Coronary Heart Disease and Biomarkers and Its Clinical Application

Guodong Wang¹, Jungang Zhang¹ and Aiyuan Zhang¹*

¹Department of Cardiology, Weifang People’s Hospital, Weifang, Shandong, P.R. China.

Authors’ contributions

This work was carried out in collaboration between all authors. Author GW wrote the first draft of the manuscript and author JZ helped to manage literature searches. Author AZ gives some professional advice. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJMMR/2015/19089

Authors:

(1) Gaetano Santulli, College of Physicians and Surgeons, Columbia University Medical Center, New York, NY, USA.

Reviewers:

(1) Adham I. Ahmed, University of Palestine, Palestine.

(2) Paulo Roberto B. Evora, University of São Paulo, Brazil.

Complete Peer review History: http://sciencedomain.org/review-history/10662

Received 25th May 2015

Accepted 30th July 2015

Published 23rd August 2015

ABSTRACT

Cardiovascular disease is a major health concern globally. Genetic testing is an attractive tool for cardiovascular disease prediction because it is a low-cost, high-fidelity technology with multiplex capability. Developments in genomic discovery have yielded valuable new candidates in the quest for better biomarkers and novel therapeutic targets. This brief review focuses on recent trends in biomarkers of cardiovascular disease. DNA microarrays, single nucleotide polymorphism chips, linkage analysis, genome-wide association studies, and other strategies have increased our knowledge of metabolic diseases of the heart. This review also examines the potential applications and challenges of using genetic information for predicting cardiovascular disease.

Keywords: Biomarker; cardiovascular diseases; GWS; SNP.

1. CORONARY HEART DISEASE AND BIOMARKERS

Biomarker is an indicator of a biological state, which is a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention. Typically it can be applied as disease process screening,

*Corresponding author: Email: 710792934@qq.com;
diagnosing, or monitoring tools and it may also be used to determine disease susceptibility and eligibility for specific therapies.

CHD (Coronary Heart Disease) is the leading cause of morbidity and mortality worldwide which has genetic and environmental determinants [1]. Generally, the lifetime risk of experiencing a CHD event is around 50% in men and 33% in women; however, clinical CHD events are rare in the first four decades of life, around 96% of all events occur after 50 years of age. Although events are rare in individuals younger than 50 years, the development and progression of subclinical atherosclerosis begins from as early as the second decade of life [2]. CHD is usually caused by a build-up of fatty deposits on the walls of the coronary arteries. The fatty deposits, called atheroma, are made up of cholesterol and other waste substances. The risk of developing atherosclerosis is up to environmental and genetic factors, which includes smoking, less regular exercise, high blood pressure, high blood cholesterol level and diabetes. Genetic variants play a role in conferring risk for disease in high blood pressure, high blood cholesterol level and diabetes [3].

The predictive capacities of the major cardiovascular risk factors, including age, sex, cigarette smoking, high blood pressure, elevated total cholesterol (TC) level, low high density lipoprotein cholesterol (HDL-C) level, and diabetes mellitus, have been well established [4]. The Framingham risk score was developed in an effort to assist clinicians in risk assessment and treatment planning via combined using this major risk [5]. While the Framingham risk score is considered a standard and generally acceptable approach to risk prediction [6], newer biomarkers, which reflect inflammation, endothelial function, fibrin formation and fibrinolysis, oxidative stress, renal function, ventricular function, and even myocardial cell damage, have also been associated with cardiovascular risk, and their predictive values have been studied.

In metabolic level, altered levels of blood lipids and coagulation factors, and higher blood pressure (BP) were recognized as induction factor for established CHD as early as the 1940s [7,8]. Many biochemical alterations were found being associated with increased risk of CHD. These observations generated interest in the possibility that one or more of these factors might be causally involved in the development of atherosclerosis and that measurement of these factors might be biochemical markers to predictive of CHD risk [5,9]. It is reported that over 200 risk factors had been associated with CHD by 1981 [10]. Among these associated factors with CHD, LDL cholesterol (LDL-C) and blood pressure have retained their pre-eminence based on randomized controlled intervention trials which showed high levels of both of these factors to be causally related to CHD.

Many of the genes were unregulated in failing hearts, relative to those in the non-failing hearts, which were associated with fatty acid metabolism, whereas those down regulated genes were linked to glucose metabolism [11,12]. The differential gene expression caused investigation of the potential treatment effect for failing hearts by using a class of drugs such as trimetazidine and ranolazine, which can shift the lipid and glucose metabolic states in myocardial cells [13].

Gene expression studies have undiscovered the importance of regulation of lipid metabolism for atherosclerosis and CHD. Using mouse models, numerous genes were identified involved in lipid metabolism, who are differentially expressed during early stages of progression of the atherosclerotic lesion [14]. In cardiovascular diseases such as ischemic and non-ischemic cardiomyopathy and atherosclerosis, it is unlikely that a single biomarker can serve sensitively and specifically as the therapeutic or diagnostic biomarker for the disease.

2. GENETIC APPROACHES TO STUDYING CHD

For a long time, common genetic variants have been thought to contribute to susceptibility to the polygenic form of CHD according to the hypothesis of ‘common disease common variant’. Linkage analysis and association studies have been the mainstay of genetic studies attempting to reveal the heritable basis of CHD. Linkage analysis is an approach to finding genetic biomarkers of cardiovascular diseases. It allows the identification of disease DNA markers by examining the patterns of heredity in large high-risk families and the occurrence of disease phenotypes among family members [15].

Linkage analysis has proved very successful in mapping genes for monogenic disorders. Some forms of CVD exhibit a simple pattern of inheritance suggestive of a single causal gene that confers a large effect on phenotype. For many of these mendelian forms of CVD, direct DNA sequencing and/or linkage analysis have successfully yielded the causal gene and mutation. In 1985, Lehrman
and colleagues directly sequenced the low-density lipoprotein receptor (LDLR) gene in a patient with homozygous familial hypercholesterolemia and uncovered a 5k bp deletion that eliminated several exons, representing the first demonstration of a mutation for Mendelian CVD [16]. After that, a series of CVD related genes including long QT syndrome, severe hypercholesterolemia, Mendelian forms of hypertension, Marfan’s syndrome, and several forms of congenital heart disease are discovered as mendelian trait [17]. The linkage is successful in single mendelian gene, but it has been difficult and much less successful cases for complex diseases by multi genes due to reasons including phenotypic heterogeneity and locus heterogeneity of complex diseases and the low statistical power of this method to detect modest genetic effects.

Several groups have used linkage analysis to study complex CHD by examining DNA markers including microsatellite polymorphisms or single nucleotide polymorphisms (SNP) exist in the human genome, in DNA samples from collections of families with premature CHD. These studies have identified regions of a number of chromosomes that potentially harbour CHD genes. However, these findings in general showed poor cross-replicated in the various listed linkage studies. It is concluded that the poor cross-replicated results may be due to different population ethnicities, different software algorithms and varying phenotypic criteria for CHD.

3. CANDIDATE GENE ASSOCIATION STUDIES

Comparing to linkage analysis, association studies involve direct comparison of the frequency of the genetic variant between those with a clinical condition and those without in unrelated individuals and with greater power than linkage studies. Candidate gene association studies often base selection of candidate genes on assumptions about biologically relevant genes. Almost all genetic association studies focused on the genes which related to known pathological pathways or risk factors such as hyperlipidaemia, high blood pressure and inflammation. These genes encode known protein to be involved in the disease pathogenesis; the variation in these genes may alter its biological function, consequently leading to disease. Lists of published CHD-related candidate gene variants are available online [18]. However, a lot of publications for candidate genes association research are reported, very few of these findings have been replicated [19]. Poor replication of candidate gene association research might be due to false positive findings in the original studies or false negative results in subsequent studies, which may resulted from different kinds of reasons. The critical reason is inadequate sample sizes, which cause increased the rates of type I and type II errors. Moreover, the candidate gene association approach has two major limitations. First, pathogenesis of CHD are not fully elucidated, the candidate genes of CHD selected for association analysis probably are biased. Second, such studies usually focus on exons, introns and immediate flanking regions of the candidate genes. However, these regions represent only a small fraction of the genome, whereas the intergenic regions contain many DNA elements (eg, enhancers, silencers) that regulate gene expression. Although sequence variations in some of such DNA elements can potentially confer susceptibility to complex diseases, they are not covered in candidate gene studies. Considering the limited sampling size and the candidate gene association analysis itself limitation, many studies of candidate gene were inconsistent associations [20,21], however, least 8–10 of these associations could be real, which includes candidate associate loci APOE and LPL [22].

4. GENOME-WIDE ASSOCIATION STUDIES

These limitations of candidate gene association studies are largely overcome by the genome-wide association study (GWAS) approach, which provides an effective approach to discovering new genetic biomarkers. In recent years, studies using the GWAS approach have led to a major breakthrough in identifying genetic determinants for complex CHD by stringent significance thresholds and large sample sizes to reduce the type I error (false positive) rate and reduce the type II error (false negative) rate. Genetic variants, such as those on chromosome 9 (interval 9p21) and chromosome 4 (4q25), have been linked to increased risk for CVD [23]. McPherson et al. [24] applied genome-wide association scanning and discovered a 58 kb interval on chromosome 9p21 that was consistently associated with coronary heart disease in six independent cohorts. The associations between SNPs on 9p21 and CHD were the most strongly replicated findings in the majority of the assessed ethnic groups [25,26].
More than 1/4 of all GWAS the CVD associate markers are related with fasting plasma glucose and diabetes, lipid profile and BP studies represent a considerable part of CVD GWAS as well.

Over the past few years, GWAS have reported a large number of novel genetic variants associated with CVDs [27,28]; nevertheless, many challenges remain. It is needed to perform GWAS on populations with diverse geographic ancestries, which have undergone more mutations and greater recombination events. This type of studies could give greater degrees of genetic variation and shorter stretches of linkage disequilibrium allowing better localisation of genome-wide association signals [29].

5. RESEQUENCING AND OTHER ‘OMIC’ APPROACHES

Both of linkage analysis and associate analysis focus on the DNA level. For proceeding biological function, the most genes need to be transcribed into RNA and translated into protein to carry out its biological function. High throughput technologies of transcriptomics and proteomics are new powerful tools to investigate new biomarkers for CHD in RNA and protein level.

Gene expression microarray technology provides a way to qualitative and quantitative scan whole transcriptome, so that subtle differences of gene expression can be monitored in different samples. DNA genetic biomarkers represent the static genetic information, expression profiles are dynamic display how the genetic markers play their roles in transcriptome. The transcriptomic profiling cardiovascular tissue samples may reveal gene functional network to identify novel marker genes and may aid identification of drug targets and validation of candidate drugs for the management of atherosclerosis. HMG-CoA reductase, the target of statin drugs, shows higher expression atherosclerotic plaque [30]. And statin can inhibit the expression of inflammatory cytokine interleukin-1β, normally present at high levels among subjects with CAD [31].

The proteins come from the translation of mRNA. Alternative splicing of mRNA and post-translational processing of proteins will increase the complexity of proteinomics. Proteomic studies of CAD will present more detail mechanisms of CAD. For example, proteome profiling of isolated plasma lipid fractions have yielded new insight into the composition of LDL and high-density lipoprotein particles, identifying 3 novel proteins and identifying unique patterns of LDL associated apolipoproteins in subjects with type 2 [32-34].

6. DISCUSSION

Cardiovascular disease is the first cause of death both in worldwide [35]. An estimated 17.5 million people died from CVDs in 2012, representing 31% of all global deaths (http://www.who.int/mediacentre/factsheets/fs317/en/). The majority of cardiovascular disease (CVD) is caused by risk factors, which includes high blood pressure, cholesterol, overweight/obesity, tobacco use, lack of physical activity and diabetes. Regarding of attribution of deaths, the leading CVD risk factor is raised blood pressure and followed by tobacco (http://www.world-heart-federation.org/fileadmin/user_upload/documents/Fact_sheets/2012/PressBackgrounderApril2012RiskFactors.pdf). Moreover some cardiovascular risk factors related to gender [36].

Rapid advances in genomic, transcriptomics, and proteomic technology provide researchers with valuable tools for understanding the genetic predisposition to atherosclerotic cardiovascular disease and have led to the development of successful therapeutic interventions for atherosclerotic cardiovascular disease; for example, reducing plasma concentrations of atherogenic lipoproteins (with statins) and blocking renninangiotensin-aldosterone system activity (with ACE inhibitors and angiotensin-receptor blockers).

It is common, in observing different genomic and genetic biomarkers studies using differing technologies–microarrays, SNP chips, or linkage analysis–that different genes or polymorphic loci are found to be associated with the same cardiovascular pathology. Certainly, an important factor to consider when comparing different genetic biomarkers studies is the selection of patient cohort. Depending on the heterogeneity or the size of the population being analyzed, the genetic biomarkers detected may be significantly different. Ideally, a larger, more heterogeneous cohort can result in a more widely applicable biomarker discovery. However, this increase in heterogeneity may also compromise the ability to find potentially more specific biomarkers that are
associated with particular extreme phenotypes. Ultimately, the key to success in genotypic characterization and expression studies will be the exquisite phenotyping of groups of patients of interest. Future systems biology studies may shed light in this regard, and provide a more complete picture of the genetic mechanisms underlying each CVD. The human genome contains more than 22 000 genes, developments in the technical capabilities underlying high-throughput genomic approaches, coupled with a focus on patient phenotyping and advanced computational strategies, will continue to add incremental value to our current understanding of heart diseases, and have the potential to revolutionize the management of patients through earlier intervention and more effective interdiction on the processes of disease progression.

Recently, research of microRNA turned into a hot spot and new bio-marker for CVD. microRNAs act as regulators of gene expression through binding complementary sequences within the mRNAs of their target genes to regulation mRNA degradation or repression translational. miR-1 was the first miRNA reported to be dysregulated in patients with CAD– its level in left ventricular endocardium was approx. 2.8-fold higher in patients with CAD than in healthy controls [37], and more than 60 microRNA are listed as cardiovascular disease related. miRNAs have emerged as promising candidates for diagnostic and prognostic biomarkers for diverse CVDs [38]. In contrast to mRNAs, circulating miRNAs are surprisingly stable. One of the advantages of using miRNAs as biomarkers over currently used peptide/protein markers is the potential for quantification of specific miRNAs using qRT-PCR. Research on miRNAs as biomarkers is still in its early stages. They probably will not replace current diagnostic tools, such as imaging, protein biomarkers, etc., but rather would complement them to make diagnoses/prognoses/stratification more precise and accurate. Up to now, several miRNA based biomarkers of CVD have been investigated, but most still require validation in further studies. technical challenge relates to the low amount of total RNA in blood, sample handling, RNA processing and miRNA quantification which makes it virtually impossible to measure the concentration and quality of the isolated RNA, hence development of novel serum-based biomarkers is often rather cumbersome [39].

### Table 1. Mapped genetic loci for myocardial infarction or coronary artery disease

| Unique # | Chr   | SNP            |
|----------|-------|----------------|
| 1        | 1p13  | rs599839       |
| 2        | 19p13 | rs6511720      |
| 3        | 1p32  | rs11206510     |
| 4        | 6q25  | rs3798220      |
| 5        | 6q26  | rs10455872     |
| 6        | 9q34  | rs579459       |
| 7        | 11q23 | rs964184       |
| 8        | 8q24  | rs17321515     |
| 9        | 2p21  | rs4299376      |
| 10       | 19q13 | rs2075650      |
| 11       | 12q24 | rs3184504      |
| 12       | 10q24 | rs12413409     |
| 13       | 9p21  | rs4977574      |
| 14       | 21q22 | rs9982601      |
| 15       | 1q41  | rs17465637     |
| 16       | 10q11 | rs1746048      |
| 17       | 6p24  | rs12526453     |
| 18       | 2q33  | rs6725887      |
| 19       | 3q22  | rs9818870      |
| 20       | 1p32  | rs17114036     |
| 21       | 6q23  | rs12190287     |
| 22       | 7q32  | rs11556924     |
| 23       | 13q34 | rs4773144      |
| 24       | 14q32 | rs2895811      |
| 25       | 15q25 | rs3825807      |
| 26       | 17p13 | rs216172       |
| 27       | 17p11 | rs12936587     |
| 28       | 17q21 | rs46522        |
| 29       | 7q22  | rs10953541     |
| 30       | 10p11 | rs2505083      |
| 31       | 11q22 | rs974819       |
| 32       | 10q23 | rs1412444      |
| 33       | 6p24  | rs6903956      |

The future biomarkers will be multi-marker panels characteristic of the complexity of the underlying pathophysiology of the disease process. Up to now, at least 33 genetic loci were mapped by GWAS for Myocardial Infarction or Coronary Artery Disease (Table 1, [17]). In fact, only a small portion of familial and sporadic atherosclerosis results from single-gene defects in lipid metabolic pathways [40-41], even lipid related abnormalities cause a lot of disorders (Fig. 1, [42]). However, the multi-marker risk prediction approach is controversial. A multimarker risk prediction approach, including several new biomarkers simultaneously, also has been studied with the goal of improving the accuracy and clinical utility of cardiovascular risk
prediction [43-44]. Some studies have suggested that adding several newer biomarkers can substantially improve risk classification [44], but others have observed only minimal improvement in the ability to classify cardiovascular risk by adding biomarkers [43].

CONSENT
It is not applicable.

ETHICAL APPROVAL
It is not applicable.

COMPETING INTERESTS
Authors have declared that no competing interests exist.

REFERENCES
1. Lloyd-Jones D, Greenlund K, Haase N, Hailpern S, Ho M, Howard V, Kissela B, Simone GD, Ferguson TB, Flegal K, et al. Heart disease and stroke statistics—2009 update: A report from the American Heart Association Statistics Committee and Stroke Statistics Sub-committee. Circulation. 2009;119:480–486.
2. Lloyd-Jones DM, Larson MG, Beiser A, Levy D. Lifetime risk of developing coronary heart disease. Lancet. 1999;353:89–92.
3. Sanja Kovacic, Mirjana Bakran. Genetic susceptibility to atherosclerosis. Stroke research and treatment 2012; 2012: Article ID 362941. Available: http://dx.doi.org/10.1155/2012/362941
4. Kim HC, Greenland P, Rossouw JE, Manson JE, Cochrane BB, Lasser NL, Limacher MC, Lloyd-Jones DM, Margolis KL, Robinson JG. Multimarker prediction of coronary heart disease risk: The Women’s Health Initiative. J Am Coll Cardiol. 2010; 55:2080-91.
5. Wilson PW, D’Agostino RB, Levy D, Belanger AM, Silbershatz H, Kannel WB. Prediction of coronary heart disease using risk factor categories. Circulation. 1998;97:1837–1847.
D'Agostino RB, Sr. Grundy S, Sullivan LM, Wilson P. CHD risk prediction group. Validation of the framingham coronary heart disease prediction scores: Results of a multiple ethnic groups investigation. JAMA. 2001;11:286(2):180-187.

Jones ES, Tobertso PW. Coronary artery disease, hypertension, and hypercholesterolaemic xanthomatosis. Br. Med. J. 1984;1:1137.

Liu H, Liu W, Liao Y, et al. CAD gene: A comprehensive database for coronary artery disease genes. Nucleic Acids Res. 2010;39:D991-D996.

Mayer B, Erdmann J, Schunkert H. Genetics and heritability of coronary artery disease and myocardial infarction. Clin Res Cardiol. 2007;96:1-7.

Ioannidis JP, Ntzani EE, Trikalinos TA, Contopoulos-Ioannidis DG. Replication validity of genetic association studies. Nat. Genet. 2001;29:306–309.

Hirschhorn JN, Lohmueller K, Byrne E, Hirschhorn KA. Comprehensive review of genetic association studies. Genet. Med. 2002;4:45–61.

Casas JP, Cooper J, Miller GJ, Hingorani AD, Humphries SE. Investigating the genetic determinants of cardiovascular disease using candidate genes and meta-analysis of association studies. Ann. Hum. Genet. 2006;70:145–169.

Hall J. Translating genetic discoveries to improvements in cardiovascular care: The path to personalized medicine. J Cardiovasc Trans Res. 2008;1:37–40.

McPherson R, Pertsemlidis A, Kavaslar N, et al. A common allele on chromosome 9 associated with coronary heart disease. Science. 2007;316:1488–1491.

Schunkert H, Gotz A, Braund P, et al. Repeated replication and a prospective metaanalysis of the association between chromosome 9p21.3 and coronary artery disease. Circulation. 2008;117:1675–84.

Zhou L, Zhang X, He M, et al. Associations between single nucleotide polymorphisms on chromosome 9p21 and risk of coronary heart disease in Chinese Han population. Arterioscler Thromb Vasc Biol. 2008;28:2085–9.

Preuss M, König IR, Thompson JR, et al. Design of the Coronary ARtery Disease Genome-Wide Replication And Meta-Analysis (CARDIoGRAM) Study: A Genomewide associationmeta-analysis involvemore than 22 000 cases and 60000 controls. Circ Cardiovasc Genet 2010;3(5):475–83.

Yang Q, Köt gens A, Dehghan A, et al. Multiple genetic loci influence serum urate levels and their relationship with gout and cardiovascular disease risk factors. Circ Cardiovasc Genet. 2010;3(6):523–30.

Mantillo TA, Collins FS, Cox NJ, et al. Finding the missing heritability of complex diseases. Nature. 2009;461:747–53.

Tuomisto TT, Korkeela A, Rutanen J, Viita H, Brasen JH, Riekkinen MS, et al. Gene expression in macrophagerich inflammatory cell infiltrates in human
atherosclerotic lesions as studied by laser microdissection and DNA array: Overexpression of HMG-CoA reductase, colony stimulating factor receptors, CD11A/CD18 integrins, and interleukin receptors. Arterioscler Thromb Vasc Biol. 2003;23:2235-40. [PubMed: 14576072].

31. Waehre T, Yndestad A, Smith C, Haug T, Tunheim SH, Gullestad L, et al. Increased expression of interleukin-1 in coronary artery disease with downregulatory effects of HMG-CoA reductase inhibitors. Circulation. 2004;109:1966–72. [PubMed: 15051633].

32. Davidsson P, Hulthe J, Fagerberg B, Olsson BM, Hallberg C, Dahllof B, et al. A proteomic study of the apolipoproteins in LDL subclasses in patients with the metabolic syndrome and type 2 diabetes. J Lipid Res. 2005;46:1999–2006. [PubMed: 15995172].

33. Karlsson H, Leanderson P, Tagesson C, Lindahl M. Lipoproteomics II: Mapping of proteins in high density lipoprotein using two-dimensional gel electrophoresis and mass spectrometry. Proteomics. 2005;5:1431–45. [PubMed: 15761960].

34. Karlsson H, Leanderson P, Tagesson C, Lindahl M. Lipoproteomics I: Mapping of proteins in low density lipoprotein using two-dimensional gel electrophoresis and mass spectrometry. Proteomics. 2005;5:551–65. [PubMed: 15627967].

35. Gaetano Santulli. Epidemiology of cardiovascular disease in the 21st Century: Updated numbers and updated facts. JCaD. 2013;1:1-2.

36. Lennep J, Westerveld HT, Erkelens DW, Wall EE. Risk factors for coronary heart disease: Implications of gender. Card Res. 2002;53:538–549.

37. Yang B, Lin H, Xiao J, Lu Y, Luo X, Li B, et al. The muscle-specific microRNA miR-1 regulates cardiac arrhythmogenic potential by targeting GJA1 and KCNJ2. Nat Med. 2007;13:486–491.

38. Wronska A, Kurkowska-Jastrzebska I, Santulli G. Application of microRNAs in diagnosis and treatment of cardiovascular disease. Acta Physiol. 2015;213:60–83.

39. Charan Reddy K. Regulatory Noncoding RNAs in cardiovascular disease: Shedding light on ‘Dark Matter’. JCV. 2015;3(1):301-307.

40. Miller DT, Ridker PM, Libby P, Kwiatkowski DJ. Atherosclerosis: The path from genomics to therapeutics. J Am Coll Cardiol. 2007;49:1589–1599.

41. Breslow JL. Genetic differences in endothelial cells may determine atherosclerosis susceptibility. Circulation. 2000;102:5–6.

42. Kant R. Emerging risk biomarkers in cardiovascular diseases and disorders. J Lipids; 2015. Article ID 971453. Available: http://dx.doi.org/10.1155/2015/971453.

43. Wang TJ, Gona P, Larson MG, Tofler GH, Levy D, Newton-Cheh C, Jacques PF, Rifai N, Selhub J, Robins SJ, Benjamin EJ, D'Agostino RB, Vasan RS. Multiple biomarkers for the prediction of first major cardiovascular events and death. N Engl J Med. 2006;355(25):2631-9.

44. Zethelius B, Berglund L, Sundström J, Ingelsson E, Basu S, Larsson A, Venge P, Arnlöv J. Use of multiple biomarkers to improve the prediction of death from cardiovascular causes. N Engl J Med. 2008;359(20):2107-16. DOI: 10.1056/NEJMoa0707064.