

Metabolic profiling in heart disease

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Abstract

Identifying the individuals who will suffer a myocardial infarction as a result of coronary artery disease (CAD), in order to direct available therapeutic resources efficiently, remains an important limiting factor in the delivery of clinical care in the cardiovascular arena. Metabolite profiling is an example of a new type of diagnostic test that shows considerable promise. Nuclear magnetic resonance spectroscopy or chromatographic separations can be used to generate a "molecular fingerprint" of a serum sample, and cutting-edge pattern recognition techniques can then be applied to identify molecular signatures associated with the presence of occlusive atherosclerotic lesions and, perhaps in the future, those associated with increased risk of myocardial infarction. Such approaches may improve clinical management of CAD and at the same time provide novel insights into the metabolic disturbances that underlie the disease.

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The need for improved diagnostic capability

Coronary heart disease remains the single biggest cause of morbidity and mortality in the UK, with almost 40 000 premature deaths (defined as death before age 75 years) attributed to it each year [1]. Over the past two decades, we have seen considerable improvements in therapeutic options (particularly with the widespread use of cholesterol-lowering drugs of the statin class [2]). However, the maximum benefits of these improved therapies are difficult to achieve in practice because of difficulties in selecting targets for preventative interventions (whether pharmaceutical in nature, or public health interventions such as dietary modification).

Existing diagnostic tests are generally focused on detecting cardiac ischemia (resulting, for example, in chest pain, shortness of breath or edema) or coronary artery stenosis, and as a result do not necessarily direct

preventative treatments to the majority of individuals who would otherwise go on to suffer a myocardial infarction. Optimizing strategies to make sure both that the majority of those who will suffer a myocardial infarction are receiving the most aggressive treatment, and that the minimum number of people who would not otherwise suffer an infarction are treated, has therefore become a major task facing the health-care profession. Any steps that improve identification of these individuals will pay a large public health dividend.

The aim of any diagnostic strategy is to identify the smallest subgroup of the population under study that still contains all the individuals who will go on to suffer myocardial infarction. Unfortunately, this definition is often clouded by the idea of searching for "high-risk" individuals. If you mistakenly optimize your diagnostic strategy simply to identify those at greatest risk (without concern for the number of such people you can find), the size of the "high-risk" group will shrink as the extent of the risk is increased, resulting in the majority of myocardial infarction events occurring in people outside the high-risk category. As a result, much of the benefit of aggressive treatment will be missed.

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“Pronostics”: profiling diagnostic technology

Over the past decade, it has become increasingly clear that improved diagnostic and prognostic capability can be achieved by combining simple risk factors. For example, the risk-scoring methods derived from the Prospective Cardiovascular Münster Study (PROCAM) or Framingham studies are considerably superior to the individual measurements that compose them ([3] and references therein).

Taking this idea further, it should be possible to collect very much larger profiles (containing many thousands of data elements related to an individual) and search within those profiles for signatures that predict future myocardial infarction events with high sensitivity and specificity. Recent rapid advances in genomics, proteomics, and “metabonomics” have made such profiling diagnostics (or “pronostics”) technically feasible. The key to such approaches is to measure the concentrations of as many different molecules as possible (whether they are mRNA, protein, or low molecular weight metabolites), ideally choosing the analytes at random rather than through any pre-existing idea that they may be associated with the disease endpoint. Mathematical modeling tools can then be applied to trawl through the resulting mountain of data to extract any molecular signature that reliably associates with the endpoint under investigation.

Metabolite profiling

Using techniques such as nuclear magnetic resonance (NMR) spectroscopy or gas chromatography followed by mass spectrometry (GC–MS), it is possible to generate such a profile of low molecular weight metabolites. Neither NMR nor GC–MS can generate an “ideal” profile (with every metabolite represented, identified, and quantitated), and guidelines on selecting the most appropriate method depending on the question under study have been published elsewhere [4]. For disease diagnosis (which does not depend on identifying any specific metabolites in the profile, but merely on reproducible differences in the profile between cases and controls), NMR spectroscopy may be the superior approach, but too few studies have yet been published to permit definitive conclusions to be drawn.

Profiling metabolites is a particularly attractive approach to developing a prognostic test for coronary artery disease (CAD). In general, metabolite profiling is more straightforward than genomic or proteomic profiling, because of the high reproducibility of the tools for chemical analysis that are used to generate the profile. For example, NMR spectroscopy yields a replicate reproducibility of about 1% across the

information-dense region of the spectrum [5], which is superior to conventional biochemical assays such as enzyme-linked immunosorbent assay (in which the reproducibility is typically 5–10%) and more than 10-fold better than gene array methodologies or labour-intensive proteomic profiling based on differential gel electrophoresis and mass spectrometry.

More specifically, there is already a well-known metabolic component to the pathogenesis of CAD. Dysregulated lipid metabolism and increased plasma cholesterol concentrations play an important part in disease development, as demonstrated by numerous genetically modified mouse models of atherosclerosis in which enzymes regulating lipid metabolism have been disrupted [6]. Similarly, the constellation of factors that compose the metabolic syndrome are believed to represent a risk factor for CAD in the human population [7]. On this basis, it seems inherently plausible that even a simple or imperfect metabolite profile might be able to offer improved clinical diagnosis of CAD.

Metabolic signatures of occlusive atherosclerosis

To test the above proposal, we performed a small-scale study [8], comparing NMR-derived serum metabolite profiles of individuals with severe angiographically-defined CAD and those from individuals apparently free from stenotic lesions. For each group, serum samples were prepared from approximately 40 individuals who presented at Papworth Hospital, Cambridge, UK, and were subjected to 600 MHz one-dimensional proton NMR spectroscopy, using previously published conditions [9]. The resulting spectra were phased, baseline-corrected and processed into 207 integral regions representing the average intensity across small regions of the spectra (*Figure 1*).

Simple inspection of the resulting profiles highlights the exceptional degree of similarity in the serum metabolome between individuals. However, even cursory analysis reveals differences between samples (yellow inset boxes in *Figure 1*). The important question is whether any of these differences represents a reproducible signature that distinguishes the cases from the controls. To test this rigorously, we used a range of pattern-recognition methodologies (reviewed in depth elsewhere [10]) to identify any signature within the profile that was reproducibly associated with CAD. Encouragingly, this yielded a clear separation between the two groups [8], based largely on subtle compositional differences between the lipoprotein particles [5,8].

These findings suggested that an NMR-derived metabolic profile could usefully distinguish severe

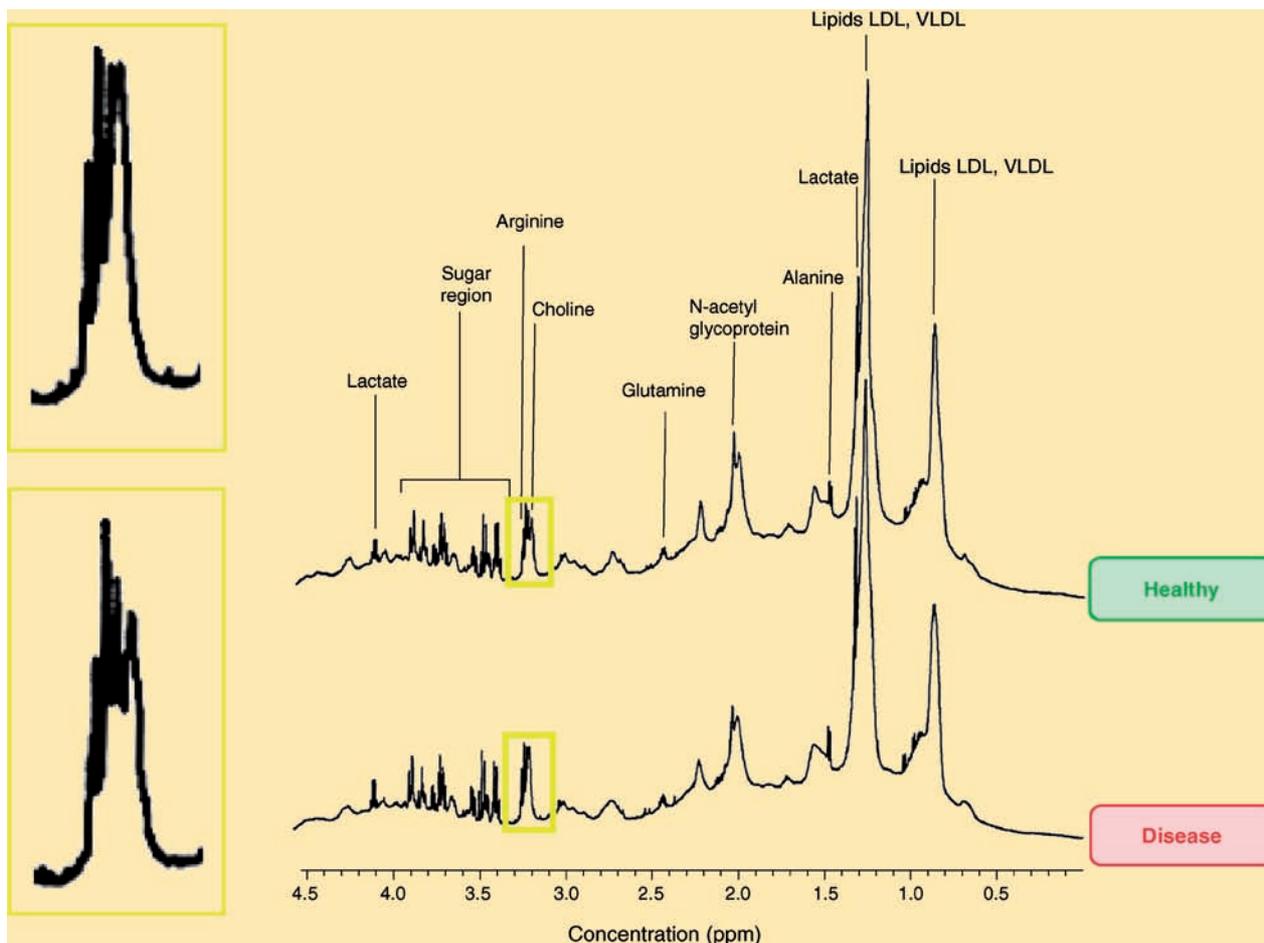


Figure 1. Serum metabolic profiles. Two metabolic profiles, derived by one-dimensional 600 MHz proton nuclear magnetic resonance spectroscopy of serum samples as previously described [9], are shown. The lower profile represents serum from an individual with no angiographic evidence of heart disease, and the upper profile represents serum from an individual with severe (at least 70% stenosis of all three major coronary arteries) heart disease. Grossly the profiles are similar, but careful examination reveals potentially important differences (insets).

disease from normal coronary arteries. At the same time, we compared the profiles from individuals with varying degrees of disease severity, and the results suggested that some information regarding severity was encoded within the profile [8], although considerably larger studies will be required to establish the extent to which any clinically useful discrimination could be achieved.

Unfortunately, the one-dimensional proton NMR procedure that we adopted for these studies, which was chosen to improve our diagnostic capability, provides little indication about the precise molecular nature of the differences in lipid composition. Further studies are now under way using profiles derived from GC-MS, and altered concentrations of particular fatty acids are now being identified which may shed new light on the pathogenesis of CAD.

Moving to a physiological endpoint

There is little doubt that clinical management of CAD could be improved by the existence of a serum-based

test that reliably predicted the outcome of coronary angiography (allowing the available angiography resource to be more efficiently targeted); however, ultimately, it is prediction of cardiovascular events (stroke and acute myocardial infarction, for example) that is most urgently required. Angiographic stenosis may predict chronic angina, but is only a small part of the story regarding unstable disease, in which plaque composition rather than size or location may be the biggest determinant of the risk of myocardial infarction [11,12]. None of the studies to date have provided any indication as to whether metabolic profiling can provide improved prediction of such events, and addressing such questions will be an important component of future research in this area.

Encouragingly, however, we have already demonstrated the existence of a metabolic signature associated with hypertension (a physiological rather than anatomical endpoint) [13], and Sabatine and colleagues recently reported a metabolic signature associated with myocardial ischemia [14]. On the

basis of these early results, there is reason to be optimistic about the use of metabolic profiling to provide clinically useful prognostic information about hard cardiovascular endpoints.

The future for metabolic profiling in heart disease

Metabolic profiling (and, indeed, profiling diagnostics in general) is an emerging field, and much remains to be discovered. The results of pilot-scale studies have provided an impetus to further investigations. A dedicated, large-scale clinical study of metabolite profiling for the diagnosis of heart disease, the Metabonomics and Genomics in Coronary Artery Disease (MaGiCAD) study [15,16], recently completed recruitment of more than 1300 individuals and will considerably expand our understanding of metabolic and genetic factors in CAD, with early results expected by the end of 2006. Similarly, the very large Human Serum Metabolome (HUSERMET) project [17] has included cardiovascular disease as one of its component studies. Over the coming years, it will become evident whether and to what extent metabolite profiling can provide clinically useful diagnostic capability in the management of CAD.

Almost as important will be comparison of different profiling methods. Today, it seems very likely that a prognostic test for CAD will be developed over the coming years, but it is unclear whether such a test will include a metabolic, genetic, or even an immunological profile. Ultimately, such questions may depend as much on the relative cost of capturing the various profiles as on the scientific rationale for their inclusion. The simplest (and least expensive) profile that contains sufficient information to yield a reliable diagnosis will probably form the basis of any clinical prognostic test; other, more costly, methods will be restricted to the research laboratory, where they seem likely to yield exciting new insights into the pathogenesis of cardiovascular diseases.

Conclusion

Early pilot-scale studies suggest that metabolite profiling may provide clinically useful information about the existence and extent of CAD, although any relationship to cardiovascular events remains entirely unknown. Novel metabolic markers of CAD are now emerging from such studies, and may yield important new insights into the pathogenesis of the disease. However, research in this area is still at an early stage,

and larger studies are required to obtain a proper definition of both the clinical and scientific utility of metabolite profiling in cardiovascular disease.

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