequent cardiovascular events in addition to established risk factors in the general population [11, 25].

This has led to a shift in the view of cardiac troponins, from specific identifiers of myocardial infarction to general indicators of myocardial damage [26]. Numerous sources of elevated troponin levels have been identified in persons angiographically free from coronary artery disease [27,28]. Most involve an oxygen supply/demand mismatch, such as in tachycardia, physical exertion, severe aortic stenosis, left ventricular hypertrophy, severe heart failure or anemia, whereas the cardiomyocyte damage mechanism may be less obviously ischemia-related in other cases, such as sepsis, myocarditis, pericarditis, diabetic ketoacidosis, or myocardial contusion [27,28]. Troponin levels are also increased in persons with renal disease, which presents a challenge when using troponins for risk prediction in that setting [29].

Increased circulating troponin levels are generally assumed to reflect severe cardiomyocyte injury or death [30], but may also signify leakage of unbound sarcoplasmic troponin through damaged membranes [31], or may be the result of troponin assays detecting cleaved troponin peptides [32], degrad-

ed as a result of a higher rate of normal troponin turnover or because of acute cardiomyocyte injury. In ischemia/reperfusion injury, two proteases suggested to be responsible for troponin cleavage and contractile dysfunction are calpain (a Ca^{2+} -activated protease) [33] and matrix metalloproteinase-2 [34].

2.2. Natriuretic peptides and ST2

Atrial and brain natriuretic peptides (ANP, BNP) are primarily produced in the heart; ANP mainly in the atria and BNP mainly in the ventricles, although all chambers have the possibility to produce both peptides [35]. BNP and the N-terminal fragment of its prohormone (NTproBNP) are mainly thought of and used clinically as indicators of left and right ventricular pressures, but may in fact rise in response to numerous stimuli, including sepsis, burn injury, or strenuous physical exercise [36]. In fact, in one study of patients with shock, NTproBNP did not correlate with ventricular filling pressures [37], but high NTproBNP levels nevertheless predicted mortality in this setting.

Higher NTproBNP levels have repeatedly been demonstrated to predict mortality also in a variety of other settings, such as in persons with acute coronary syndromes [38–40], stable coronary disease [41,42], and in the general population [25,43,44], independently of most established risk factors. A dose-response relation has been demonstrated; the higher NTproBNP, the higher the risk [41,43]. The main use of BNP or NTproBNP in risk prediction today is in patients with heart failure [45,46] or dyspnea in the emergency setting [47].

NTproBNP is one of the few myocardial biomarkers that have been demonstrated to improve discrimination of risk for cardiovascular events above and beyond established risk factors in apparently healthy persons [11,25]. When interpreting levels of natriuretic peptides, it should be noted that persons with renal disease may have higher levels (due to reduced clearance

or increased production) [48], and obese people may have lower levels [49].

In response to stretch, cardiomyocytes also produce the interleukin-1 receptor family member ST2. Higher levels of the truncated, circulating form of ST2 have been demonstrated in patients with acute heart failure, and predict mortality in patients with acute coronary syndromes [50], dyspnea [51] or acute [52] or chronic heart failure [53]. ST2 expression is stimulated by interleukin-33 produced by stretched fibroblasts, and may thus be a marker for the cross-talk between fibroblasts and cardiomyocytes in response to mechanical overload, and may have a role similar to BNP [52]. The role for ST2 in risk prediction in asymptomatic individuals remains to be determined.

2.3. Hormones

Growth-differentiation factor-15 (GDF-15; also known as macrophage-inhibitory cytokine-1, placental bone morphogenetic protein, placental transforming growth factor- β , and nonsteroidal antiinflammatory drug-activated gene-1) is a member of the transforming growth factor- β cytokine superfamily. It is produced in several tissues, including activated macrophages and cardiomyocytes. In response to ischemic injury and other stressors, cardiomyocyte production of GDF-15 increases [54], and it has been suggested to have antiapoptotic, antihypertrophic, and antiremodeling effects. In one case-control study, higher plasma GDF-15 levels predicted subsequent cardiovascular events [55]. Higher circulating GDF-15 levels also predict adverse outcome in persons with acute myocardial infarction [56,57] or chronic heart failure [58]. The cellular source of the circulating GDF-15 in different settings remains to be determined, but the cardiomyocyte is a primary suspect.

Another interesting biomarker is chromogranin-A (CgA), which is a prohormone that is cleaved to form several peptides with regulatory properties [59], some of which may be cardioinhibitory [60]. CgA is stored in the same granulae as BNP in cardiomyocytes of heart failure patients [60], and heart failure patients have increased circulating levels of CgA [60]. High circulating CgA levels after myocardial infarction predict worse prognosis [61,62]. No studies have investigated the predictive capacity of CgA in the general population.

Osteoprotegerin is a soluble member of the TNF receptor superfamily with many effects, including bone metabolic, endocrine and immune functions, and its is produced in cardiomyocytes [63]. Higher levels predict adverse prognosis in acute coronary syndromes [64,65] as well as in the general population [66,67].

2.4. Other cardiomyocyte biomarkers

Heart-type fatty-acid-binding protein (H-FABP) is a cytosolic protein produced by cardiomyocytes. Elevated H-FABP levels are observed in heart failure patients [68], and may signify adverse prognosis in patients with heart failure [68] or acute coronary syndrome [69,70].

More than a decade ago, elevated levels of circulating myosin light chains were observed to predict adverse events in patients with chest pain [71]. The sub-type myosin light chain-I (MLC-I) has been demonstrated to predict adverse prognosis in patients with chest pain [72] or heart failure [68]. Although other cardiomyocyte biomarkers such as creatine kinase type MB (CK-MB) [73,74] and myoglobin [75,76] are successfully used clinically to diagnose myocardial infarction and predict mortality in patients with chest pain, their use has been overshadowed by the introduction of troponin measurements. It should be noted that although troponin-I has been demonstrated to be a useful predictor of cardiovascular events in the general population [25], CK-MB and myoglobin have not been investigated with that question in mind. These biomarkers may hence require more study in that setting, as discriminative capacity of a biomarker for risk of adverse events determined in the setting of acute chest pain cannot be directly transposed to the general population. Myoglobin is likely the least promising of these two for the purpose of risk prediction in the general population, because of its rapid clearance from peripheral blood and because it is not cardiomyocyte specific but is also produced by oxidative skeletal muscles. The roles for MLC-I and H-FABP in risk prediction in the general population are also unknown. See the Figure for a schematic illustration of some of the relations described.

3. Myocardial biomarkers reflecting the non-cardiomyocyte compartment

3.1. Inflammatory, macrophage and fibroblast substances

Relations of circulating inflammatory biomarkers to subsequent cardiovascular events have been known for a decade, and have been demonstrated numerous times for C-reactive protein, but also for other biomarkers and less specific measures such as the erythrocyte sedimentation rate [77]. The main source of the circulating

Fig. 1. Sources of and relations between some promising myocardial biomarkers.

C-reactive protein is the liver, as is the case for several other inflammatory biomarkers. The contribution of the inflammatory molecules produced in the myocardium to the circulating levels is not known, but is likely small for many of these biomarkers. Non-cardiomyocytes are the main producers of inflammatory biomarkers in the myocardium, with the exception of a few, including interleukin (IL)-6 [78].

Circulating levels of IL-6 predicts subsequent stroke, coronary events [79], and heart failure [79,80] in general population samples. IL-6 produced in peripheral arteries contribute significantly to circulating IL-6 in heart failure patients [81], but the contribution of IL-6

produced in the myocardium [78] to circulating levels in the general population is not known.

In heart failure patients treated with intravenous immunoglobulin, left ventricular systolic function improved and circulating interleukin-10 levels increased [82], indicating a protective role for this anti-inflammatory cytokine. The predictive capacity in the general population of this and other interleukins having the myocardium as a major source of origin remains to be clarified.

Macrophages have been invoked in many disease processes, especially in fibrosis development. Hence, the cardiac macrophages have been suggested to play

a role in cardiac fibrosis and dysfunction by stimulating fibroblasts, and myocyte chemoattractant protein (MCP)-1 [83], transforming growth factor (TGF)- β [83] and galectin-3 [84] have been suggested as relevant markers of this process. Higher circulating galectin-3 levels have been demonstrated in heart failure patients, and these may signify an adverse prognosis [85]. Connective tissue growth factor (CTGF) is a cysteine-rich protein induced by TGF- β in connective tissue cells. CTGF can trigger many of the cellular processes underlying fibrosis, such as cell proliferation, adhesion, migration and the synthesis of extracellular matrix [86,87]. The role of most these biomarkers in prediction of events remains to be clarified.

Macrophages in failing hearts have been demonstrated to produce tumour necrosis factor- α (TNF- α), but no such production is found in normal hearts [88]. Circulating TNF- α [79] and production of TNF- α by peripheral blood mononuclear cells [80] predict incidence of heart failure and coronary events in the general population. The importance of the TNF- α produced by the myocardium vs. other sources is unknown, as is its role in risk prediction. Its role as a drug target in heart failure patients has not been successful hitherto [89].

3.2. Extracellular matrix peptides

Derangements in the cardiac extracellular matrix and its turnover have recently come into focus as key elements in the pathogenesis of cardiac remodeling and disease, such as cardiac fibrosis, ischemia, arrhythmias and remodeling/failure, and have emerged as possible tools for monitoring disease and targets for intervention. All macroscopic cardiac changes involve extracellular matrix remodeling. Collagen breakdown in the matrix is under the control of a balance between the matrix-degrading matrix metalloproteinases (MMPs), and their inhibitors, the tissue inhibitors of metalloproteinases (TIMPs). Extracellular matrix metabolism can be assessed by measuring circulating levels or activity of MMPs and TIMPs, or by assaying levels of procollagen peptides derived from assembly and/or breakdown of collagen fibrils [90]. This approach is molecule-specific, but organ-unspecific, as these biomarkers are produced by many different organs, including but not exclusive to the myocardium. Correlations between cardiac and circulating levels of some of these matrix biomarkers are excellent [91–93], but the markers best reflecting the cardiac matrix metabolism remain to be established among the nearly forty identified MMPs, TIMPs and procollagen peptides. Thus, the relations

described below cannot be said to exclusively reflect myocardial sources of the markers, until further evidence is at hand.

All major cardiovascular disease risk factors – such as age, male sex, smoking, dyslipidemia, diabetes, hypertension, obesity and inflammation – have been related to altered levels of circulating MMPs, TIMPs or procollagen peptides. These relations have mainly been established in small patient samples, but for MMP-9 and TIMP-1 also in large community-based samples [94–96]. In two such samples, circulating MMP-9 and TIMP1 levels have also been related to LV hypertrophy [94–96], and TIMP-1 levels inversely related to LV systolic function [94,96].

Several of these markers have been demonstrated to predict adverse outcome in various patient settings. In persons with angina pectoris, higher circulating MMP-9 levels predict rapid coronary luminal diameter reduction [97] and fatal cardiovascular events [98]. Also higher TIMP-1 levels predict an increased risk of myocardial infarction [99] and mortality [99,100] in angina patients. The increased extracellular matrix turnover indicated by these circulating marker levels may be causal for both plaque growth (collagen accumulation) and plaque instability (collagen depletion in the fibrous cap of a vulnerable plaque).

During an acute coronary syndrome, a cascade of MMPs are expressed and activated in the myocardium, mainly in and near the infarcted area, which gives rise to collagen degradation and replacement and TIMP activation. Some matrix markers (mainly known for TIMP-1 and the collagen propeptides PIIINP and PICP) can be found in peripheral blood during many weeks after an acute coronary syndrome. High circulating levels of PIIINP predict mortality [101,102], systolic dysfunction [103] and heart failure [102] after a myocardial infarction; and high PICP levels also portend an adverse outcome after myocardial infarction [104,105].

In persons with chronic heart failure, elevated PIIINP levels have repeatedly been demonstrated to predict poor outcome [91,106,107], and higher PICP [91,106] and ICTP [106] levels have also indicated a poor prognosis in a few reports. In both the settings of an acute coronary syndrome and heart failure, subsequent normalization of circulating PIIINP levels corresponds to a better prognosis [103,106].

Studies of the predictive value of matrix biomarkers in the general population is limited. In one small study of vitamin D-deficient Bangladeshi East Londoners, plasma MMP-9 level was a predictor of subse-

quent ischemic heart disease and/or hypertension incidence [108].

The use of extracellular matrix regulators as drug targets is promising. Several conventional drugs influence extracellular matrix metabolism, most notably thrombolytic therapy, which powerfully stimulates MMP expression and collagen degradation [109–112]. Notable are also MMP-9-lowering effect of statins [113–116] and matrix effects of thiazolidinediones [117, 118], angiotensin-converting enzyme inhibitors [119–124], angiotensin II-receptor antagonists [125, 126], and spironolactone [106, 124]. In a small study of heart failure patients, thalidomide treatment lowered MMP-2 and also improved LV systolic function [127].

Pharmaceutical companies have shown great interest in synthetic MMP inhibitors because of potential for use in a broad spectrum of diseases, including cancer, periodontitis, pulmonary disease and cardiovascular diseases. An early clinical trial with an MMP inhibitor (batimastat)-eluting coronary stent did not show beneficial effects of the treatment [128]. The most promising setting and timing for the right (specific or broad-spectrum) MMP inhibitors remain to be identified.

4. Taking myocardial biomarkers from bench to bedside

A number of issues should be considered when choosing myocardial biomarkers for risk prediction. Properties sought in a new myocardial biomarker should include scientific proof of a primary role for the biomarker as mediator of a cardiac disease process; demonstration of a correlation between circulating levels and myocardial levels of the marker; consistent evidence that circulating levels of the biomarker are elevated in persons who subsequently suffer a clinically relevant adverse outcome; stability of the biomarker in stored blood samples; reasonable physiological variability; and accessibility of assays with small measurement error. Criteria for usefulness of a new biomarker have been proposed [10], which include a precise, accessible and reasonably priced assay; the observation that information carried in the biomarker is not known from other clinical data; and demonstration of a beneficial decision making by using the biomarker.

To this list should be added the need for demonstration of added power of a new marker to discriminate between persons who will have an adverse outcome from those who will not, above and beyond a mod-

el with established risk factors [11]. Discriminative capacity has traditionally been investigated using the area under the receiver operating characteristics curve of sensitivity and specificity (the C-statistic). Investigating differences in C-statistics between two models is a conservative method, and will often leave the investigator with a case of a statistically significant and independent relation of a new marker to an outcome, with a better model fit, but with a non-significant difference in C-statistics between a smaller model with established risk factors and a bigger model which also incorporates the new marker [129]. As a solution to this problem, two new, simple measures of added discriminatory capacity have been proposed [11], the net reclassification improvement (NRI) and the integrated discrimination improvement (IDI). NRI measures the reclassification of persons from one risk category to another by addition of the new marker to a model with established risk factors. If all persons risk are on average better classified (the persons ends up in a more correct risk class) by the model with the new marker, NRI is positive. The NRI is highly attractive from a clinical point of view, because it assumes that there exist *a priori* meaningful risk categories, which means that the investigator must decide which the most clinically relevant risk thresholds are (likely those thresholds that influence treatment decisions). If a person's risk is more correctly classified according to these thresholds by measuring a new marker, the marker by definition has clinical importance. The IDI is similar, but does not consider risk thresholds.

Two myocardial biomarkers have passed these tests of usefulness for risk prediction in the general population to date, troponin-I and NTproBNP [25]. Among the biomarkers reviewed in this article and those that were not mentioned, more may find usefulness by this definition in the future; if not in the general population, then perhaps in specific patient settings. If a new biomarker does not pass the tests mentioned above, it may still be useful for the understanding of pathophysiology, but it should not be marketed as a clinically useful marker for risk prediction.

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