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Review

Cardiovascular disease detection using bio-sensing techniques

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ABSTRACT

Universally, cardiovascular disease (CVD) is recognised as the prime cause of death with estimates exceeding 20 million by 2015 due to heart disease and stroke. Facts regarding the disease, its classification and diagnosis are still lacking. Hence, understanding the issues involved in its initiation, its symptoms and early detection will reduce the high risk of sudden death associated with it. Biosensors developed to be used as rapid screening tools to detect disease biomarkers at the earliest stage and able to classify the condition are revolutionising CVD diagnosis and prognosis.

Advances in interdisciplinary research areas have made biosensors faster, highly accurate, portable and environmentally friendly diagnostic devices. The recent advances in microfluidics and the advent of nanotechnology have resulted in the development of improved diagnostics through reduction of analysis time and integration of several clinical assays into a single, portable device as lab-on-a-chip (LOC). The development of such affinity based systems is a major drive of the rapidly growing nanotechnology industry which involves a multidisciplinary research effort encompassing nanofluidics, microelectronics and analytical chemistry. This review summarised the classification of CVD, the biomarkers used for its diagnosis, biosensors and their application including the latest developments in the field of heart-disease detection.

Contents

1. Introduction

The World Health Organization (W.H.O.) lists cardiovascular disease (CVD) as the leading cause of death globally [1]. In the United Kingdom for every 1000 people, 11 are affected by some form of CVD which in turn is directly responsible for 200,000

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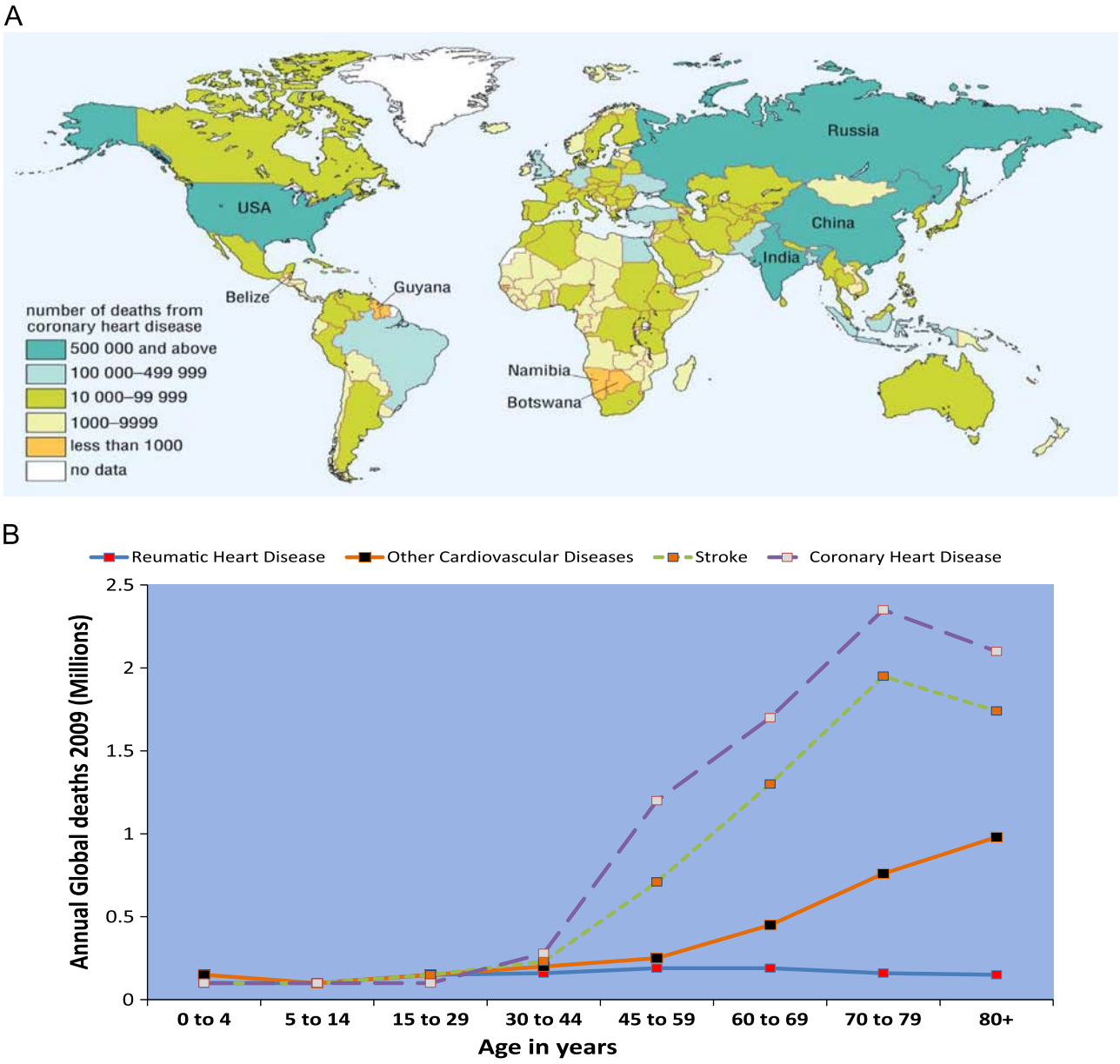

Fig. 1. A map of the world showing numbers of deaths from coronary heart disease (A) [4]. Global number of deaths per year due to different types of cardiovascular diseases and their age distribution (B) [6].

deaths annually, slightly more than 1 in 3 deaths [2]. According to the British Heart Foundation (BHF) there are two main forms of CVD which are coronary heart disease (CHD) and stroke. About half of all CVD deaths (48%) are from CHD and more than a quarter (28%) are from stroke [3]. Fig. 1A shows the global distribution of the problem [4]. Although many advances have been made in the prevention, early detection and treatment of these conditions, they still remain the leading cause of death worldwide [5]. There are indications that these figures will keep on rising and by 2030 it is estimated that almost 23.6 million people will die from CVDs, mainly from heart disease and stroke [1]. Fig. 1B shows the current global number of deaths due to different cardiovascular diseases and the incidence at different age groups [6]. It is clear that cardiovascular disease prevention is an important social and clinical issue, but it is also an economic one.

It is important to detect patients with high risk of acute myocardial infarction early. This can help in cutting cost by screening the hospital admissions process and focusing resources to those that are specifically at risk. Biomarkers and biosensors are playing a key role in the diagnostic revolution of cardiovascular disease [7,8]. Although significant studies have been done on various biosensors for detecting CVDs, of late they have had moderate commercial success. However, advances in interrelated research areas have made it possible to develop faster, accurate, portable and environmentally friendly diagnostic devices [9]. There have been various developments in the field of biosensors such as lab-on-a-chip (LOC) technology and various devices formats and these have been shown to be capable of detecting and quantifying cardiac markers [10–14]. Hence the recent advances in microfluidics technology can also improve diagnostics by reducing the application time and integrating several clinical analyses into a single, portable device as the LOC technology [15–16]. The development of protein chips is a major aim of the rapidly growing nanotechnology industry and requires the use of multidisciplinary research effort [17–20]. This paper will focus on the current and cutting edge developments in the field of heart-disease diagnostics

using biosensors and review the disease, the biomarkers associated with cardiovascular conditions, biosensing systems and their diagnostics application.

2. Classification of cardiovascular disease

Cardiovascular disease is a term that includes several medical conditions of the heart and blood vessels and includes coronary heart disease (CHD), which is the condition known when the heart's blood supply is blocked or interrupted by a build-up of fatty substances in the coronary arteries. The walls of the arteries can become clogged up with fatty deposits called atheroma in a process known as atherosclerosis [1]. The condition that affects the blood vessels supplying the brain is known as cerebrovascular disease (CeVD). This is caused mainly by hypertension and in the long term may permanently change the structure of the blood vessels transforming them into stiffened, deformed, narrow, and uneven which in turn makes them more vulnerable to blood pressure fluctuations. Peripheral arterial disease is another condition affecting the blood vessels supplying the arms and legs. Bacterial infections can also affect the heart such as rheumatic heart disease, which is caused by *Streptococcal* bacteria resulting in damage to the heart valves and muscle through rheumatic fever, congenital heart disease caused by birth defects and malformations of heart. Deep vein thrombosis and pulmonary embolism are delocalised blood clots from the leg veins, which can dislodge and move to the heart and lungs. Heart failure and stroke are the two ultimate results of most unattended cardiovascular diseases. Further details of the classifications of cardiovascular diseases can be found at [4] and these are summarised in Fig. 2.

3. Biomarkers for cardiovascular diseases

Biomarkers that can identify individuals most at risk of cardiovascular diseases have been studied and found to be linked with inflammation or cardiac tissues damage [21–23]. Many of these are well established biomarkers and currently used in the disease diagnosis [Creatine-kinase MB (CK-MB), Myoglobin, Troponin I, and T], while others are still under investigated [C reactive protein (CRP), B-type natriuretic peptide (BNP)] [24–25]. These biomarkers which are detected in the patients' blood can provide clinical evidence and help in the disease prognosis. However, their use may be limited in some cases due to lack of providing insight on the location of the atherosclerotic plaque, its composition, and its liability to rupture and develop other major heart complications. The use of multi markers detection in the diagnosis of cardiovascular disease is more beneficial in its diagnosis since the specificity and sensitivity of the biomarkers vary with the disease condition. Even though biomarkers have their limitations, these are far outweighed by the benefits of providing rapid and more accurate diagnosis [23,25]. Biomarkers are also important to follow the disease progression since the level of certain markers change according to the heart disease stage.

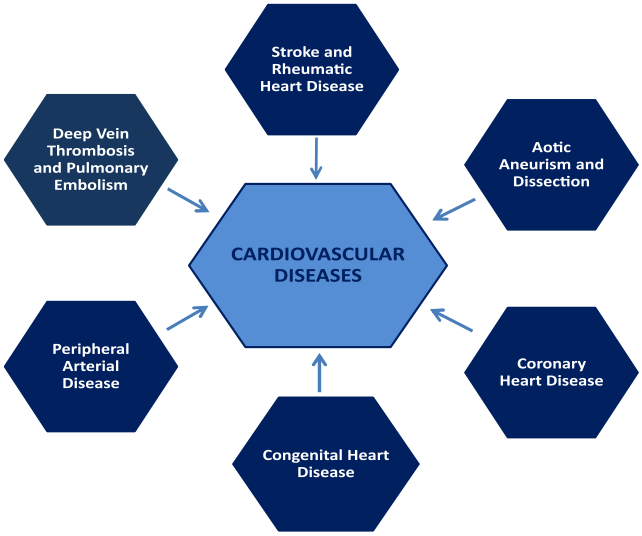

Fig. 2. Different types of cardiovascular diseases and their origin.

A cardiac marker is defined as a biological analyte that can be detected in elevated levels in the blood upon the onset or progression of CVD including myocardial damage [26]. It is important that an ideal cardiac marker exhibit several important characteristics due to the high risks of misdiagnosis that may result in fatalities. These include high clinical sensitivity and specificity, quick release for early diagnosis, suitable diagnostic window, ability to be assayed quantitatively, reasonable costs, and time-efficiency [27]. Currently no single biomarker have been reported to be able to deliver all the characteristics listed above [28], hence, the need for multi-marker approach. Table 1 shows the classifications of biomarkers used for the detection of CVD. However, new biomarkers are being discovered due to the advances taking place in proteomics and genomics and these may play an integral part in the future diagnosis of CVDs.

Table 2 lists some of the biomarkers used to diagnose different type of cardiovascular disease with their normal and disease levels. From the tables listed, there are wide arrays of biomarkers which have been identified as suitable for use in the diagnosis and monitoring of specific antecedents to cardiovascular diseases. However, only a selected few are dominant in the point of care (POC) setting. Due to the different conditions and symptoms associated with CVDs, there is a need to discover and develop new biomarkers which can be used to better diagnose and manage acute myocardial infarction. The study of the pathophysiology as mentioned earlier can play a pivotal role in the discovery and development of new biomarkers. Jaffe et al. [22] and Braunwald [28] give overviews of some cardiac biomarkers that may have potential to identify the patients at risk. Braunwald [28] identified key prospects in markers like myeloperoxidase, metalloproteinase-9, soluble CD40 ligand, pregnancy-associated plasma protein A, choline, ischaemia-modified albumin, unbound free fatty acids, glycogen phosphorylase isoenzyme BB (GPBB) and placental growth factor. Some of these biomarkers demonstrated promise and therefore, further evaluation is required to establish their potential use, but others have been dismissed as too general. Growth differentiation factor 15 (GDF-15) is one of the markers showing high potential, which is a growth factor β cytokine. It was originally used to predict miscarriage in pregnancy because of its

Table 1
Classifications of biomarkers for the detection of cardiovascular diseases [28].

Cardiovascular biomarker classification group	Selected example
Inflammation	CRP, TNF-α
Oxidative stress	Oxidised LDL, Myeloperoxidase
Extracellular matrix remodellers	MMP, TIMP
Neurohormones	Renin, Angiotensin, Arginin vasopressin
Myocyte injury	cTnT, cTnI, CK-MB, Myoglobin
Myocyte stress	Natriuretic peptides ANP, CNP and BNP
Potential new markers	Copeptin, Growth differentiation factor-15

KEY – CRP: C-reactive protein, TNF-α: tumour necrosis factor alpha, LDL: low density lipoproteins, ANP: atrial natriuretic peptide, BNP: brain natriuretic peptide, CNP: C-type natriuretic peptide, MMP: metalloproteinases, TIMP: tissue inhibitors of matrix metalloproteinases.

Table 2
Normal and disease levels of heart disease markers according to the type of CVD.

Biomarker	Type of CVDs	Normal levels	Disease levels	Reference
Tissue necrosis factor (TNFα)	Inflammation	4.8 pg mL^{-1}	48 pg mL^{-1}	[29]
Intercellular adhesion molecule-1 (ICAM-1)	Cardiac risk	227 ng mL^{-1}	513 ng mL^{-1}	[30]
Interleukin-6 (IL-6)	Inflammation and cardiac risk	4.0 pg mL^{-1}	138 pg mL^{-1}	[29]
Interleukin-1β (IL-1)	Cardiac risk	< 70.0 pg mL^{-1}	120 pg mL^{-1}	[31]
Serum amyloid A (SAA)	Cardiac risk	3.7 mg L^{-1}	2200 mg L^{-1}	[32]
Fibrinogen	–	2.5 g L^{-1}	5.6 g L^{-1}	[33]
Troponin I	Acute myocardial infarction	0.01 ng mg L^{-1}	0.1 ng mg $L-^{-1}$	[34–35]
Troponin T	Acute myocardial infarction	0.05 ng mg L^{-1}	0.1 ng mg L^{-1}	[34,36]
Myoglobin	Acute myocardial infarction	70 ng mg L^{-1}	200 ng mg L^{-1}	[35]
Myeloperoxidase	Inflammation	–	> 350 ng mg L^{-1}	[37]

high concentrations in the placental tissue. Since then, it was discovered that increased levels of GDF-15 can also be found in activated macrophages to counteract pathological or environmental stress. Kempf et al. [38] identified the best risk stratification strategies and found that there was synergy when GDF-15 was combined with BNP as the result improved the identification of high-risk patients compared with the individual markers. Copeptin has also been identified as a potential marker; a non-functional protein which is located on the same precursor as arginine vasopressin [39]. Increased levels of arginine vasopressin have been correlated with risk of CVD, similar to copeptin which shares the same precursor with it. Copeptin was shown to be able to predict adverse outcome especially when it was used in combination with the amino-terminal of the BNP precursor. It has also been proven to be able to distinguish patients with an acute myocardial infarction from those with other causes of chest pain. Unlike arginine vasopressin which is stable in serum the copeptin is vulnerable with a short half-life and a great affinity for binding to other proteins, which makes it difficult to measure.

4. Current methods for diagnosis of CVDs

There is no standard method in which CVD is diagnosed; this is in part due to the disease being clinically silent until serious complications occur [40]. The current diagnosis of CVD is dominated by either expensive imaging techniques or risky invasive techniques. Sophisticated imaging techniques like the magnetic resonance imaging (MRI) or ultrafast computerised tomography (CT) require expensive equipment and highly skilled staff. The same problem applies to invasive techniques such as coronary or cerebrovascular angiography. The invasive procedures are known to have associated risks making them not ideal for mass screening. Some affordable, user friendly and less invasive diagnostic approaches have been developed as alternatives but these come with their own limitations. A more clinical diagnostic approach is presented by scoring methods like the Framingham study or the Prospective Cardiovascular Münster Study (PROCAM) which considers several simple risk factors and classical symptoms of myocardial ischaemia. The combinations of several obtained risk factors is believed to create a synergy and thus give better predictive power but a huge number of patients at high risk go undetected [40].

Current protocol stipulates medical professionals to focus their resources on all people with chest pains reporting at emergency department as having a potential AMI [21]. This puts strains on precious resources, and can lead to situations where patients with a milder CVD like angina are unnecessarily admitted and tested for possible heart attack. For this reason much recent research has focused on identifying individuals most at risk of primary cardiovascular events at the point of care [23]. This makes it quick to administer effective prophylaxis, such as aspirin, beta blockers and statins, as well as give appropriate advice on lifestyle choices on an outpatient basis, and has the advantages of freeing up hospital beds and admitting only patients with a high probability of having an imminent AMI [21,25]. This section will continue with some of the currently used diagnostic techniques for cardiovascular diseases. It will highlight the advantages and challenges associated with the current methods and will then give examples and discuss the need for the shift to point of care methods.

4.1. Electrocardiography

Electrocardiography is the most commonly used diagnostic apparatus for cardiovascular diseases, as it is affordable and widely available. It is based on the electrical changes that occur temporally as the heart completes its usual cycle. These changes can be monitored in healthy state and vary when compared to those in certain disease states [41]. There are two major tests that are carried out using the electrocardium which are the resting and the exercise testing. Resting testing is commonly used to rule out rather than rule in CHD as it has relatively low sensitivity [42]. The exercise testing is also known as the stress testing and is performed under conditions that aim to exasperate stenosis. Patients will usually perform the ECG under pharmacologically induced stress or a treadmill. This procedure is important in the diagnosis of angina [42]. Exercise tests are usually performed following an indicative resting ECG or to aid risk assessment and disease state following AMI to allow the clinician to tailor rehabilitation and management for the individual patients. The clinician will monitor the patient's condition not only by examining the graphical data but by observation of the patient's physical condition [40].

4.2. Imaging techniques

Magnetic resonance imaging (MRI) is an imagining technique that has significant benefits in the diagnosis of CVD as it is capable of providing information on plaque composition. According to Adame et al. [43] there are many applications of MRI in CVD diagnosis, contrast-enhanced magnetic resonance angiography (CE-MRA) uses materials such as gadolinium which has desirable magnetic properties to give greater contrast to vasculature clearly denoting stenosis. Another MRI technique such as vessel wall MRI (VW-MRI) which can be used to detect vascular remodelling has proven to be an excellent diagnostic tool [43]. MRI can also give insight into the pathological state of an atheromatous plaque as well as elements of composition and thickness of the fibrous cap thus it can be used to more definitively identify patients at high risk of AMI or CVA [33]. The major drawback of MRI is the cost. It is not suitable for patients with metallic stents or pacemakers as it utilises a strong magnet [43].

Computed tomography also commonly known as a CT scan is routinely used in the diagnosis of cardiovascular diseases. It combines multiple X-ray images obtained from a ring of rotating X-rays with the aid of a computer to produce cross-sectional views of the body. Cardiac CT is a heart-imaging test that uses CT technology to visualise the heart anatomy, coronary circulation, and great vessels [44]. The quality of the results obtained is similar to those of the MRI. A modified variation of CT which is becoming more popular with the diagnosis of cardiovascular diseases is the ultra-fast computer tomography (UFCT), also called the electron beam CT. It varies from conventional CT scans because it does not have a rotating X-ray assembly; it has a ring of fixed crystal detectors and another fixed ring of tungsten X-ray targets, which act together. A major disadvantage of the CT scan to its rival the MRI is that it uses X-rays which have long been associated with cancer.

4.3. Immuno techniques

The analysis of cardiovascular markers can be carried out using immunology based methods like immunoassays. These utilise the specificity of immuno reactive components mostly antibodies, in binding to a specific analyte, to qualitatively and quantitatively confirm the presence or absence of the antigen. The most common immunoassays are the membrane-based immunoassays such as the lateral flow devices (LFD) and the enzyme-linked immuno sorbent assays (ELISAs). Many LFD devices still use the same technology that was patented more than 25 years previously. However, better materials, detection through the use of portable readers, and more precision has made the technology very attractive as point of care devices. Multiplexing and simultaneous identification and quantification of multiple biomarkers in single line is also the future trend for LFDs. ELISA is a method for detecting and quantifying a specific analyte in a complex mixture. In this method, the visualisation of the target antigen is realised through a colour-generating enzyme, covalently linked to a specific antibody. The colour formation is proportional to the concentration and can be used for quantification by analysing the absorbance. The ELISA method was made possible because of scientific advances in a number of related fields, including the production of antigen-specific monoclonal antibodies by Kohler and Milstein [45], and the ability to chemically link antibodies to biological enzymes whose activities can be measured as a signal. Besides the ELISA, there are several other immunoassays formats that were developed earlier including radioimmunoassay which use antibodies labelled with radioisotopes. It was first used before alternatives were sought because of health risks associated with radiation. Several variations have been made to the original ELISA with most changes being made on the method of transduction. A general term 'immunoassays' is more universally accepted.

It is important to understand the structure and function of antibodies as they play a major role in immunoassays. Antibodies are produced by B-cells as part of the immune response system that identify and neutralise foreign objects such as bacteria and viruses known collectively as pathogens. Antibodies have been used as recognition elements in immunoassay and subsequently immunosensor development because of their high affinity, high specificity, versatility and commercial availability [46]. Antibodies derived from separate cell lines that recognise various regions on the immunogen are termed as polyclonal antibodies, and those derived from single cell line are monoclonal antibodies. There are five major classes of antibodies, which are, IgG, IgD, IgE, IgA, and IgM. Of all these the IgG is the most abundant class in serum which has been used in immunoassay development for half a century.

There are four main types of immunoassay protocols for the detection of proteins: direct, indirect, competitive, and sandwich immunoassays. The basic principles for the assays are similar and they include, the capture of the analyte of interest, blocking of non-reacted surface, and recognition of the analyte. Direct immunoassay is the simplest form of analyte detection. It involves immobilising the analyte of interest to the surface. This is followed by a washing step, drying and blocking before a specific labelled antibody for the analyte is added for detection. Fig. 3 shows the illustrations of a direct, indirect and sandwich immunoassays. The other format called indirect immunoassay has the ability to improve the sensitivity of the detection. In this method, the analyte of interest is bound to a specific antibody after immobilisation onto the surface. A labelled secondary antibody against this primary antibody is then incubated for detection purposes. It is important that the secondary antibody be raised in another species than the primary antibody to avoid non-specific binding.

By far the most common type of immunoassay detection is achieved by applying two specific antibodies for the analyte in a sandwich format immunoassay. Antibody-sandwich immunoassays may be the most useful of the immuno-sorbent assays for detecting antigen because they are frequently between 2 and 5 times more sensitive than those in which antigen is directly bound to the solid phase [47]. The antigen being investigated is actually sandwiched between two antibodies which are the coated antibody immobilised on a surface and the detection antibody which is usually conjugated to a marker that can cause a quantifiable response which is proportional to its concentration. Auto-analysers which enable automated routine biochemical tests in hospital laboratories for biomarker diagnosis are used today to perform immunoassays [48].

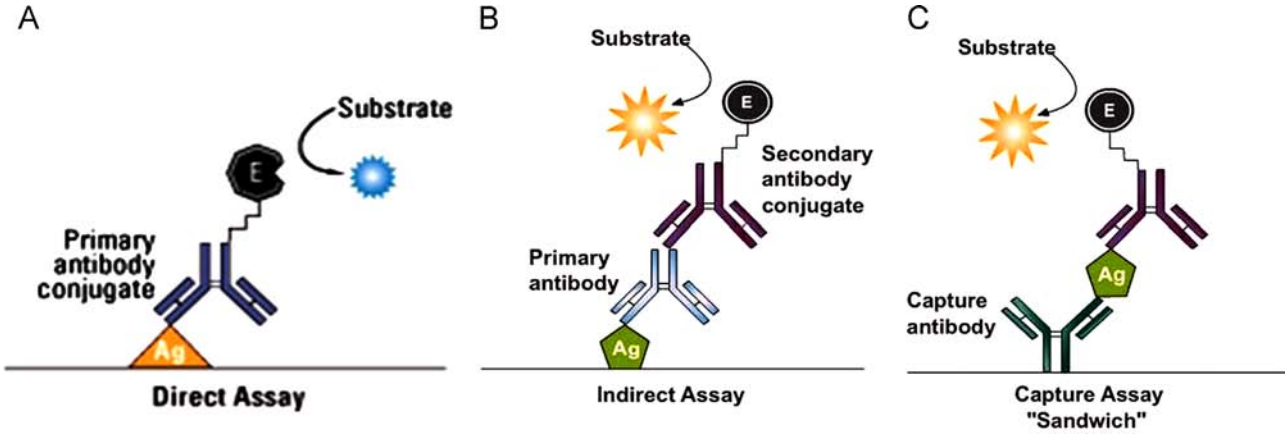

Fig. 3. Schematic illustration of direct (A), indirect (B) and sandwich (**C**) immunoassays to detect a biomarker.

5. Biosensors

A biosensor can be defined as a device which converts chemical information into a quantifiable electrical signal. It is an analytical device that is made up of a biological material such as micro-organisms, tissue, enzymes, antibodies or biologically derived material that acts as the recognition molecules [49,50]. These have to be utilised in conjunction with or attached to a transducer that can give an electronic signal proportional to the concentration of a specific analyte or group of analytes. The recognition of the analytes can be through a binding process, for instance involving an antibody in case of affinity based biosensors or biocatalytic reaction in the case of enzymes. The signal can be an end-point measurements or continuous depending on the regulatory and market requirements. The high expectation of the market especially for miniaturised, cost effective and environmentally friendly devices has resulted in biosensors becoming relevant in a wide range of analysis. The analytical capabilities of biosensors have been improved as faster microprocessors are constantly being developed [51]. Biosensors are classified according to the type of transducer employed, hence these are categorised into electric, radiant or optical, thermal, magnetic, and mechanical, frequency transducer. Biosensors offer an almost unlimited range of applications in many different disciplines due to the incorporation of a biological sensing component with its selectivity and specificity [52]. Today, the applications of biosensors span from applied research to commercially available sensors. There are a wide range of applications from agriculture to the measurement of blood metabolites routinely in the laboratory or as point-of-care devices [49,50]. Electrochemical transduced biosensors based on potentiometric and amperometric devices are the most described in the literature. However, affinity based devices have generally proved more amenable to optical detection methods. Fig. 4 shows the various applications of different biosensors with their systems and examples of sensor chips.

Immunosensors are biosensors with immuno-reagents as the biological sensing elements or receptor [53,54]. These are mainly antibody or antigen immobilised in close contact with physico-chemical transducers for example electrodes and optical fibres. The measurement of the analyte in the sample is achieved by the selective transduction of the binding of the receptor–target for example antibody–antigen reaction, resulting in a quantifiable electrical or optical signal. Immunosensors have made it possible for continuous detection to be achieved for a wide variety of analytes. The electrochemical immunosensor is particularly suited for situations where on-site monitoring capabilities are required and they have the advantages of being sensitive and selective due to the use of immunochemical interactions [49]. The limitation of the immunochemical sensors rises from the fact that the regeneration of the immuno surface is often challenging. There is also the issue of cross-reactivity or interference, which can be both an advantage or a disadvantage depending on the application of the immunosensor as well as the type of the target analyses. Below the most widely used transducers for cardiovascular disease biomarkers detection are described.

5.1. Optical transducers

Optical techniques are one of the best established methods used for detecting biochemical analytes. They usually comprise of a light source, modulating agent, and a photo-detector for processing the optical signal [55–57]. Optical transducers include fluorescence, interferometry and spectroscopy of optical waveguides and surface plasmons resonance (SPR). Many commercially available platforms use fluorescence labels as the detection system, but the instruments used for signal readout are usually expensive and more suitable for laboratory settings. SPR is a good example of an optical transducer system and it is based on a phenomenon that occurs when light is reflected off thin films of metal and can be verified by the arrangement based on the Kretschmann configuration [58]. SPR is one of the first examples used to demonstrate the functionality of affinity sensors based on optical transducer and is still widely used. Antibodies or other type of receptors are usually immobilised to the surface of a thin metal film as gold

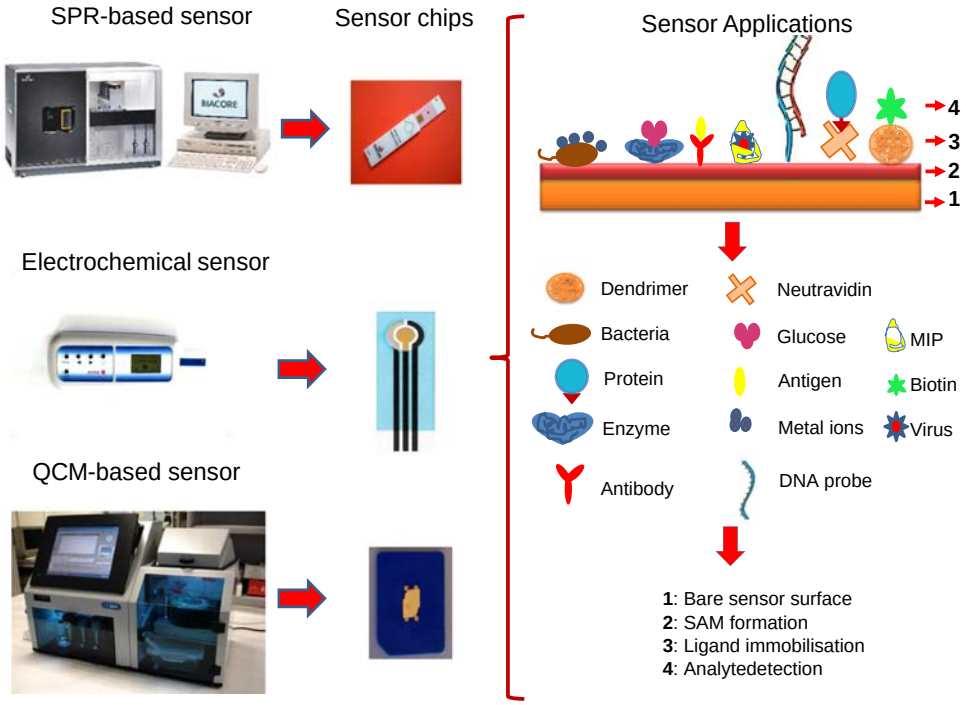

Fig. 4. Detection of various biomarkers using examples of biosensor platforms.

deposited on the reflecting surface of a glass prism. Affinity capture of the analytes by the immobilised receptor to the surface will elicit a change in the refractive index as variations in light intensity, reflected from the back of the film proportionally to the mass of antigens bound to the surface. When light is shone onto the grating at a certain angle of incidence, the momentum of the photons is converted into a collective motion of the electrons in the metal called surface plasmons [59]. This results in a decrease in the intensity of the reflected light. The angle of incidence at which resonance occurs depends on the optical thickness (essentially refractive index) of the layer that is within 300 nm of the diffraction component surface (the evanescent field), and thus, the SPR-based sensors facilitate direct label-free detection. SPR measurements are often made with full instruments systems comprising the chip in a flow cell to allow for controlled delivery of reagents and sample. SPR sensors have been developed based on immunosensors principle for biomarkers of cardiovascular diseases including for C-reactive protein [60], myoglobin and cardiac Troponin I [61], and Troponin T [62]. The SPR commercial scene is still dominated by the BIAcore system® (GE Healthcare), but more recent a range of new SPR instruments have been commercialised [63], as an example, the AutoLab Spirit® (Metrohm Autolab B.V.) and the SPR-2/4 system (Sierra Sensors, GmbH).

Other optical based immunosensor systems such as an SPR based fibre optic sensor [64] and fluorescence resonance energy transfer (FRET) based immunosensors for cardiovascular disease detection have also been reported [65]. FRET involves utilising the distance-dependent chemical transduction method of fluorescence resonance energy transfer and requires two fluorophores termed the donor and the acceptor. When in close proximity, the donor absorbs energy from the excitation source and non-radioactively transfers the energy to the acceptor, which in turn emits fluorescent energy. This distance-dependent property is utilised to detect conformational changes when antibodies combine with their respective antigens.

5.2. Piezoelectric/acoustic transducer

The quartz crystal microbalance (QCM) is an extremely sensitive mass sensor, capable of measuring mass changes in the nanogram range. QCMs are piezoelectric devices fabricated of a thin plate of quartz with electrodes affixed to each side of the plate. Piezoelectric ceramic materials can be produced by a less expensive process, however, the ceramics are limited by their lack of long term stability compared to single crystal materials which are less sensitive but when carefully handled have a significantly long-term stability [66]. Single crystal materials are quartz, tourmaline and gallium phosphate. Of the two it is the quartz crystals which are the common type of single crystal materials used in analytical application due to their electrical, mechanical, and chemical properties.

The resonant frequency of the quartz crystal depends on several parameters, for instance the size, the density, the shear modulus, but also the cut. QCM is a special sensor that has acoustic impedance detector by mass loading. Piezoelectric also known as acoustic biosensors such as QCM and other surface acoustic devices are classified as direct label-free and real time detection sensors [67]. Quartz crystal immunosensors are dependent on the immobilisation of antigen or antibody at the surface of a piezoelectric material [68]. The natural vibration frequency of the support is changed when there is a significant immunochemical recognition reaction. The applications of QCM are diverse and many commercial piezoelectric detectors are now available. They have been proven to show the possibility of developing faster diagnosis assays compared to traditional detection methods for cardiovascular diseases. Wong-ek et al. [69] developed a QCM sensor for cTnT using carboxylic polyvinyl chloride immobilisation layer on the sensor surface and achieved a detection limit of 5 ng ml^{-1} for cTnT.

Surface acoustic wave (SAW) transducers use piezoelectric substrate, such as quartz and are based on the mechanical waves created by an applied field that propagate through the substrate and are then transformed back to an electric field for measurement. Sensing layers immobilised on the transducer surface will bind the analyte of interest producing a change in the acoustic wave signal output and provide a quantitative signal [70,71].

5.3. Electrochemical transducers

Electrochemical transducers have been the most widely used system in the development of biosensors. This is due to the benefits it offers including miniaturisation, high specificity and sensitivity as well as the success story of the blood glucose biosensors, which has emerged as the leading commercial biosensor of all time. Electrochemical transducers can be classified into three main types: amperometric, potentiometric and conductemetric/impedance. Biosensors and immunosensors based on electrochemical transduction have been widely developed for the detection of a range of analytes [9,72–74]. The detection of cardiovascular biomarkers using immuno-electrochemical assays has been attempted for cardiac troponins [75], myoglobin [76] and CRP as a potential biomarker [77].

Immunoassay-based electrochemical sensors have been developed to exploit the high degree of specificity and affinity of antibodies for specific antigen. Their mechanism catalysis of substrates by an enzyme conjugated to an antibody produces products such as ions, pH change or a given chemical, metabolite, or modified protein can act as the antigen, and an EC response linked to antibody binding with the antigen [78]. The direct immuno-electrochemical detection without the use of labelling can be performed by cyclic voltammetry, chronoamperometry, impedance spectroscopy, and by measuring the current during potential pulses, a process called pulsed amperometric detection. These methods are able to detect a change in capacitance or resistance of the electrode induced by binding of protein. Despite having a lot of variety in the label free immunoelectric sensor platforms, it is the labelled amperometric immunosensor, which are widely developed and used [79]. In this biosensor format, the antibody or analyte of interest is immobilised on a membrane or directly on the sensor surface and the analyte is measured by signal derived from a conjugated tag that is attached to either the antibody or analyte [80]. Two types of immunoassays are generally employed and include competitive or sandwich assay depending on the analyte size. Both are detected by coupling the electrode to the antibodies raised against the biomarker of choice and the use of enzyme label that can generate electrical signal when catalysing its substrate solution via low molecular weight mediators. These are redox species that transfer electrons between the electrode and redox active biomolecules via an alternate oxidation/reduction reaction [73]. There are several of such species including peroxidases, phosphatases, ureases, and glucose oxidase mediated electron transfer [80]. Peroxidase is an excellent enzyme label in this application due to its properties. By using a suitable substrate such as 3, 3′, 5, 5′-tetramethylbenzidine hydrochloride (TMB) and hydrogen peroxide, the enzyme is capable of producing an oxidised product that can be electrochemically reduced to produce electrons. These electrons are responsible for generating a catalytically enhanced current which is easily detected if a constant potential is applied. These approaches are essentially conventional binding assays which use enzyme label to detect the reaction and involve multistep procedures of incubation and washing that are often found in traditional immunoassay formats based on

microtitre plates. Improvements in the assay development for immuno-electrochemistry for example the use of gold nanoparticles have made this a common research area with potential commercial outcomes [81–82].

In recent years more interest in impedance detection has been observed. This is due to it being a label-free technique and can be applied for real time detection [83–84]. Impedance based biosensors measure impedance changes in solution when the target analyte interacts with the bio-recognition molecule immobilised on the electrode surface [85].

6. Detection of cardiovascular diseases using biosensors

Effective intervention of cardiovascular disease strongly relies on a rapid turnaround time. This is the time taken between acquiring the blood sample from the patient until the results are obtained. The standard for chest pain diagnosis should be between 30 min to one hour, with 30 min or less being the optimum and this is not always possible if tests are performed in a centralised laboratory. Laboratory analysers are an invaluable resource but have several limitations, they are more suited for a hospital setting where there are trained personnel who can operate the machine and interpret the results accurately as well as phlebotomists who can take blood and supply the sample. Recent market demand devices to be more compact and can be used independently by the end user. Therefore, hand held biosensor devices are the ideal tools for this setting where testing can be conducted on-site aiding in the diagnosis of the condition and illuminating nonurgent hospital admissions. There are no known over the counter devices for cardiovascular disease diagnosis but a step towards this has been achieved through the point-of-care devices [86]. Bench top analysers are an example of such devices and are usually modified main laboratory analysers that have been miniaturised. These analysers are able to test a single sample for a multitude of cardiac markers with the ability to use a variety of analytical methods including enzyme activity measurements, spectrophotometric substrates, haematological particle counting, immunoassay and biosensors [87]. A summary of such commercially available devices as the Triage® cartridge (Alere Inc., USA), RAMP® cardiac marker system (Response Biomedical, Canada/Roche, Germany), Cardiac reader (Roche, Germany), Alpha DX (First Medical, USA), Stratus (Dade Behring, Germany), Vidas CK-MB (bioMérieux, France), i-STAR® (Abbott, UK) have been reviewed [86]. Some of these instruments can work for other antigens not just cardiac markers and are designed to handle blood/serum samples, and therefore does not require sample pre-treatment. The major drawback is that the cost associated with some systems is still

Table 3
Heart disease markers detection on different biosensor platforms and their detection range reported in the literature.

Biomarker	Sample	Biosensor	Sensing method	Linear range	Detection limit	Ref
TNF-α	Buffer	Optical	Fluorometric	1–0.0016 ng mL^{-1}	–	[88]
CRP	Serum	Optical	Fluorometric	10–10^5 ng mL^{-1}	–	[89]
CRP	Blood	Optical	Fluorometric	–	20 ng mL^{-1}	[90]
CTnI	Serum	Optical	Electrochemiluminescence	–	0.002 ng mL^{-1}	[91]
CRP	Serum	Optical	SPR	2–5 × 10^3 ng mL^{-1}	10^3 ng mL^{-1}	[92]
Arteriosclerotic, cytokinic, inflammatory, lipidic, and oxidative stress biomarkers	Blood	Optical	Fluorescence microscopy and ellipsometry	–	–	[93]
cTnT	Serum	Optical	SPR	0.03–6.5 ng mL^{-1}	0.01 ng mL^{-1}	[94]
cTnT	Serum	Optical	SPR	0.05 and 4.5 ng mL^{-1} ng mL^{-1}	0.05 ng mL^{-1}	[95]
Cytokines	Serum	Optical	Fluorometric	0.01–10 ng mL^{-1}	0.01 ng mL^{-1}	[96]
Myoglobin Creatine kinase mb (CKmb) cTnI Fatty acid-binding protein (FABP)	Serum	Optical	Chemiluminescence	–	1.2 ng mL^{-1} 0.6 ng mL^{-1} 5.6 ng mL^{-1} 4 ng mL^{-1}	[97]
BNP, cTnI, myoglobin, CRP	Serum	Optical	Optical fibre	–	0.1 ng mL^{-1} ng mL^{-1}, 7 × 10−3 ng mL^{-1}, 70 ng mL^{-1}, 700 ng mL^{-1}	[98]
CRP	Serum and plasma	Optical	Photonic microring resonator	10–10^2 ng mL^{-1}	3 × 10−2 ng mL^{-1}	[99]
IL-6	Buffer	Optical	Photonic crystal resonant	0.001–0.01 ng mL^{-1}	–	[100]
cTnT	Blood	Optical	Optomagnetic	0.03–6.5 ng mL^{-1}	0.03 ng mL^{-1}	[101]
cTnT	Buffer	QCM	Acoustic impedance detector	–	5 ng mL^{-1}	[69]
cTnT	Serum	Electrochemical	Faradaic, screen printed electrodes	0.1 and 10 ng mL^{-1}	0.2 ng mL^{-1}	[102]
Myoglobin	Blood	Electrochemical	Faradaic, Fe graphite electrodes		5 ng mL^{-1}	[76]
NT-proBNP	Buffer	Electrochemical	Faradaic, nanostructural gold and carbon nanotubes composite	0.02–100 ng mL^{-1}	0.006 ng mL^{-1}	[103]
cTnT	Buffer	Electrochemical	Faradaic, ITO electrodes	1–100 ng mL^{-1}	–	[104]
CRP	Serum	Electrochemical	Faradaic, magnetic beads with carbon electrodes	–	5.4 × 10^{-11} ng mL^{-1}	[105]
CRP	Serum	Electrochemical	Faradaic, nanotextured polystyrene (PS) electrode	0.001–10^3 ng mL^{-1}	–	[106]
cTnI and CRP	Serum	Electrochemical	Faradaic, poly (dimethylsiloxane)–gold nanoparticle composite microreactors	–	0.01 ng mL^{-1} and 0.5 ng mL^{-1}	[107]
Lipoprotein-associated phospholipase A (2)	Serum	Electrochemical	Faradaic, iridium-modified carbon electrodes	0–150 UmL^{-1}	–	[108]
CRP	Buffer	Electrochemical	Impedance spectroscopy	–	1 fg mL^{-1}	[108]
CRP	Serum	Electrochemical	Impedance spectroscopy	–	100 fg mL^{-1}	[108]
CRP	Serum	Electrochemical	Impedance spectroscopy	–	1 fg mL^{-1}	[109]
BNP-(NT-pro–brain natriuretic peptide)	Serum	Electrochemical	Impedance spectroscopy	–	1 ag mL^{-1}	[109]

high and not competitive for home users. The systems also require larger sample of blood than that used in the glucose biosensor device which deter their use as over the counter tests.

Research in the area of cardiovascular disease diagnosis and the development of the optimal biosensor is still thriving and this is evident from the recently published literature. There is wide range of biosensor-based cardiovascular disease devices being developed using different sensing platforms for single or multiple biomarkers detection with serum, buffer or blood samples. Table 3 summarises the current literature for biosensor developments for cardiovascular disease detection.

7. Conclusions

Biosensor technology using biomarkers is playing a critical role in the diagnostic revolution of cardiovascular disease. Development of highly specific and sensitive biosensor platforms using well established surface chemistries and nanomaterials are important for multiple disease marker detection and the precise diagnosis of heart disease. The stability of proteins on biosensor surfaces is crucial to the feasibility of any commercialisation prospects and can make or break the business viability of any biosensor product. The storage conditions and transportation of bio reactive products also play an important role in their functionality and shelf life. Environmental factors like humidity, temperature, and air exposure all offer potential obstacles in the functionality of biomaterials. The use of self-assembled monolayers (SAMs) has been demonstrated to have a positive impact on the stabilisation of the immobilised proteins [110–111].

Simultaneous analysis of multiple markers with a single test using small volumes of blood sample greatly enhances the applicability of the device in disease stage quantification with minimised diagnostic expenses. This is because most clinical results from cardiac markers are more useful if they are part of a panel of several markers providing several a set of results from a single sample. This helps the doctors to give a more accurate diagnosis as well as lowering turnaround times and maximising output from the samples. Combination of current technologies such as microfluidics, proteomics, polymer sciences with biomarker discovery and biosensor development can also provide miniaturised, easy-to-use, reliable and cost-effective point of care sensing tools. Moreover, the integration of the immunosensors to a micro fluidic device capable of controlling sample and substrate movement will progress the work further towards it becoming a commercial product.

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