Metabolomics in coronary heart disease

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Abstract

Improvements over the last decade in treatment of vascular disease, to reduce future myocardial infarction events, have not been matched by improvements in diagnostic technology, to predict who will suffer from such events. As a result, targeting limited healthcare resources so that increasingly effective drugs are given to the people most at risk remains the weak link in clinical cardiovascular care. It is now more than a decade since the promise of metabolic profiling to aid diagnosis of vascular disease was first uncovered, but such techniques have yet to deliver real improvement to the clinical management of heart disease. Here, we review the progress that has been made, and examine what remains to be done before the promise of metabolomics as a clinical diagnostic tool can be fully realized.

Keywords: diagnostics; atherosclerosis; profiling; multivariate statistics.

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The diagnostic problem

Coronary heart disease (CHD) remains single biggest cause of morbidity and mortality in the United Kingdom, with over 25,000 premature deaths (defined as death prior to age 75) 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]), with dramatic improvements in outcome now evident in the statistics [2]. However, the maximum benefits of these improved interventions 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 (MI). Optimizing strategies to make sure that the majority of those who will suffer an MI are receiving the most aggressive treatment (while treating the minimum number of people who would not otherwise suffer an MI) has therefore become a major task facing the healthcare profession, and any steps that improve identification of these individuals will pay a large public health dividend.

The scale of the problem is best illustrated by the diagnostic performance of the gold standard: coronary angiography. Five-year follow-up data from the MaGiCAD cohort [3,4] shows that individuals with angiographically defined CHD have an annual death rate from cardiovascular causes of around 3%. This compares to about 0.3% in the general population of the same age and gender distribution, from whom the MaGiCAD subjects were drawn. At first

sight, a ten-fold enrichment of the at-risk individuals looks impressive, but the problem is that a little less than 0.1% of the relevant population has an angiogram. As a result, less than 1% of the deaths from cardiovascular causes occur in the population selected to undergo angiography. Indeed, the majority of cardiovascular deaths occur among people unaware that they had CHD and who are receiving no treatment for it.

Faced with a problem of this magnitude, it becomes clear that improvements in prognosis will require a step-change in the nature of the tests that are used (Fig. 1). Firstly, discrimination of cases from healthy subjects will need to improve: sensitivities and specificities of 90%, or even 99% are insufficient as part of a population screening paradigm. Secondly, the test needs to be suitable for use on a large fraction of the population. Clearly, angiography would not meet that criterion, since it is both invasive (carrying risk of morbidity and even mortality) and costly. In addition to a dramatic improvement in performance, then, a new test that could significantly address this key problem is also going to have to be non-invasive, high throughput and low cost.

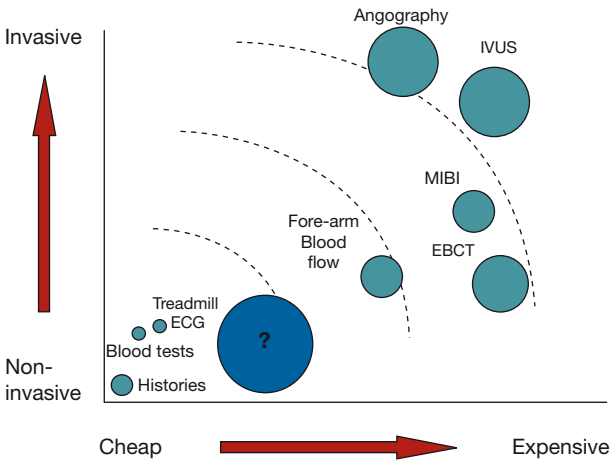

Fig. 1 *Mapping diagnostic technologies used for risk-stratification and clinical management of CHD. Different technologies are positioned on the map according to a qualitative assessment of their cost (x-axis) and the extent of intervention required for the patient (y-axis). The area of the circle for each technology is proportional to the average diagnostic performance for predicting future myocardial infarction (MI) events. Current diagnostic modalities show a trend of increasing cost and intervention with increasing diagnostic power. The darker grey circle "?" represents an ideal future technology, with high diagnostic power but low cost and minimal intervention for the patient. Currently multi-analyte metabolic profiling cannot be placed on the graph, because the power for predicting future events has not been reported in any study, but would in any case be placed considerably further to the right than this ideal future technology. IVUS intravascular ultrasound, MIBI methoxy isobutyl isonitrile, EBCT electron-beam computed tomography.*

Using profiling assays to meet the challenge

It is inherently unlikely that any single measure (certainly any single molecular measure) can yield the required degree of diagnostic performance. Several solutions to this problem have been adopted. For example, physiological rather than molecular measures effectively integrate multiple pathways into a single measurement. Hence, measuring blood pressure reflects properties of many cell and organ systems, and provides a better indication of future cardiovascular risk than most molecular measures. A similar integration is also achieved with measures such as low-density lipoprotein (LDL)-cholesterol, where assays for classes of lipoprotein particles provide a better snapshot of lipid metabolism than any single lipid component. The limitation is that a single measure, even integrating multiple pathways, has insufficient information density to achieve reliable risk stratification.

An alternative approach is to measure different markers in sequence, using low cost, non-invasive methods as an initial screen followed by more intensive work-up of the selected sub-population. This is at the heart of current clinical strategy for the management of heart disease. Patients are prioritized for more aggressive diagnostic work-up or therapeutic intervention on the basis of "risk factors," such as elevated plasma lipids, the presence of diabetes or hypertension or, more recently, inflammatory markers such as hsCRP. The limitation of the approach, however, is usually the performance of the initial "risk factor" screens—few if any approach even 90% sensitivity and specificity and consequently the majority of CHD events occur outside the "high-risk population" they define.

The solution to both these problems may lie in profiling diagnostics. The principle here is that many measures are made at once, on the whole population, without stratifying on the basis of individual risk factors. This profile is then sufficiently information-dense to allow a clinically useful prediction of future risk of a CHD event [5,6].

There are multiple profiling approaches, each focused on a different type of analyte: genomics (genetic polymorphisms), transcriptomics (mRNA) proteomics (proteins), immunomics (antibodies) and

metabolomics (metabolites). There are advantages and disadvantages to each, but for cardiovascular disease, with defects in lipid metabolism at its core, metabolomics seems particularly promising. Unlike genetic profiling (which can only identify baseline risk, since your genotype is fixed at conception), metabolomics offers the promise of integrating genetic and environmental influences, which are both known to contribute to the development of CHD in almost equal measure.

Metabolic profiling and CHD

During the 1990s, Professor Jeremy Nicholson and his team at Imperial College, London, perfected the methodology required to apply nuclear magnetic resonance (NMR) spectroscopy directly to complex biological fluids, in order to obtain a fingerprint of the collection of low-molecular weight metabolites present. This approach exploited the exquisite reproducibility of NMR spectroscopy (with co-efficients of variation between replicate measures as low as 1%), although the lack of sensitivity restricts the method to examining the most abundant metabolites (perhaps as many as 1000 of the most abundant metabolites contribute to the spectra), and considerable additional work is required to assign changes in the spectrum to particular compounds.

Following a demonstration that this technique was able to robustly and completely discriminate between mice of different strains [7], it was applied to blood samples collected from subjects with and without CHD, defined by angiography [8]. The results were very striking: the two groups were complete distinguished on the basis of their NMR-derived metabolic profiles. The metabolite(s) responsible for this discrimination were not uniquely identified, but the key region of the spectrum association with the separation (around chemical shift [∂]3.22ppm) indicated the involvement of choline-like species [8].

Despite such a positive first study, attempts to exploit these findings have been slow. This, in part, may be explained by a poorly designed study [9] that used samples from different sources combined into a single cohort. As a result, the variability between samples was larger, diluting the signal due to differences in CHD health status. Despite this issue, statistically significant separation of the groups was achieved, and the spectral regions responsible were the same as in the original study. Inexplicably, this was interpreted by the authors as a negative outcome, when (to the extent possible given the limitations inherent in the design) it replicated all the key findings of the original study and additionally demonstrated that the diagnostic power of the NMR-derived metabolite profile, even in this mixed cohort, was greater than any combination of existing risk factors [9].

A final resolution to these opposing conclusions came in 2011, with the publication of a study using a different technique to obtain a metabolic profile. Using chromatography linked to mass spectrometry, Wang et al. [10] conclusively demonstrated that metabolites of choline differ between individuals with CHD and those without, consistent with both the 2002 and 2006 publications [8,9]. Moreover, they went on, in mice, to demonstrate that these metabolic differences are due to the interaction between the host metabolome and gut flora, and that these differences actually contribute to the development of atherosclerotic disease [10].

Separately, a Finnish group published the largest clinical metabolomics study reported to date, with NMR spectroscopy of serum on more than 4,300 subjects [11]. In contrast to the earlier studies, their definition of CHD was based on carotid intima-media thickness in asymptomatic subjects, and therefore examined the metabolic phenotypes associated with sub-clinical atherosclerosis. This study also concluded that the multi-metabolite signature obtained by NMR spectroscopy was strongly associated with vascular disease, and provided a clinically useful extension to conventional risk factor measures in predicting the presence of atheroma.

Indeed, all the studies published to date demonstrate a significant association between the metabolite profile and CHD, irrespective of the methodology used to derive the profile or the phenotypic definition of cardiovascular disease [8–14]. The strength of the association varied, with the quality of the study design a major factor in the outcome.

However, none of these studies have properly quantified the additional diagnostic power of the multi-metabolite profile compared to conventional (and very low-cost) analytes such as HDL-cholesterol, triglycerides and hsCRP combined with demographic information such as gender, age and body mass index, and physiological measures such as hypertension. It will be important to understand the extent to which the apparent improvements in diagnosis stem from the

extra information in the metabolite profile rather than from the improve statistical analysis framework that 'omics' studies typically employ compared to conventional risk factor modeling.

Additionally, these studies use phenotypic definitions based on imaging techniques, and are therefore associated with a structural, rather than functional, definition of the disease. Improving clinical management of CHD, however, requires better prediction of events rather than simply less invasive methods to diagnose the presence of atheroma. The presence and severity of vessel stenoses is a poor predictor of long term outcome: by 5 years after angiography the annual risk of a clinical event among those with multiple, severe stenoses is almost back to the risk among individuals with no detectable stenoses [3]. It is completely unknown at present whether the metabolite profile, either alone or in combination with other readily available clinical data, can better predict future risk of a cardiovascular event.

Exploiting metabolic profiling to improve clinical management of CHD

Having burst onto the scene with a powerful demonstration of the association of the metabolic profile with angiographically defined CHD in 2002 [8], it has taken more than a decade for the robust nature of that association to be demonstrated by multiple laboratories in different studies using a range of techniques (both analytical and statistical) [10–14]. The next challenge is to translate this basic science into improved clinical management of CHD.

All the current methods of generating a multi-analyte metabolite profile depend on expensive hardware with limited throughput. They do not (and for the foreseeable future seem unlikely to) meet the criteria set out in Fig. 1. While metabolite profiling may have good diagnostic performance and is non-invasive, it is neither high throughput nor low cost.

Over a longer timescale (perhaps another decade) advances in analytical technology, for example using sensors based on nanotechnology, may allow extensive multi-analyte profiles to be captured quickly and cheaply. In the shorter term, however, it may be possible to exploit the new information by extracting from the multi-analyte profiles just those measurements that contribute the greatest fraction of the diagnostic performance. Conventional clinical chemistry assays for choline metabolites or for triglyceride species selectively associated with atherosclerosis may find utility in a clinical setting.

The key next step, then, will be to test the ability of the most promising new biomarkers to emerge from these metabolomics studies to predict clinical events. The predictive power of both NMR and gas chromatography-mass spectrometry (GC-MS) derived metabolite profiles for events up to five years after testing will be evaluated in the MaGiCAD cohort during 2012, which should provide the first indication of the likely clinical utility of these biomarker signatures. More likely, the best predictions will come by combining the best metabolites with other measures (both conventional risk factors and the winner's from other 'omics' investigations into CHD, such as genomics, transcriptomics and proteomics). As we move into this new phase of pragmatic testing, it will be as important to determine the relative contribution of different analytes to the power to predict clinically relevant events so that available resources can be targeted most efficiently. Simply demonstrating that metabolite profiles, or individual analytes that compose them, are different between those with CHD compared to disease-free controls will no longer be sufficient.

Conclusions

Early, encouraging pilot scale studies suggesting that metabolite profiles differ in those with heart disease [8] have, despite early concerns [9], now been extensively replicated [10–14]. It is now time to take the next step, and address some important questions: how much additional diagnostic information can metabolite profiles contribute compared to existing risk factor modeling? Can metabolite profiles predict clinically relevant events rather than just replicate the diagnostic power of invasive imaging techniques? And does enough predictive power reside in a small enough fraction of all the analytes to allow a cost-effective screening test to be developed, without relying on the impractically expensive technology used to generate multi-analyte metabolic profiles today?

Based on the growing amount of data available, it is tempting to guess that all three questions will be answered in the affirmative. The challenge for the next decade is to deliver that promise into the hands of clinicians as quickly and cost-effectively as possible. •

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References

1. England and Wales, Office for National Statistics (2010) Deaths registered by cause, sex and age. www.statistics.gov.uk
2. Smolina K, Wright FL, Rayner M, Goldacre MJ (2012) Determinants of the decline in mortality from acute myocardial infarction in England between 2002 and 2010: linked national database study. British Medical Journal 344:d8059
3. http://www.magicad.org.uk
4. Mosedale DE, Smith DJ, Aitken S, Schofield PM, Clarke SC, McNab D, Goddard H, Gale CR, Martyn CN, Bethell HW, Barnard C, Hayns S, Nugent C, Panicker A, Grainger DJ (2005) Circulating levels of MCP-1 and eotaxin are not associated with presence of atherosclerosis or previous myocardial infarction. Atherosclerosis 183:268–274
5. Grainger DJ (2006) Metabolic profiling in heart disease. Heart Metabolism 32:22–25
6. Goonewardena SN, Pravette LE, Desai AA (2010) Metabolomics and atherosclerosis. Curr. Atheroscler. Rep. 12:267–272
7. Gavaghan CL, Holmes E, Lenz E, Wilson ID, Nicholson JK (2000) An NMR-based metabonomic approach to investigate the biochemical consequences of genetic strain differences: application to the C57BL10J and Alpk:ApfCD mouse. FEBS Lett. 484:169–174
8. Brindle JT, Antti H, Holmes E, Tranter G, Nicholson JK, Bethell HW, Clarke S, Schofield PM, McKilligin E, Mosedale DE, Grainger DJ (2002) Rapid and noninvasive diagnosis of the presence and severity of coronary heart disease using 1H-NMR-based metabonomics. Nature Med. 8:1439–1444
9. Kirschenlohr HL, Griffin JL, Clarke SC, Rhydwen R, Grace AA, Schofield PM, Brindle KM, Metcalfe JC (2006) Proton NMR analysis of plasma is a weak predictor of coronary artery disease. Nature Med. 12:705–710
10. Wang Z, Klipfell E, Bennett BJ, Koeth R, Levison BS, Dugar B, Feldstein AE, Britt EB, Fu X, Chung YM, Wu Y, Schauer P, Smith JD, Allayee H, Tang WH, DiDonato JA, Lusis AJ, Hazen SL (2011) Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. Nature 472:57–63
11. Würtz P, Soininen P, Kangas AJ, Mäkinen VP, Groop PH, Savolainen MJ, Juonala M, Viikari JS, Kähönen M, Lehtimäki T, Raitakari OT, Ala-Korpela M (2011) Characterization of systemic metabolic phenotypes associated with subclinical atherosclerosis. Mol Biosyst 7:385–93
12. Sabatine MS, Liu E, Morrow DA, Heller E, McCarroll R, Wiegand R, Berriz GF, Roth FP, Gerszten RE (2005) Metabolomic identification of novel biomarkers of myocardial ischemia. Circulation 112:3868–3875
13. Vallejo M, García A, Tuñón J, García-Martínez D, Angulo S, Martin-Ventura JL, Blanco-Colio LM, Almeida P, Egido J, Barbas C (2009) Plasma fingerprinting with GC-MS in acute coronary syndrome. Anal Bioanal Chem 394:1517–1524
14. Chen X, Liu L, Palacios G, Gao J, Zhang N, Li G, Lu J, Song T, Zhang Y, Lv H (2010) Plasma metabolomics reveals biomarkers of the atherosclerosis. J Sep Sci 33:2776–2783