Review Article

The use of biomarkers for assessment of cardiovascular disease risk

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Abstract

Cardiovascular disease (CVD) is the most prominent cause of morbidity and mortality in the world and its financial burden on society is escalating. Accurate diagnosis and prevention is therefore a health priority. Many traditional factors are known to increase the risk of CVD including, but not limited to; hypertension, sedentary lifestyle, diet, hypercholesterolaemia, smoking and obesity, but these alone are insufficient to identify all patients at risk of CVD. There is substantial focus on identifying individuals at high-risk of developing CVD while they are asymptomatic and subclinical, allowing preventative treatments to be implemented and thereby reduce clinical manifestations and their serious sequelae. Identifying high-risk patients also allows avoidance of over-treatment in low-risk presentations, therefore yielding more efficient use of medical resources. Surrogate biomarkers for CVD potentially augment clinical risk stratification by identifying key mechanisms in CVD initiation and progression. By using surrogate biomarkers, the effectiveness of treatments can be monitored in relation to a point along the disease timeline, without measuring the true outcome of interest (i.e. CVD event). This review aims to provide an overview of some of the leading surrogate biomarkers currently in use for assessing CVD risk.

Keywords: Cardiovascular disease risk, biomarkers, subclinical atherosclerosis, endothelial function, arterial stiffness

Introduction

Cardiovascular disease is the current primary cause of mortality worldwide. The World Health Organisations’ (WHO) published statistics state, in 2004 there were...
17.1 million CVD related deaths, representing 29% of global mortality\(^1\). Additionally, approximately 20 million people survived a CVD event and required further medical interventions. WHO predict that by 2030, this will rise to 23.6 million deaths per year, with CVD remaining the leading cause of global mortality\(^1\). In view of this escalating responsibility, it is imperative to develop successful means of screening the population to reduce CVD events and deaths.

CVD affects the heart and blood vessels and has many clinical manifestations including: coronary heart disease (CHD) (myocardial infarction), cerebrovascular disease (stroke), and peripheral arterial disease (intermittent claudication). Lifestyle factors including smoking, sedentary lifestyles and unhealthy diets, all typical of the Western World but also rising in the Indian subcontinent and Middle East, increase the likelihood of CVD in later life. Clinical CVD is generally rare in children\(^3\). Regardless of the low incidence of symptomatic CVD in childhood, it is now understood atherosclerosis develops from early childhood or even prenatally\(^3\), and progresses throughout life.

**Atherosclerosis**

Atherosclerotic plaque development, progression and eventual rupture is driven by inflammation. The thrombotic potential of a plaque is the most dangerous component of atherosclerosis, not the extent of arterial stenosis created\(^4\). A ruptured plaque characteristically exhibits thinning of the fibrous cap, subsequently followed by true rupture, uncovering the underlying thrombogenic lipid core\(^5\). Ordinarily, arterial wall endothelial cells are able to resist leukocyte adhesion and aggregation, promoting fibrinolysis. When influenced by CVD factors, endothelial cells begin to express adhesion molecules which selectively recruit various classes of leukocyte. Blood monocytes are the most abundant inflammatory cells present within plaques, adhering to the dysfunctional endothelial surface. Once adherent to the activated endothelium, chemokines induce monocyte entry into the intima via chemotactic stimuli. Having entered the intima, monocytes evolve into macrophages which express scavenger receptors, allowing them to engulf modified lipoprotein particles. The macrophage cytoplasm enlarges with lipid particles generating the typical frothy appearance of foam cells found within atherosclerotic lesions. Macrophages proliferate within the intima structure, the release of growth factors and cytokines sustaining and amplifying the inflammatory process. Atheromatous plaques also contain a small proportion of T-lymphocyte cells which are able to exert decisive regulatory roles through instruction to the more abundant, monocytic effectors of the innate immune response.

**Biomarkers**

Biomarkers act as indicators of normal or pathogenic processes, or can be used to monitor pharmacological responses to therapeutic interventions. These characteristics require the biomarker to be objectively measurable in order to facilitate evaluation of change or variance. This review focuses on biomarkers that can be used as tools for screening and prognosis of CVD development and as surrogate end points (SEPs), with a particular emphasis on endothelial function and arterial compliance. A biomarker can be used to substitute a hard clinical end point, characteristic or variable which reflects how a patient feels, functions or survives\(^6\). SEPs are designed to predict clinical benefit, harm or lack of effect based on epidemiological, therapeutic, pathophysiological or other scientific evidence\(^7\). SEPs can be used to understand the disease process and to evaluate therapy effectiveness and safety in clinical trials by tracking closely with, but without measuring, the true outcome of interest, namely a CVD event.
Experimental studies using SEPs can be conducted over a shorter time frame, using fewer patients and with reduced costs in terms of both resources and CVD events\(^6\).

For assessing CVD risk, it is extremely unlikely that a single biomarker will act as a universal surrogate due to the complex pathophysiology involved\(^7\). Instead, accurate risk assessment necessitates use of a combination, or panel of biomarkers. Imaging technologies are generally able to assess disease presence or absence with a high degree of sensitivity and specificity, but technical difficulty may limit their effectiveness, along with high costs and limited availability of diagnostic equipment and skilled operators. Soluble biomarkers such as low density lipoprotein cholesterol (LDL-C), have high availability, ease of collection and storage, and lower costs, however they may not provide the high sensitivity derived from imaging procedures\(^7\).

**Imaging**

Imaging modalities improve understanding of CVD pathophysiology, early diagnosis, progression monitoring, optimum therapy selection and effectiveness based on clinical presentation\(^8\). Patient comfort and compliance are essential considerations; hence non invasive imaging has clinical indications for stratifying patients at intermediate CVD risk to justify early interventions. Imaging techniques require high spatial resolution to detect and contrast a defect, but also have to overcome blood flow and cardiac/respiratory movement\(^9\). Imaging agents require high sensitivity and specificity, possessing both a signal detection compound identifiable by appropriate imaging hardware, and an affinity ligand which specifies the molecular/cellular target\(^9\).

Current imaging techniques mostly focus on identifying luminal stenosis, wall thickness and plaque volume\(^10\), however the constituents of atherosclerotic plaques including fibrous tissue, calcification, the lipid-rich necrotic core and intraplaque haemorrhages\(^5\), also need to be evaluated to progress appropriate and effective treatments.

**Invasive Imaging Techniques**

Invasive imaging modalities are available for identifying coronary arterial stenosis, but can only be applied to individuals requiring clinical investigation of established disease. X-ray angiography has the ability to reveal the presence of advanced lesions, disruption of plaques, luminal thrombosis and calcification\(^10\) and is the current gold standard for diagnosing coronary, carotid and peripheral artery stenosis. Angiography does not image the vessel wall or give information about plaque composition, vulnerability or histological status, which in turn identifies pathophysiology, and thus does not reveal information regarding future prognosis and development of symptomatic CVD\(^11\).

**Intravascular Ultrasound**

Intravascular ultrasound (IVUS) allows for both real-time atheroma imaging and normal imaging producing a “cross-sectional, tomographic perspective of the vessel and atherosclerotic disease”\(^10\). IVUS applications include angiographical detection of unrecognised disease and determination of lesion severity.

**Coronary Angioscopy**

Coronary angioscopy employs an angioscopic catheter to directly envision a plaque surface, identifying luminal surface colour, thrombus status, and other macroscopic features such as tears, ulceration and fissures\(^12\). This technique facilitates identification of plaque rupture and thrombus formation which cannot be identified by X-ray angiography or IVUS.

**Computerised Tomography Angiography**

Computerised Tomography Angiography (CTA) is currently considered the gold-standard technique in vascular lumen evaluation, providing accurate coronary risk stratification and identification of stenotic lesions\(^13\).
Non-Invasive Imaging Techniques
Non-invasive imaging modalities can evaluate both the structure and function of the arterial wall(2).

Computerised Tomography
Computerised Tomography (CT) facilitates coronary plaque evaluation and therefore stratification of coronary disease risk. Dual-source computed tomography (DSCT) can visualise both the vessel lumen and wall, identifying the presence and size of non-calcified plaques with high sensitivity and specificity(13). Yang et al, (2010) have shown that DSCT carries diagnostic accuracy when determining coronary risk stratification, when compared with less desirable invasive procedures such as coronary angiography and IVUS(13).

Magnetic Resonance Imaging
Magnetic Resonance Imaging currently has the greatest utility in characterisation of plaque biophysical and biochemical parameters and can be repeated over time as it does not use ionizing radiation. MRI use in imaging epitopes identifies molecular markers commonly found in vulnerable plaques, confirming varying levels of plaque biological activity(14).

Ultrasound
High-resolution ultrasonic B-mode imaging is employed using a transducer frequency of approximately 10 MHz to obtain high quality measurements. The ultrasonic pulse duration and beam dimensions are determinants of the practical imaging resolution(15). As described later in this review, ultrasound can be used for evaluation of cardiovascular risk by assessment of endothelial function through flow mediated dilatation (FMD) testing, and for assessment of sub-clinical atherosclerosis by measurement of intima-media thickness (IMT).

Endothelial Function
The endothelium is now recognised as one of the most important regulators of vascular function(16,17). Given that the earliest changes in development of atherosclerosis are most likely to involve changes in endothelial function, this has become an attractive target for evaluation of CVD risk and progression. Accordingly, several techniques are available for evaluation of endothelial function. Endothelial dysfunction precedes structural changes responsible for thickening of the arterial wall, resulting in decreased arterial compliance, increased pulse pressure, alterations in shear stress and atherosclerotic plaque development.

Endothelial dysfunction is characterized by reduced vasodilator bioavailability, including nitric oxide (NO), with a parallel increase in vasoconstrictor bioavailability including endothelin-1, determining overall endothelium-dependent vasoconstriction(18). A series of proinflammatory, proliferative and procoagulatory mechanisms are over activated, contributing to all stages of CVD(19). Endothelial dysfunction is associated with traditional CVD risk factors such as hypercholesterolaemia, hypertension and diabetes and more recently identified factors including obesity, hyperhomocystinaemia and systemic inflammation(20).

Assessment of Endothelial Function
An artery’s ability to dilate in response to chemical or physical stimuli, or measurements of biomarkers of endothelial activation, dysfunction or damage has been used to evaluate endothelial function. Various invasive and non-invasive methods of measurement have been developed, validated and used clinically. These modalities test different aspects of endothelial function, including endothelial injury and repair(18) and provide insight into CVD pathophysiology, early disease detection, risk quantification and response to interventions designed to halt disease progression.

In coronary vessels or the brachial artery, endothelial responses can be evaluated by assessing vasodilator response to an infusion of a chemical, often acetylcholine(21,22). The
disadvantage of this modality is the invasive process which poses restrictions on frequent study, coronary assessment being contra-indicated unless a coronary angiogram is required.

**Flow-Mediated Vasodilatation (FMD)**

Non-invasive measurement of peripheral vasculature endothelial function can be evaluated by measuring brachial artery FMD\(^{(23)}\) as shown in Fig 1. FMD is the current gold standard technique to measure endothelial function, and uses high-resolution ultrasonographical measurements of endothelium-dependent changes in the brachial artery following post-occlusive reactive hyperaemia. The principle behind FMD is that shear stress stimulates the artery to produce a NO-dependent response, the FMD measurement providing a direct indicator of NO bioavailability. Brachial artery FMD bears a close link to coronary artery vasomotor response to acetylcholine stimulation\(^{(24)}\) and has the ability to independently predict CVD events in both at risk and healthy individuals\(^{(11,25)}\). Following treatment for CVD risk factors, FMD levels are seen to improve, verifying it is a reliable surrogate marker of cardiovascular health before and after treatment interventions\(^{(20)}\). FMD requires a number of factors to be considered prior to clinical application including; equipment costs, staff training and accurate image analysis.

**Fig 1:** Ultrasound assessment for evaluation of flow mediated dilatation (FMD). Brachial artery diameter changes are measured before and after arterial occlusion for 5 minutes. The ultrasound probe is held in a constant position using a stereotactic clamp. Analysis of brachial diameter can be carried out using edge-detection of near and far walls.

**Laser Doppler Imaging**

Laser Doppler imaging (LDI) is gaining increasing use as a measure of endothelial function in the microcirculation. The hypothesised initial site of CVD endothelial damage is in the microcirculation, hence this modality could prove invaluable\(^{(26,27)}\). LDI can be combined with iontophoresis, which allows delivery of vasodilator ions of soluble salts, across the skin (typically the forearm). The iontophoresis principle is that positively or negatively charged molecules will migrate across the skin under the influence of a weak electrical current. Once iontophoresed, the vasoactive chemicals reach the microvessels within the skin, and the increased blood perfusion is measured using LDI. A more detailed description in terms of methodological issues and set-up can be found in the review by Turner and colleagues\(^{(28)}\). This reproducible technique demonstrates
correlations between abnormalities in the microvascular bed, CVD risk factors and established CVD\(^{(29)}\). The demonstrated association between microvascular function and coronary flow reserve within healthy individuals has verified that assessments made in the skin give an accurate global measure of microvascular function\(^{(30)}\).

**Endothelium-Derived Biomarkers**

Many biomarkers are available as having possible use in CVD risk stratification and only some will be mentioned here. E-selectin, thrombomodulin and Von Willebrand factor are endothelium-derived biomarkers. Soluble E-selectin levels within the plasma are indicative of endothelial cell activation and/or damage\(^{(31)}\). CVD risk factors such as low HDL-C and diabetes are associated with high plasma E-selectin, and decrease with treatments such as statins and ACE-inhibitors. E-selectin is a predictor of re-stenosis in patients with intermittent claudication\(^{(31)}\). Thrombomodulin is the thrombin receptor on vascular endothelial cells, which contains an extracellular domain released into the circulation during the course of endothelial damage. Platelet aggregation and adhesion are determined by Von Willebrand factor, plasma levels having an inverse correlation with endothelial function, as measured by FMD, with inflammatory cytokines able to induce its release. Von Willebrand factor has implications for use as a predictor of CVD\(^{(32)}\) as well as being an independent predictor of ischaemic stroke\(^{(33)}\). The Atherosclerosis in Risk Communities (ARIC) study reported intracellular cell adhesion molecule 1 (ICAM-1) concentrations were indicative of coronary events and carotid atherosclerosis development, with a further association between ICAM-1 and soluble E-selectin concentration\(^{(34)}\). The Woman’s Health Study concluded that P-selectin levels were predictive of cardiovascular events\(^{(35)}\) and the Atherogene Study showed that in populations with coronary heart disease, levels of ICAM-1 and E-selection were increased in those who went on to develop cardiovascular events\(^{(36)}\).

**Endothelial Progenitor Cells**

Endothelial progenitor cells (EPCs) derived from bone marrow contribute to re-endothelialisation of injured blood vessels and neovascularisation of ischaemic lesions\(^{(37)}\). A decrease in EPC number is an independent predictor of CVD morbidity and mortality, suggesting an integral role in the pathogenesis of atherosclerosis and CVD\(^{(38)}\). Interestingly, EPC number and function is not only influenced by CVD risk factors, but also certain clinical interventions, including lifestyle modifications and pharmacological interventions, an inverse correlation existing between number of EPCs and endothelial function\(^{(39)}\). Furthermore, a reduced EPC number in patients with CVD correlates with impaired migratory functions and capacity for neovascularisation, supporting this evidence\(^{(40)}\). It is therefore surmised, by a mechanism not yet fully evaluated, that the number and function of EPCs directly influence development of atherosclerosis, displaying an important relationship between the role of EPCs and CVD disease.

Currently there is not a precise definition of an EPC, although identification is conducted through location of unique surface markers of haematopoietic and endothelial lineages including CD\(^{34}\), AC1\(^{(33)}\) and VEGFR-2\(^{(37)}\). The true definition of an EPC is still subject to confusion as there is no gold standard marker for identification. In addition there is no established protocol for EPC analysis, which is a necessary consideration when analysing EPC studies and comparing results.

**Arterial Compliance**

Arterial compliance is the buffering capacity and distensibility, which together play intrinsic roles in vascular biomechanics and homeostasis\(^{(41)}\). One of the first signs of CVD is a change in arterial compliance\(^{(42)}\). Measuring arterial stiffness non-invasively
provides information on both functional and structural changes occurring within the artery vessel wall at the aortic, muscular conduit artery, peripheral branch and microvascular levels\(^{(42)}\).

**Assessment of Arterial Compliance**

**Pulse Wave Velocity**

Pulse wave velocity (PWV) is defined as the distance a pulse wave will travel over a given time period, usually measured at diastolic pressure\(^{(41)}\). PWV is measured by recording an arterial wave form at two separate sites in the arterial tree, typically the carotid and femoral artery\(^{(42)}\). The time delay between the carotid and femoral arterial sites divided by the distance between the sites is used to calculate the PWV in the conduit artery system, the higher the reading the stiffer the artery.

**Applanation Tonometry**

This non-invasive, simple modality yields accurate results using a hand-held tonometry probe. The method principally employs partial compression of typically the radial artery against a hard structure, the sensor being able to detect the force placed upon the arterial walls to generate an actual pulse recording\(^{(43)}\). The central aortic pressure can be estimated using the properties of transfer function between the aorta and radial artery. Conventional cuff measurements using systolic and diastolic pressure values are used to calibrate the radial waveform and an average waveform is calculated from the series of contiguous pulses\(^{(44)}\).

**Arterial Compliance and CVD Prediction**

In normotensive individuals, aortic stiffness could be used as a predictor of future hypertension even after correction for traditional risk factors\(^{(45)}\). Longitudinal studies have shown the independent predictive value of arterial compliance for CVD events\(^{(42)}\). The greatest quantity of evidence has been generated for aortic stiffness, through measurements of carotid-to-femoral PWV. Independent predictive value from measurement of aortic stiffness was found in CVD deaths, fatal and non-fatal coronary events and strokes in the population\(^{(46,47)}\).

**Intima-Medial Thickness**

Intima-medial thickness (IMT) is a generalised marker of arterial-wall injury and has a close relationship with cardiovascular risk in adults, making it attractive for use in primary CVD prevention\(^{(15)}\). Measurement is most frequently obtained using simple B-mode ultrasound of the common carotid artery (CCA), carotid bulb, and internal carotid artery (ICA)\(^{(3)}\). Carotid IMT (cIMT) has use as a risk predictor and surrogate marker of CVD, which has been approved by the United States Food and Drug Administration for clinical use in the USA. cIMT by definition, is the combined thickness of the intima and media layers of the carotid artery. This measurement varies with anatomical site, age, sex and ethnicity. Typical measurements are approximately 0.50 and 1.00 mm for the middle 90% of the general population aged between 45 and 65 years of age\(^{(48)}\). As cIMT reflects the total thickness of the intima and media, it also reveals any pathological process within them, such as intimal thickening due to early atherosclerosis, or vascular hypertrophy due to hypertension\(^{(49)}\).

A single cIMT measurement is not generally valid for cardiovascular risk assessment. cIMT varies with the precise anatomical site within the carotid system and the ultrasonic interrogation angle due to atherosclerosis being circumferentially asymmetrical\(^{(45)}\). cIMT validity is increased by taking multiple measurements and combining these appropriately. Accurate interpretation of cIMT readings determines they are best evaluated in terms of an ethnic, age and gender matched population. Values which exceed the 75\(^{th}\) percentile in this reference population or exceed 1mm in thickness are said to be at increased CVD risk. Annually, cIMT levels increase by approximately 0.01mm in the general population, with increments often several
times greater in high-risk patients. However, in order to study changes in cIMT over time, precise measurements and reliable methodology are required.

**IMT and CVD prediction**

The Atherosclerosis in Risk Communities (ARIC) study evaluated CVD-free subjects, cIMT and traditional CVD risk factor measurements were collected. Subjects who showed no evidence of atherosclerotic thickening were then used as controls. cIMT was shown to increase with advancing age, BMI, systolic and diastolic blood pressure, smoking, total cholesterol, triglyceride levels and LDL-C. A significant inverse relationship was noted between HDL-C and cIMT, and evidence was also found which indicates cIMT correlates with the extent of alteration in cardiac risk factors, including that cIMT values are higher in smokers than non-smokers, and that values steadily amplify with increased smoking duration and quantity\(^{50}\).

Diabetes is associated with increased cIMT levels independently of other cardiac risk factors\(^{51}\), and HbA1c levels also independently relate to cIMT\(^{52}\). Epidemiological studies have shown a predictive value of cIMT for future cerebrovascular and cardiovascular events. The Atherosclerosis in Risk Communities (ARIC) study measured cIMT levels on the far wall of the carotid artery at three sites along each side of the extra-cranial carotid tree. A positive correlation exists between mean cIMT levels and CVD events which increase in a graded manner. This trend was noted to decrease slightly when adjustments for conventional CVD risk factors were taken into account. These results are concordant with results of the Kuopio Ischaemic Heart Disease Risk Factor Study, where the risk of acute CVD events increased with increasing cIMT, a finding which remained significant after calculating controls for age, smoking, systolic blood pressure and HDL:LDL cholesterol ratio\(^{53}\). The Rotterdam Study strengthened the evidence from these two studies, the risk of CVD event increasing with cIMT increases\(^{54,55}\).

The latter three studies focused on the middle-aged to elderly age group, the Carotid Atherosclerosis Progression Study (CAPS) however demonstrated the predictive value of cIMT in subjects over a 19 to 90 year age range: for each increase in common cIMT reading, the risk of CVD event increased even after adjustments for age, sex, BMI, systolic and diastolic blood pressure, antihypertensive therapy, LDL-C, lipid-decreasing medications, smoking and diabetes\(^{56}\). Moreover, the risk was found to be higher for subjects younger than 50 years than for those over 50, although the risk is absolute for the older subjects. This could be due to a lower referent cIMT value in younger subjects which makes relative risk higher or due to a decreased ability of cIMT to predict vascular effects in older subjects.

In conclusion, surrogate biomarkers provide a powerful mean for understanding the CVD spectrum. Inflammation is involved at every stage of CVD, from initiation to eventual plaque rupture when inflammation has exhausted the endothelial defence mechanisms, which are no longer able to provide protection. It is clear that damage to the endothelium and therefore endothelial dysfunction is key to CVD. Many techniques can be used to evaluate the mechanisms and mediators of this disease, critical to an improved understanding of atherosclerosis and its clinical complications, with a view to providing a rational means for accurate disease prediction and prevention.

This review has focused on some of the current surrogate biomarkers of CVD, but there are potentially many others suitable for clinical use and future development. As CVD is multifactorial, a single biomarker is not able to fully evaluate the disease process; instead a panel of biomarker techniques will be the most effective means of assessment. Each individual technique brings its own series of
advantages and disadvantages, but with time and further developments in terms of disease understanding and technique advancements, a panel of biomarker techniques is increasingly being accepted as the only way to accurately identify CVD risk in subclinical patients. Ultimately the optimum method of identifying high-risk populations will be determined allowing preventative measures to have greater beneficial effect.

The ultimate questions to be asked when identifying and evaluating biomarkers for potential use in clinical settings are whether or not they improve risk predictions for a patient when combined with already existing clinical risk factors, and whether they can accurately identify normal physiological as well as pathophysiological developments. We are by no means close to a definitive answer to whether surrogate biomarkers will be used within routine clinical settings, but there is a clear mandate for further investigation of the many identified techniques with this potential in mind.

References
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