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Clinical Biochemistry Markers in Early Detection of Cardiovascular Diseases

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Annotation: Despite concerted efforts to prevent and reduce cardiovascular disease (CVD), CVD remains a leading cause of morbidity and mortality worldwide. In 2019, 697 000 people died of heart disease in the United States alone. The evidence base for controlling traditional CVD risk factors (hypertension, hypercholesterolemia, diabetes, lifestyle modification, and smoking) is well established, but significant research has been directed to studying additional tools, such as novel biomarkers and imaging techniques. Cardiovascular disease risk assessment using comprehensive biomarker analysis holds promise as a more effective approach for stratifying those at high risk for CVD. Atherosclerotic cardiovascular disease (ASCVD) accounts for a large proportion of the CVD burden, characterized by lipid accumulation in the arterial wall, inflammatory response, oxidative stress, and endothelial dysfunction. As a result, lipoproteins, inflammatory biomarkers, oxidative biomarkers, and endothelial dysfunction biomarkers have been proposed as additional tools to predict CVD. Hormonal imbalance

biomarkers, as well as genetic- or kidney-derived biomarkers, have also been proposed. Aside from traditional and novel CVD risk factors, additional biomarkers have also been investigated, including cardiac and digestive biomarkers. A recent umbrella review identified 408 unique biomarkers but highlighted the lack of consensus about which additional biomarkers to use in clinical practice. Populations of interest were heterogeneous in terms of age, sex, and CVD risk assessment. Many studies have assessed the potential of various biomarkers to predict CVD and mortality, but most have focused on individuals known to be at high risk for CVD, such as those with a prior CVD history or with symptomatic coronary/intermittent carotid artery territory or peripheral artery disease. Therefore, this systematic review and meta-analysis aimed to assess and summarize the current evidence on the ability of advanced or novel lipoproteins/biomarkers to predict incident CVD among middle-aged adults without a prior CVD history or symptoms, with particular focus on the following issues: 1) novel advanced protocols and assays.

1. Introduction

Cardiovascular diseases (CVDs) are ranked among the preeminent causes of morbidity and mortality across the globe, with an alarming upsurge in their prevalence. The projections indicate that CVDs will remain the primary killer disease worldwide by the end of this decade. Over the years it expanded its scope to focus on heart failure, cardiomyopathy, arrhythmias, valvular heart disease, and heart surgery. The spectrum of CVD includes a broad range of major clinical heart and circulatory disease conditions such as ischemic heart disease, heart failure, cerebrovascular disease, peripheral artery disease, and congenital heart disease. This spectrum of diseases can be evaluated fundamentally using hemodynamic, electrocardiographic, chemical, microscopic, macroscopic imagery, and genetic studies. There is currently a great burden on healthcare systems due to the rising threat of CVDs.

The prevention of CVD demands maintaining a proper lifestyle and monitoring of various risk factors such as age, gender, family history, diabetes, smoking habit, hypertension, and dyslipidemia. An ideal biomarker is expected to be highly sensitive, specific, easily measurable, usable in point-of-care, low cost, easy to read and interpret, reproducible among patients, and a good assay platform. Moreover, this biomarker could preferably be a protein or lipid or small molecules in the body fluids, as these can accurately divulge abnormality in a pathological state. CVDs remain an intimidating challenge to identify a suitable biomarker in readily accessible body fluids such as blood, saliva, and sweat. Body fluid biomarkers have high potential not only in further evaluation of disease severity based on quantitative measurement but also in improving the accuracy of disease diagnosis and prognosis. Majority of the existing clinical biochemistry

markers for CVDs are molecular blood markers such as creatine kinases (CKs), B-type natriuretic peptide (BNP), cardiac troponins (cTns), homocysteine (Hcy), and Myeloperoxidase (MPO). There are two approaches for biomarker detection: knowledge-based detection and unbiased detection.

2. Overview of Cardiovascular Diseases

Cardiovascular diseases (CVDs) are defined as diseases that affect the heart and blood vessels. They include hypertension, coronary artery disease, heart attack, arrhythmia, congenital heart disease, heart valve disease, cardiomyopathies, heart failure, peripheral arterial disease, and vascular diseases. CVDs affect more than 32% of adults for which it is considered the leading cause of morbidity and mortality worldwide, producing immense health and economic burdens. It results in a humongous healthcare burden costing hundreds of billions of dollars in direct and indirect costs. The global annual cost of CVDs is estimated to burden the world economy by US$ 1 trillion [2]. Prevention of CVD demands maintaining a proper lifestyle and monitoring risk factors. A proper diet containing sufficient vitamins, essential fatty acids, and antioxidants for preventing atherosclerosis, together with avoiding alcohol, smoking, stress, chronic diseases, and regular workouts should collectively aim at both primary and secondary prevention (e.g., recognizing at-risk individuals and monitoring progression). Prior to recognition of cardiac biomarkers detection, CVDs characterized by heart failure, ischemia, or injury were diagnosed or suspected based on clinical history, physical examination, and electrocardiogram (ECG) variation. On arrival to chest pain (CP) units, standard serial ECG monitoring is acquired to detect the presence of ST-segment elevation myocardial infarction (STEMI) and urgent coronary angiography (CA) is performed. In those with non-ST elevation acute coronary syndrome (NSE-ACS), further risk stratification is required to identify individuals at high risk for significant underlying CAD. The elevated flow load on left chambers and vessels from stress testing in conjunction with an abnormal late enhancement (LE) study in graded exercise myocardial perfusion imaging is used to help stratify interim risk in otherwise at low-risk individuals with normal troponin. In highly specialized centers, cardiac magnetic resonance (CMR) imaging probe is employed to inspect markers of ischemic cascade with greater accuracy. In the absence of cardiac biomarkers, a high risk of CAD is classified as men aged 45 years or older, women aged 55 years or older, or patients having a family history of premature CVDs (before age 55 years in male and 65 years in female relatives). Risk factors of CVD comprise elevated levels of low-density lipoprotein (LDL) cholesterol, triglycerides, or lipoprotein(a), hypertension, diabetes, smoking, sedentary lifestyle, or systemic lupus erythematosus in women. Further risk stratification may be performed by measuring ankle-brachial index, coronary artery calcium (CAC), or cardiac computed tomographic (CT) angiography.

3. Importance of Early Detection

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality worldwide. CVD refers to disorders of the heart and blood vessels ([2]). The prevention of CVD demands maintaining a proper lifestyle and monitoring of risk factors. Continuing and past physical inactivity are major modifiable risk factors for CVD. Hypertension, dyslipidemia, and diabetes are other foremost risk monitoring parameters for CVD. Non-modifiable risk factors include age, gender, ethnicity, and genetic background. The detection of a suitable biomarker in body fluids has high potential in further evaluation of disease severity and hence can improve the accuracy of CVD diagnosis and in prognosis, therapy planning, and follow-up.

Globally, 3.8 million deaths occur from CVD each year, mainly from heart attack, stroke, or congestive heart failure. The early diagnosis of CVD has a pivotal role in the patient’s health. Besides effort and lifestyle change, unknown heart problems can be detected by regular health check-ups at clinics. Routine check-ups include measuring anthropometric parameters, lipid profiling, blood sugar testing, electrocardiogram (ECG), and echocardiography. It is tedious for patients to undergo these tests routinely, especially ECG recording, which require skilled

professionals and costly equipment. Hence, there is a pressing need in both the clinical sector and general society for a simple, early detection strategy for the live samples in blood, serum, and urine to assess the possibility and availability of CVD induced biomarker molecules.

4. Clinical Biochemistry: An Overview

Cardiovascular diseases (CVDs) are a group of life-threatening diseases, including a variety of heart diseases, congenital heart defects, hypertension, arrhythmia, Harvery disease, coronary artery disease, atherosclerosis, cardiomyopathy, valvular heart diseases, pericarditis, and heart failure. CVDs are the number 1 killer disease globally, considered an epidemic and pandemic like COVID-19. CVDs produce an immense health burden and devastating cost in the economy. In the USA, the total cost for CVDs in 2018 is projected to be $204 billion, while the healthcare burden in Europe due to heart disorders is costing 210 billion euros annually. CVDs are not only a problem in developed countries but also an emerging threat in developing countries. India is regarded as the diabetic capital of the world, leading to a rapid rise in CVDs within a short time. The World Health Organization (WHO) and national health agencies worldwide are screening for risk factors for CVD and initiating preventive measures [2].

To prevent CVDs, a proper lifestyle along with regular monitoring of risk factors like high blood pressure, inflammation, diabetes, and other lifelong risk exposures is required. Early and accurate diagnosis of CVDs can prevent disease progression and death. Hence, the early accurate diagnosis of CVDs has a pivotal role in patients' health. The primary diagnosis of acute coronary syndrome (ACS) relies upon the clinical history, physical examination, explorative ECG variation, and informative cardiac biomarker tests. The diagnosis of possible ACS patients may be confirmed using echocardiogram, treadmill test, and coronary angiography. Essential cardiac biomarker testing is highly valuable for the speedy triage of possible ACS patients, which can be easily conducted in any labs within a few hours.

Detection of suitable biomarkers in the body fluids has high potential for evaluating the disease severity and also has the potential to improve the CVD diagnosis accuracy and prognosis. Clinical biochemistry markers are online simulators providing lab tests and simulated results. A biomarker (derived from biological marker) is defined as "any measurement that observe or reflect an interaction that take place in a biological system and a potential hazard". An ideal biomarker to detect CVDs and AE-CVDs is expected to be highly sensitive, specific, applicable cost-effectively, and a novel biomarker should be taken for validation based on sensitivity, accuracy, reproducibility, ease of use by clinicians, and patient acceptance. An effective biomarker detection technique should be rapid, more affordable without the need for expensive instruments, and patient-friendly along with detection at pM level. There are two approaches for biomarker detection, knowledge-based detection and unbiased detection. Knowledge-based detection involves understanding biological processes to look for suitable targets and improving the assay methods. Unbiased detection is the applying of existing technologies for comprehensive characterisation of biomolecular profile such as proteomic, lipidomic, metabolomic, genetic, and epigenetic.

5. Key Biochemical Markers

In addition to traditional risk factors, circulating markers of cardiovascular disease (CVD) are ripe for evaluation; numerous proposed markers of CVD have been previously reviewed. Atherosclerotic CVD is thought to be characterized by a multifaceted process that involves lipid accumulation, inflammation, oxidative stress, and endothelial dysfunction. These processes have given rise to putative circulating biomarkers of CVD that might be used for detection. The proposed lipoprotein markers are lipoprotein (a) [Lp(a)], ApoB/ApoA ratio, and LDL/HDL ratio. Inflammatory biomarkers include hs-CRP, homocysteine, and suPAR. Proposed oxidative biomarkers include myeloperoxidase and ROS. Additionally, proposed endothelial dysfunction biomarkers are pentraxin-3, asymmetrical dimethylarginine (ADMA), and angiopoietin. Cardiac biomarkers of CVD that have undergone scrutiny include the troponins, most notably hsTn, and

the natriuretic peptides, particularly NT pro-BNP. Furthermore, digestive biomarkers such as GGT, alkaline phosphatase, AST, and ALT have been evaluated concerning CVD risk. Most studies have examined the predictive power of these biomarkers among those at high risk for CVD; much less attention has been given to guidance on the predictive ability of these biomarkers in asymptomatic, middle-aged individuals. Thus, there is an urgent need to determine the relationship between incidence of CVD events and traditional risk factors in the middle aged, as well as novel CVD biomarkers. Effective characterization of the relationship between these risk factors and CVD events in the middle aged might identify currently undetected individuals who are at elevated risk for CVD events but who would not be identified using standard practices [1]. Obstructive coronary artery disease is caused by the atherosclerosis. In the accumulation of plaque, inflammation, and neovascularization, hyperplasia of smooth muscle cells and structural modifications of extracellular contracts take place. As coronary artery stenosis (CAS) worsens, patients may have ischemic cardiomyopathy. Increased metabolic demand and a reduced supply of perfusion are both involved in the ischemic mechanisms. Current clinical practice guidelines recommend non-invasive and invasive examinations. Although, the non-invasive studies have been developed highly sensitive and specific for the detection of CAS, the clinical use still has serious limitations: they are relatively expensive, involve radiological exposure and may cause allergic reactions and risks of nephrotoxicity. Therefore, new non-invasive methods with high performance for early detection of CAS are urgently required by epidemiological characteristics of CHD and progression eastward [3].

5.1. Lipid Profile

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality worldwide. CVD may be less likely to occur if preventive strategies are implemented in at-risk populations. Vascular diseases' initiation and progression require modification of biological or environmental risk factors, including the lipid profile. The lipid profile consists of different measurements, including cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides, and high-density lipoprotein (HDL) cholesterol. Restricting lifestyle or using lipid-lowering medications lowers the risk of coronary artery disease and vascular deaths. Identifying risk factors is vital in determining revascularization in CAD and most heart diseases. Measurement of the lipid profile is instrumental in cardiovascular risk stratification [4].

Epidemiological studies show that elevation in total cholesterol is directly associated with increased mortality. The American College of Cardiology/American Heart Association, National Cholesterol Education Programme Adult Treatment Panel (NCEP ATP-III), and European guidelines on CVD prevention recommend screening for lipids beginning at age 20 years, every 5 years for cardiovascular risk assessment, and annually for individuals with other risk factors (e.g., diabetes, hypertension, smoking, obesity). Lipid measurement is a need in the outpatient clinic as well as in the emergency department. Measurement of the lipid profile is indicated upon presenting with chest pain, but due to the prevalence of dyslipidemia, taking a full lipid profile and risk stratification in all patients will not only identify preventable causes but also reduce CVD burden from the country. Obesity, diabetes, hypertension, smoking, and family history of CAD should also trigger the clinician to order a lipid profile. However, it is difficult to diagnose dyslipidemia without serum sampling in the emergency setting.

Assessment of the lipid profile requires blood sampling and separation of serum. Currently, these tests are carried out in laboratories requiring techniques that are accurate but time-consuming. In developing countries like India, this time may extend to days before results are available. Point-of-care tests can practically be used at or near the site of patient care for target analytes that may help in cardiovascular disease risk stratification and therapeutic monitoring. A variety of point-of-care testing compatible portable meter devices have come up where blood is applied to test strips and results are shown on the meter screen. Such testing options include handling of whole blood, simple and rapid analysis, and need minimal intervention. These analyze samples at a very low volume and time and require minimal maintenance. The lipid profile is one of the most

analyzed parameters in point-of-care testing.

5.2. High-Sensitivity C-Reactive Protein (hs-CRP)

The C-reactive protein (CRP) is a fundamental indicator of inflammation. It is commonly synthesized in the liver, and its elevation occurs secondary to systemic inflammation, especially during the acute phase of inflammatory reactions. The concentrations of CRP in blood do not exceed 10 mg/L, but they significantly increase in responses to inflammatory factors, sometimes exceeding 500 mg/L. The available methods for CRP measurement are immunoturbidimetric assays and immunoassays utilizing polyclonal or monoclonal antibodies against CRP. The CRP molecule belongs to pentraxin family proteins, whose members share conserved structures and are involved in various physiological functions. CRP is composed of five identical non-glycosylated subunits and is a soluble 115 kDa globular protein. Practically, CRP may be interpreted as a biomarker of ongoing inflammation regardless of its origin, but it is not specific to a given disease or mechanism of inflammation.

Nonetheless, as a pentamer protein, it cannot easily cross the endothelium and vascular basement membrane. It cannot bind to active immune mechanisms in the vascular bed; therefore, it is solely a marker presenting a disease or a pathological process in its early phase. Therefore, a high level of CRP indicates the presence of an inflammatory process. When concerns the chronic inflammatory process (e.g., atherosclerosis), there is a gradual degradation of pentameric (pCRP) isoform to monomeric form (i.e., mCRP) in the local environment. The consecutive modification of mCRP isoforms that provide calcium sites on the protein structure mediates various pleotropic pro-inflammatory and pro-coagulant functions. It binds to damaged endothelium, activating platelet aggregation. It binds to surface phospholipids, eliminating apoptotic cells and aggregates sedimented by the complement, initiating innate immunity mechanism. mCRP can also bias the Th response, especially in chronic inflammation conditions, and induce a shift towards the pro-inflammatory response.

Others characterized that the presence of risoforms of CRP complements the role of the molecule in the systemic inflammatory process and plaque destabilization. Test measurement is simple, rapid, and cheap, and hsCRP is stable, while basal hsCRP levels depend on many factors such as age, sex, obesity, renal and liver function, and articular diseases. These factors do not exclude hsCRP determination in people with cardiovascular risk, provided they obtain instructions on possible physiological factors affecting the result. Observational cohort studies found no clear evidence of hsCRP level associated with cardiovascular risk in patients with established coronary artery disease or diabetes mellitus and in those regarded as being at risk of atherosclerosis. Expansion assessment of hsCRP applied by the American Heart Association and the Centers for Disease Control and Prevention. In average-risk people, in the absence of symptomatic CVD and inflammatory conditions, hsCRP assessment should be considered in primary prevention at 40 years and older. If the result is <1 mg/L, the risk is classified as low, and risk assessment can be repeated every 5 years. Blood sample collection for this assessment should follow standard laboratory criteria.

5.3. Troponins

Aim of this study was to evaluate the predictive value of cardiac troponin I and T for subsequent death in end-stage renal disease (ESRD) patients on hemodialysis (HD). The level of cardiac troponin I (cTnI) was determined in 102 patients on purposeful dialysis. Patient data were collected using questionnaires and from medical records. The study population which had a mean age of 61 years predominantly consisted of males (74%). The troponin I cut-off limit of 0.04 ng/mL was determined as a minimum value for cTnI. Sixty-nine patients had undetectable cTnI values while in 33 patients cTnI was detectable. In HD patients with detectable cTnI values, the risk of mortality was 8.5% in 0-12 months and it increased to 52.9% in 25-35 months compared to 1.4% risk in those with undetectable cTnI. Troponin I levels on HD patients was higher than controls but with no statistically significant difference. There is a significant

relationship between death risk and cTnI levels, independent of patient's age, blood pressure, ATS score and clinical symptoms [5]. To evaluate the use of the highly sensitive Centaur TnI-ultra assay for detection of myocardial infarction (MI) and adverse events in patients presenting with symptoms suggestive of acute coronary syndrome (ACS). A total of 207 patients (70% male, mean 59 years old) were enrolled with 39 acute MIs and 68 non-MIs (NMI). Adverse events were monitored at 30 days for subjects with non-MI. Hospital admission-sensitive TnI was higher in MI group than NMI-group (5.7 vs. 0.021 ng/ml). Supportive diagnostic cut-off >0.027 ng/ml ruled-in A Cox regression analysis identified two independent predictors of 30-day adverse events. Patients presenting with a diagnosis of unstable angina (UA) (n=49) had a significant 2.651 times greater hazard (HR) of 30-day adverse events relative to patients with non-cardiac chest pain (n=56) ($p<0.035$), after accounting for hsTnI-sensitivity test results of >3.80 ng/L. The results showed that Centaur hsTnI-ultra assays can rapidly and accurately detect ACS-induced troponin elevation within and outside the diagnosis of MI.

5.4. B-type Natriuretic Peptide (BNP)

B-type Natriuretic Peptide (BNP) emerges as an important biomarker to diagnose heart failure (HF). Knowledge on the possible biological basis and overview of heart failure is presented as a basis for the broad clinical picture of this multifactorial disease. Acute heart failure (AHF) is defined as rapid onset or worsening of heart failure symptoms, and the new onset of heart failure signs. AHF is a growing epidemic and major cause for hospital admission worldwide. The standard diagnostic and prognostic procedures are mainly symptom-based, requiring subjective clinical acumen. Different methods have been developed for B-type natriuretic peptide (BNP) measurement and are broadly used in clinical practice. Structurally, this iconic molecule is studied by various biophysical and biochemical techniques to appreciate its remarkable blood- to heart-take molecular cascade. Emerging techniques provide sensitive platforms to monitor BNP in point-of-care settings where obsolete protocols cannot be applied. Discussion is presented on the future of BNP measurements based on results of decades of fundamental and clinical research, focusing on assays and biosensors to facilitate access to the broad community of non-specialized medical and scientific personnel in developing areas [6]. B-type natriuretic peptide (BNP) was discovered as a 32-amino-acid polypeptide and its cardiovascular effects were characterized. The poor prognosis of heart failure is related to the severity of the condition, reflecting the biological activity of the natriuretic peptides (NP) antagonistic to the renin-angiotensin-aldosterone system. Elevated levels of BNP and amino-terminal proBNP (5-766) are useful markers in diagnosing heart failure. Although not specific for heart failure, they improve risk stratification and management in acute coronary syndrome. Both circulating hormones were catalytically cleaved by the proteins neprosin and corin to obtain their biologically active forms. Considering differences in half-life, assay principles and clinical applicability, measures of BNP and proBNP complement each other in clinical practice [7].

5.5. D-dimer

D-dimer (DD) is a fibrin degradation product and is a marker of ongoing fibrinolysis. The test is not disease specific but measures thrombus formation and lysis. D-dimer is routinely ordered in evaluation of suspected venous thrombosis (VT) but its clinical utility in other conditions like myocardial infarction is still evolving. Myocardial infarction leads to thrombosis in the coronary vessel and unstable coronary plaques being the source of growth of thrombus within. D-dimer levels are found to be higher in acute coronary syndromes (ACS) than in other conditions. Plasma D-dimer elevation is associated with higher mortality in patients with conventional risk factors but has not commonly been included in risk scores. DD oxidised-III is a novel biomarker and studies have shown that it has superior prognostic value for acute coronary syndrome (ACS) patients [8].

D-dimer (DD) is an evolutionarily conserved fibrin degradation product formed from cross-link fibrin by Fibrinase initiated by the action of thrombin-signaled prothrombin activation factor

(PTA). Fibrinogen is cleaved by thrombin into fibrin monomers which polymerize upon binding with FXIIIa. These polymeric fibrin filaments are cross-linked between the 16th E-N and 13th D-K domains of adjacent gamma chains. The result is the formation of a 200nm long, 10-300nm wide structure called the clot. This tightly knit structure is loosened by the action of plasmin on the conversion of Plasminogen to Plasmin by the plasminogen activator. The cleaved D-D and D-xy cross-links are released to the plasma cuff. The D-D dimension is 200 nm in length and 5.4 nm in diameter [9].

The D-dimer level predicts prothrombotic states suggested by preliminary associations with venous thrombosis and other conditions associated with embolism and with intracardiac thrombosis and the rapid normalization of D-dimer values after cardioversion or warfarinization in patients with arterial fibrillation. It is important to note that PDD normal values vary from laboratory to laboratory. Diagnostic performance of the D-dimer test and collection and assay quality control procedures should be undertaken in specific climes. Prothrombotic state evaluation remains a challenging and fruitful endeavor. Evaluation of D-dimer in viral infection and neurodegenerative diseases is yet unstudied.

6. Role of Inflammatory Markers

Acute coronary syndrome is regarded as a major cause of morbidity and mortality worldwide. Research confirmed that a number of inflammatory markers induced in response to metabolic dysregulation are involved in the pathophysiology of coronary artery disease (CAD) and atherosclerosis. Inflammatory markers play a major role in atherogenesis and may therefore assist in the early detection of the ongoing process of vascular inflammation and the detection of vulnerable plaques [10]. In addition to classical inflammatory markers including reactive protein C (CRP), levels of markers such as IL-6, IL-1, IL-18, E-Selectine, fibrosis-gain factor-21 (FGF-21) or serum amyloid A (SAA) have recently been associated with CAD. Several state of the art ELISA assays enable rapid and sensitive detection of these markers [11].

CRP is an acute phase protein produced in response to inflammatory stimuli, and IL-6 has been identified as the key hepatic stimulating cytokine. CRP is detectable in human serum and plasma in concentrations of <0.5 g/L but may rise to >200 g/L under conditions of acute inflammation. A borderline elevation of CRP (1.0 to 2.9 mg/dL) is associated with an increased risk of CVD, and elevations of CRP >3 mg/dL indicate a high-risk category for CVD. CRP was elevated in non-ST-segment elevation acute coronary syndrome. Analysis of the CRP as a prognostic marker revealed that among patients presenting with unselected acute chest pain, there was a U-shaped relationship between CRP and 30-day mortality: low (<0.95 mg/L) and high (>3.5 mg/L) concentrations were both associated with increased mortality.

IL-6 was one of the earliest discovered members of the helical cytokine family. It was initially identified as a B-cell differentiation factor, but its role in the acute phase response and chronic systemic inflammation has attracted considerable interest. It is produced by a variety of cell types including macrophages, T-cell subsets, and endothelial cells. The hepatocyte-stimulating activities of IL-6 and other mediators of inflammation have been extensively studied. Most of the classic acute phase proteins, such as fibrinogen, haptoglobin, CRP, and serum amyloid A, are under the control of IL-6 and the other mediators of inflammation. Recent studies have shown that IL-6 trans-signaling plays a predominant role in mediating tissue factor expression in endothelial cells. Reduction of plaque IL-6 and CRP by GM-CSF antagonism significantly decreased blood monocyte accumulation and CVD progression. IL-6 is distributed throughout the arterial tree, and in humans, upstream cerebral atherosclerosis has been linked to IL-6 gene promoter SNP (-572 G/C).

6.1. Interleukins

Biomarkers of CVDs can include pro-inflammatory markers Interleukins. Coronary artery disease (CAD) is the leading cause of diseases due to severe morbidity and mortality globally in

both developed and developing settings. Atherosclerosis is a chronic inflammatory response of the arterial wall that plays an essential role in CAD pathogenesis. In the progression of atherosclerosis, pro-inflammatory cytokines play critical roles. Thus, atherosclerosis brings inflammatory defense response and induces the recruitment of different subclasses of lymphocytes to the arterial intima, resulting in an inflammatory microenvironment in which various pro-inflammatory cytokines such as interleukin-6 (IL-6) and interleukin-8 (IL-8) are released. Both IL-6 and IL-8 are potent pro-inflammatory cytokines implicated in the recruitment of macrophages and leukocytes to endothelial cells resulting in the perpetuation of the atherosclerotic plaque. Also, the secretion of IL-6 and IL-8 in the atherosclerotic plaque reflects the local inflammation in the plaque [12].

The release of IL-6 is crucial in the formation and propagation of the atherosclerotic lesion. In many human clinical studies, elevation of serum IL-6 was demonstrated in CAD. IL-6 was proposed as a common pro-inflammatory factor leading to traditional cardiovascular risk factors, which include overweight, dyslipidemia, hypertension, and hyperglycemia. It was reported that IL-6 levels may serve as a strong, independent predictor of the occurrence of a first acute coronary event and may significantly improve the predictive value of traditional risk factors in women over 55 years old. Results showed that serum IL-6 levels have a strong, independent correlation with the severity of liver dysfunction. Since IL-6 is believed to reflect the local inflammation in the atherosclerotic plaque, plasma levels of IL-6 have been shown to increase exponentially with plaque size. These data suggest that besides exerting as local release in the plaque, IL-6 may be released into circulation from various other sources of tissue injury [10].

6.2. Tumor Necrosis Factor-alpha (TNF-α)

Atherosclerosis can be considered an inflammatory disease as a number of ongoing inflammatory responses and immune reactions occur in the vessel wall. There is complex interactions between many inflammatory cells and infiltrated low-density lipoproteins in the arterial wall, which together constitute plaques. Plaques increase in size and complexity with time. Eventually, lesions rupture and cause thrombus formation and acute events. Atherosclerotic lesions are filled with immune cells that coordinate and affect inflammatory responses. It is believed that the inflammatory responses related to atherosclerosis are caused by local inflammation in atherosclerotic tissue. Likewise, major cardiovascular events (MCE) have also been related to a local inflammatory process. Known markers of inflammation are tumor necrosis factor-α (TNF) and interleukin-6 (IL-6). Together, TNF and IL-1 stimulate the production of IL-6 by smooth muscle cells. TNF and IL-6 are secreted by a variety of cell types and are most elevated at the site of plaques. Blood levels of TNF and IL-6 are low in non-atherosclerotic individuals. IL-6 is the main hepatic stimulus for crp production in humans [13]. 60-70% of MCE occurs in these levels. Sausages with TNF and IL-6 could be included to increase the sensitivity of crp detection and identification of non-stenotic lesions.

Major cardiovascular events (MCE) are the most common cause of death and do not always correlate with stenosis severity. There is a need of a biomarker which would allow early identification of most patients at risk as well as monitoring the effectiveness of treatments. Inflammation is also involved in CAD. Early responses to atherogenic diet include an increase in the number of foam cells, migration of monocytes and recruitment of inflammatory factors. These early changes lead to an increase in further inflammatory cytokines and chemokines. Among them the role of TNF and IL-6 is critical. TNF is a pro-inflammatory cytokine secreted primarily by macrophages and endothelial cells during an inflammatory response [14]. Other cells can also release TNF, including lymphoid cells, mast cells, cardiac myocytes, adipose tissue, fibroblasts, and neuronal tissue. The physiological plasma concentration of TNF is smaller than 1 pg/mL. However, under pathological conditions, a substantial increase, ranging from several to hundreds of pg/mL depending on the patient, type and stage of pathology, was documented. TNF is also an important pro-inflammatory cytokine for MCE. High plasma levels of TNF are known to be toxic (producing vascular dysfunction) and could lead to vascular

dysfunction.

7. Oxidative Stress Markers

With the consequence of arterial injury, either from shear stress or through the action of circulating factors exposed to atherogenic levels, an ever-increasing wave of circulating monocytes migrate into the arterial walls. These cells proliferate and differentiate into macrophages and foam cells, consuming low-density lipoprotein (LDL). In the early stages of this thickening, no clinical feature is identifiable, but concerning processes such as E- and P-selectin expression and matrix metalloproteinase (MMP) production can occur. Such asymptomatic vascular disease, similar to many other pathological states, nevertheless requires patients to undergo a treatment regime to prevent later complications [15]. Currently available clinical modalities fall short of either being sufficiently cost-effective mass screening options, or being sensitive and specific enough to identify early disease stages before a critical threshold has been crossed. Although a relentless incidence of cardiovascular disease (CVD) results in years lived with disability, many do not feel the effects. New modes of fault finding in early CVD are thus required. The mechanism(s) involved in the transition from early-stage endothelial dysfunction to established CVD is complex and incompletely understood; redox dysregulations and subsequent oxidative stress have been proposed as key mediators. In this light, anti-oxidative supplementation is a burgeoning mode of both disease treatment and prevention. This strategy poses many challenges due to the sub-systems and interfaces at which any anti-oxidative treatment can be targeted. Biomarker discovery efforts seek to identify redox state alterations caused by oxidative-eustatic imbalances, which are evident in the circulation as well as in target tissues [16]. Thus, biomarkers summarise dynamic biochemical states, indicating disease risk far earlier than time-integrated measures such as E-selectins or size-distributed arterial plaque assessment. Identified candidate screening biomarkers include oxidised low-density lipoprotein (oxLDL), myeloperoxidase (MPO) and nitric oxide. These may also serve screening criteria for entry into clinical trials testing disease-modifying therapy. This review will detail innovative enzyme assays suitable for biomarker discovery in addition to active species-directed imaging, before discussing biomarker interrogation through a combination of clinical, imaging, histological, genetic and statistical methods to ascertain current oxidative state over different time integrations.

7.1. Malondialdehyde (MDA)

Malondialdehyde (MDA), a three carbon dialdehyde, is one of the most well-studied lipoperoxidation products. It is a major product of peroxidation of polyunsaturated fatty acids that is now well recognized as a suitable marker for oxidative stress in both basic and applied research scenarios. MDA has been measured in many matrices including biological samples such as plasma, breath, exhaled breath condensate, urine and tissue. Also in the field of culinary research MDA concentration serves as an important food quality control marker. Its unique double aldehyde reactivity renders MDA a very attractive and promising biomarker, but this also challenges its measurement in complex matrices because MDA can react with numerous nucleophiles.

The reaction of MDA with amino acids, proteins and biopolymers gives rise to classical HPLC-UV detectable MDA-protein adducts. Important MDA adducts, which can be measured in tissues and biological fluids, are MDA-LysA adducts. ELISAs for MDA-protein adducts based on polyclonal and monoclonal antibodies are available. A maximum binding competition immunoassay has been developed to measure MDA-LysA adducts. This assay uses two labels in the direct binding competition assay format, allowing the use of a monoclonal antibody against MDA. The assay was validated in human plasma, but no validation protocol for mouse tissues was available. Commercial kits based on immunoassays, LC-UV and enzymatic HPLC methods have been extensively reviewed and evaluated. As real-time sensitive methods for measuring MDA in plasma and brain tissue homogenates a LC-UV/MS and a close to real-time LC/MS

assay are reported, but these have not been commercially available. Optical waveguide lightmode spectroscopy and differential scanning calorimetry have been employed to analyze lipid peroxidation and MDA levels. Gene manipulation has been used to study the metabolic formation, handling and action of MDA in rodents.

7.2. Glutathione

The peptide GSH is recognized as a potent antioxidant in cellular physiology, playing a central role in maintaining appropriate cellular bioenergetics and eliminating reactive oxygen species (ROS) by directly scavenging hydroxyl radicals and singlet oxygen, as well as providing cysteine for the synthesis of other antioxidants. The evolutionarily developmental history of GSH as a metabolic molecule is unique as it is only produced in combination of three amino-acids. Given the key role of GSH as a protective antioxidant, it was expected that GSH would contribute to amelioration of the health effects of environmental chemical exposures. However, not only is excessive intracellular GSH accumulations hazardous because of the potent nucleophilic properties of GSH, GSH efflux from the cell can also take place with consequent adverse health consequences. GSH deficiency is suggested to foster the development of cardiovascular disease (CVD) through both toxicological and epidemiological lines of evidence. First, industrial chemical exposures are documented to selectively induce oxidative damage, loss of GSH, inflammation, lesion development, and cardiovascular biology perturbations. CVD aggravates health challenges imposed by the pandemic, which is hypothesized to contribute to clinical outcomes through an initiation phase involving environmental exposures contributing to low GSH levels. Unlike wholesale inflammatory elevation of cytokines in early infection which is expected to exacerbate later stages of the disease, loss of GSH is suggested to foster viral entry and amplification of viral transcription and translation through proteolytic induction of furin and cysteine proteases in exosomes, and elevated superoxide anion production and consequent oxidative damage. Both extracellular and intracellular GSH loss have mostly been documented in toxicological and epidemiological studies, respectively. GSH-efflux may take place via GSH-transporters at the plasma membrane such as the classical multi-drug-resistance proteins or the multicomponent system which transports GSH-cystine dyamer. It may be speculated that efflux of GSH from the cytosol into the extracellular space may result in the formation of the antioxidant pool on one hand, while on the other hand, GSH is rapidly oxidized to glutathione disulfide or its punctiform oxidation products that have deleterious cytocidal properties. While how extracellular GSH levels are associated with CVD remains unknown, epidemiological studies find GSH efflux to correlate with high energy consumption supported by large geological events.

8. Genetic Markers in Cardiovascular Risk

CARDIOVASCULAR DISEASES (CVD) ATTRIBUE HIGH MORTALITY AND MORBIDITY RATES IN DEVELOPED COUNTRIES, WHEREAS HEART ATTACKS FOLLOWED BY CARDIOVASCULAR SYSTEM FAILURE STILL COMPRISE THE LEADING CAUSE OF DEATH IN EMERGING AND DEVELOPING ECONOMIES. ISOENZYMES OF CREATINE KINASE, MYOCARDIAL TROPONINS T AND I, BRAIN NATRIURETIC PEPTIDE, AND N-TERMINAL PRO-HORMONE BRAIN NTREOTELINJETIDE PRO-HORMONE BRAIN WERE FOUND SIGNIFICANT IN HUMAN STUDIES.

Despite the fact that established biomarkers of cardiovascular disease (CVD) allow clinical diagnosis of heart attacks and heart failure, there has been a significant push toward the discovery of new biomarkers using strategies such as omics. However, these efforts have not resulted in any new widely adopted clinical biomarker tests. During the past decade, studies of both genetically predetermined predisposition to CVD and pre-existing ‘end-stage’ CVD using novel biomarker discovery strategies such as proteomics, metabolomics, and transcriptomics have culminated in the identification of numerous CVD biomarkers allowing clinical detection

of heart attacks, heart failure, and cardiac arrhythmias. The successful new biomarker approaches have relied heavily on population studies. In well-designed population studies that detail not only existing risk factors, but also the multi-factorial threshold cutoff scores for these risk factors in the development of interventions by which the risk could be averted, focus should be shifted in order to augment dramatic gains in further clinical diagnostic tests complementary to existing tests in concert [17].

Coronary artery disease (CAD) and myocardial infarction (MI) are recognized as leading causes of mortality in developed countries. These vascular phenotypes, along with other cardiovascular diseases, are also strongly related to genetic background. It has been estimated that 40–60% of susceptibility to CAD can be attributed to genetic factors. As genome-wide association studies become more common, it has become evident that contemporary theories on the genetic architecture of disease must be adjusted to include variants with small effects that nevertheless account for a significant fraction of CAD risk [18]. To date, replication has been quite successful for several SNPs discovered in diverse cohorts. However, because genetic testing is recorded only for some variants, there is still a quest for comprehensive genetic tests providing CVD protection information and allowing cost-effective mass clinical applications. Comprehensive and non-invasive tests of genetic markers for inherited and sporadic primary myocardial infarction, coronary artery spasm, and coronary artery disease would be beneficial to detect the genetic predisposition to CAD at an early stage.

8.1. Single Nucleotide Polymorphisms (SNPs)

Single nucleotide polymorphisms (SNPs) are the most abundant form of genetic variation in humans, and provide the basis of wide-ranging genomic association studies to evaluate population genetic, evolutionary, and disease association hypotheses. These studies are feasible because of the availability of highly scalable and inexpensive genotyping technology. Limiting the SNPs that are densely distributed across the genome to those that are in, or near, genes can potentially reveal functional variations and regulatory mechanisms underlying phenotypic variation [19]. Additionally, understanding the molecular structures of genes and their regulatory elements will shed light on the core mechanisms by which these variations affect phenotypes. From this perspective, here is presented a systematic analysis of SNPs with an emphasis on their functional annotation in the context of coronary heart disease and related traits.

Information on 2,437 genes containing 17,576 SNPs typed in three large studies is presented, and a substantial proportion of SNPs that are in, or near, the corresponding genes is emphasized. Association with lipoprotein(a), triglyceride, low-density lipoprotein cholesterol, and risk of coronary heart disease or myocardial infarction is attributed in part to variation in the genes albumin, CCL14, CCL19, and WNT2. In addition to drawing attention to potentially protective SNPs relevant to coronary heart disease, evidence that variation in the production of apolipoprotein B-48 may differentially affect lipoprotein(a) levels is presented [20].

More generally, a substantial proportion of SNPs are shown to localize to transcription factor-binding sites annotated in whole-genome maps of regulatory elements, providing a framework for investigating the mechanism of CVD-associated SNPs. Various sterols, including cholesterol, stimulate autophagy by eliciting the oxidation of Akt at a conserved site in its pleckstrin homology domain, and thereby signal the recruitment of the autophagic machinery to endosomes that are also sites of cholesterol accumulation. This pathway is likely to be implicated in variations in CVD-associated THBS1 expression, and provides a rationale for a substantial number of SNPs identified from the trans-acting associations.

8.2. Gene Expression Profiles

Coronary artery disease (CAD) is the main cause of death in adults in western countries. The morbidity of CAD has increased gradually in China, and most subjects do not have symptoms before a cardiac event. Early recognition of the risk of atherosclerosis is important for prevention

of cardiac disease. Nowadays, little information is available on early gene expression in subjects at high risk for CAD but who do not exhibit symptoms. Evidence suggests that many acute coronary syndromes are caused by plaque disruption and thrombosis rather than stenosis severity. The composition and configuration of atherosclerotic plaques are the main factors for plaque stability. Hence, a reliable and non-invasive detection method is urgently needed in subjects who have risk factors for CAD. Peripheral blood is an accessible source compared with other tissues. Moreover, blood contains platelets, neutrophils and circulating leukocytes that are associated with processes in cardiovascular diseases (CVDs). Thus, gene expression profiling in peripheral blood could provide information on early risk factors for CVDs.

Inflammation has a key role in the pathogenesis of atherosclerosis. Multiple cytokines such as interleukin IL1B, IL6, IL8, IL10, IFNG, MCP-1, TNFalpha, MCSF, and ICAM1 are present at inflammation sites, each of which participate in the processes of atherosclerotic plaques. TNFalpha, IL1B and IFNG promote the instability or disruption of atherosclerotic plaques. Other gene expression panels such as HMOX1, VWF, ID2, MTHFR and SELL are of great interest to researchers. Almost all studies have focused on individual gene expression, which cannot provide the profile information of all inflammation-related cytokines. Technologies such as cDNA microarrays can analyze thousands of transcripts in one chip. Beckman Coulter developed the GenomeLab GeXP Genetic Analysis System that can analyze up to 35 genes in a single reaction. We carried out analytical validation of the GeXP system in blood samples. We were interested in establishing a platform for 15 CVD-related gene expression profiles and 2 housekeeping genes [21].

9. Technological Advances in Marker Detection

CVD is the leading cause of morbidity and mortality worldwide, despite advancements in healthcare. In India, nearly 33% of mortalities are related to CVD. Conventional methods for early detection of CVDs are invasive, complicated and time consuming. Moreover, false positive and false negative results are a major concern. These limitations necessitate the identification of simple, quick and inexpensive point-of-care tests or devices which can be used for mass screening of the population. Combination of digital health and artificial intelligence is a promising approach, which can revolutionize CVDs early detection, triaging and thus treatment. Estimation of a combination of multi-biomarkers is expected to improve the early detection of CVDs and accuracy of clinical diagnosis. Optical biosensors are simple, easy to use, cost effective and portable, suitable for use by non-specialists. This review presents an overview on recent advancements in optical biosensing technologies and providing insights into transducing approaches for detection of cardiovascular biomarkers in body fluids with enhanced sensitivity, specificity and multifactorial capabilities.

Cardiovascular diseases are one of the major causes of death worldwide. Traditional detection of cardiovascular diseases through clinical biochemistry markers is done using blood tests. Although these tests are performed widely, they have several limitations. Very few types of markers are assessed in routine tests because multiple tests require complex procedures and efforts to reduce false positive/negative results. Detection of multiple types of markers simultaneously would require semi-automated detection systems, which need skilled technicians and are costly. Hence, a portable and user-friendly biosensor for the accurate and fast detection of at least two types of clinically relevant markers simultaneously is highly desirable. Multi-marker detection combined with pattern analysis would be more effective. The advancement in chromatographic techniques, mass spectrometry, capillary electrophoresis, and nano material-based detection methods has opened the doors for the detection of multiple biomarkers on a single platform. Most of these techniques are still laboratory-based, costly, and complicated to operate; hence user-friendly and low-cost systems for simultaneous detection of multiple biomarkers with high sensitivity and specificity are urgently needed. This major breakthrough is crucial for effective operation and diagnosis adaptable in hospitals and clinics.

9.1. Mass Spectrometry

Cardiovascular disease remains the world's leading cause of mortality and morbidity. The recent and rapid improvements in non-invasive diagnostic imaging and imaging technologies have resulted in an increased understanding of the clinical manifestations and underlying physiological mechanisms of heart disease. However, with all of the advances in imaging modalities and techniques, there remains a need for effective cardiovascular biomarkers [22]. Cardiovascular biomarkers are secreted proteins whose blood levels vary as a consequence of heart damage, lesions, left ventricle wall tension, or derangement in myocardial metabolism.

Biomarkers are needed for the diagnosis, prognosis, therapeutic monitoring, and risk stratification of acute injury and chronic disease. Thus, there is a need for new and improved cardiac biomarkers for the early detection of malignant cardiac hypertrophy, left ventricular dilation, heart failure, and the assessment of efficacy of drug therapy in preclinical animal models. The enormous development of high-resolution mass spectrometry is encouraging a second gold age of cardiac biomarker discovery. Biopanning of a new class of proteomic mass spectrometry tags is allowing comprehensive serum proteome characterization/monitoring. Tag-assisted mass spectrometry is allowing both high-throughput and high-sensitivity monitoring of the potential biomarker ensembles. Such detection technologies will enhance the breadth and diagnostic capability of serum proteomics. Examples will be presented that illustrate the first tags used for cardiac biomarker discovery and examination of complement biomarkers for the early detection of cardiac cellular necrosis. Finally, some established biomarkers and the new frontiers will be demonstrated.

9.2. High-Throughput Screening

Cardiovascular diseases (CVD) are considered to be the leading cause of mortality across the globe affecting millions of people every year. CVD patients who experience a sudden heart attack qualify for urgent medical attention. The delay in getting such attention is one of the major influences on the patient condition and subsequent long-term health. This situation can be minimized with early detection or fast diagnosis of the disease. Troponin is considered the gold standard biomarker for CVD. This biomarker gets released to the blood for a prolonged duration when the heart muscle is damaged. In addition, several other cardiac injury markers also qualify for CVD diagnosis but these are used in clinical settings as side touch diagnosis or for differential diagnosis. These markers include creatinine kinase MB, myoglobin, and heart-type fatty acid binding protein. In addition to the increase in sensitivity and specificity testing methods, early detection methods based on new biomarkers are indeed a highly demanded urgent need for the health care system. A single CVD biomarker is not sufficient for effective diagnosis as it is still observed in at least one type of CVD. It is still prudent to have several markers to correlate each other type of CVD for the reliable diagnosis.

Similarly, there is also an urgent need for reliable biomarkers for new- or specific-type CVD. Proper screening would also include other and/or improved tools that are sufficiently sensitive for the proper timing of treatment. Such biomarkers are anticipated to have properties like high specificity to certain types of CVDs, present in blood samples around the time of diagnosis, short half-life compatible to early detection, low concentration into the blood stream compatible with avoidance of false positive cases etc [2]. Detection of such a biomarker required innovative ideas that have little feasibility upon current detection approaches in laboratories. Another approach that has shown promising evidence in cancer biomarker detection is multi marker detection combined with effective pattern analysis and multi marker detection utilizing different detection principles.

For some new non-invasive screening method, a novel screening biomarker like biomarker cocktail must be prepared by pooling multiple existing biomarkers from different methods. This might be highly desirable in the sense that the detection of markers representing each pathophysiological condition of the disease. Fish oil and other Omega 3 fatty acids lower

triglycerides, but elevated triglycerides still increase cardiovascular disease risk and disease burden, with a high area of unmet need. Many people who cannot tolerate a statin, or who still have event risk despite adequate statin treatment, may prefer a targeted drug that only lowers triglyceride levels with no other side effects. One way to achieve this goal would be to develop a highly sensitive and specific method for the detection of such biomarkers in body fluids. Body fluids like serum and/or saliva are ideal venues for simple tests as these are generally available for other blood tests.

10. Clinical Guidelines for Marker Utilization

It is acknowledged globally that Cardiovascular Disease (CVD) is the chief cause of death among adults of every gender, ethnic, and economic class. It is unanimously recognized that the early identification of this disease is likely to save a great number of lives, and Member States are earnestly requested to support and promote the communication of preventive information, especially to women and vulnerable individuals. The proportion of the preceding year's global budget devoted to raising awareness of CVD among those at particular risk, especially ethnic groups and poorer communities, is requested. It is appreciated that CVD is firmly on the global policy agenda.

Early diagnosis of CVD is a helpful solution to prevent it. Early diagnostic information is life-saving. National and international authorities emphasize utilizing such clinical biochemistry markers efficiently and specifically. Also, the guidelines governing the proper utilization of biomarkers exist in a nutshell but not in a comprehensive manner. Despite the existing guidelines, the clinical utilization of markers deserves proper enforcement and practice as per the guidelines, which has not yet happened in many laboratories.

For the early detection of CVD, a variety of modalities are available. Each of those modalities has its own guidelines governing the utilization of such already established markers. Nevertheless, those markers are unusable in a clinical laboratory without a proper guideline governing its use in day-to-day practice even by trained experts. As understanding the CVD is a lengthy process by itself every clinician cannot handle these complex markers. Thereby, every clinical laboratory must have its own guidelines governing markers in an easy to understand and processable format. Such guidelines might invariably lead to universal-like long-lasting standards of the already established markers for the cost-effective and efficient diagnosis of CVD in both developed and underdeveloped countries.

11. Case Studies and Clinical Trials

Participants with non-fatal coronary heart disease (CHD) (n=220) and matched controls (n=660) had an electrocardiography at baseline and during the follow-up attending the screening (n=628). Non-fatal myocardial infarction was classified by an expert panel according to the World Health Organization definition. Participants with a history of angina at baseline or with a history of non-fatal/chronic heart failure were excluded. Diagnosis of non-CHD events (n=199), additional exclusion criteria included a history of ischaemic heart disease and a congenitally stenosis. In the remaining participants quadrants defined by troponin I and BNP levels. The mean age was 53.7 and 51.2% were males. The follow-up period between baseline and event date was significantly longer in non-CHD events. The relative hazard of developing non-fatal myocardial infarction was raised when troponin I was set at ≥4 ng/L (multivariate-adjusted HR 3.87 95% CI 1.33-11.41). N-terminal pro-B-type natriuretic peptide was predictive at all thresholds examined with the association being strongest at ≥62 pmol/L (2.95 1.42-6.12). Troponin I and N-terminal pro-B-type natriuretic peptide are predictive of subsequent congestive heart failure events in the population without prior heart failure but future research is needed to explore their potential predictive value for heart failure subtypes [23].

Non-fatal cardiovascular disease (CVD) cases and matched controls were identified in a large prospective cohort study. Metabolic risk factors and biochemical markers were measured in

blood and urine samples collected at five-year intervals over the 15-20 years prior to diagnosis for cases and at similar time intervals for controls. Participants with CVD had slightly more unfavourable levels for most risk factors and biomarkers 15-20 years before diagnosis. Differences were sometimes statistically significant but more often more pronounced in the group with CVD. The majority of the differences remained statistically significant after correction for body mass index and lifestyle factors, indicating that risk factors and biomarkers contribute toward the increased risk of CVD in middle-aged subjects [24].

The study aimed to examine how levels and trajectories of metabolic risk factors and biochemical markers prior to diagnosis differ between people with and without CVD over a period of up to 15-20 years. Data of a large prospective cohort was used in which CVD cases and matched controls were identified from the general population. CVD case-control analyses, including 449 CVD cases and 1,347 matched controls, and linear mixed models were performed to estimate levels and trajectories of risk factors in the 15-20 years prior to diagnosis. Unfavourable levels of metabolic risk factors and biochemical markers are present long before the diagnosis of CVD.

12. Challenges in Marker Implementation

Cardiovascular disease (CVD) is the leading global cause of morbidity and mortality for both men and women. Approximately 17.9 million people die each year due to CVD, accounting for approximately 31% of deaths worldwide. Acute myocardial infarction (AMI), also known as heart attacks, is one of the most common deadly forms of CVD [2]. This condition happens when the blood flow to the heart is blocked either due to the clogging of the coronary artery by cholesterol or due to blood clot formation in the stenosis part. When heart muscles are injured during AMI, they release proteins called cardiac markers or biomarkers into the blood. It can take 6–12 hours for cardiac biomarkers to show elevated levels in serum after the incidence of an AMI attack due to the delay in their elevation. This time gap is a crucial influence on a disease's case and long-term health. If patients seek medical attention after this period, conventional cardiac markers will not be effective in the diagnosis of AMI and patients will miss the golden hour, which will severely impact their chances of survival.

After the advent of high-sensitivity troponin tests, conventional urine-based cardiac markers such as Myo, CK-MB, and LDH have experienced a partial decline in use. After troponin, no second gold standard biomarker has been identified and despite the efforts of researchers to develop reliable biomarkers, there is still an urgent need for reliable biomarkers that should be specific to the type of CVD, with sensitivity greater than 80%, required a less than 1-h laboratory turn-around time, required high analytical throughput, and ease of detection. Markers representing each of the pathophysiological conditions first exhibited during the progression of a disease may be desirable because the disease and the type of biomarkers they released into the blood identified for CVD would shape the accessibility of the detection methods. CVD patients may be asymptomatic or progress rapidly without premonitory symptoms. Hence early-stage multi-marker detection combined with pattern analysis will be effective as the early-stage diseases are likely to be initiated by dysfunction of CVD-associated proteins. Available detection methods include the use of bioanalytical methods such as mass spectrometry and optical methods; electrochemical methods, nanomaterials-based architecture, and strategies to enhance mass/heats transfer. Among these, the detection and quantification of proteins in the serum and saliva of the human body fluid is an utmost approach. Proteins make up a significant part of the human body and they circulate through the blood by binding with biomolecules. However, advancements in chromatographic techniques, such as HELEOS liquid chromatography and a tandem mass spectrometric system at atmospheric-pressure chemical ionization sources, have opened doors to enormous selectivity and sensitivity.

13. Future Directions in Cardiovascular Biochemistry

For decades, endeavors to find new biomarkers for prediction, prevention, diagnosis, and

prognosis of cardiovascular events have focused on a rather small number of molecules. Greasy analysis of lipoproteins and even more advanced methodologies to look at apoproteins and lipid structures to detect aggravating properties of particles with mass < 4 kDa of intracellular organelles aggravating atherosclerosis in the arterial wall. However, rather traditional biomarkers are now rediscovered as they do turn out to detect clinical relevant characteristics. In particular, the study of curves instead of simply looking at baseline analysis, and looking at changes in pathway activities instead of concentrations in compounds brings new valuable insights [25]. Otherwise hard to analyze constructs such as plexinD1 in an ELISA build environment show intriguing results which will have to be confirmed in ongoing studies. It remains a mystery why such apparently robust and clinically relevant observations have not yet paved their way to the clinical setting.

Technological improvements in analytical methodologies have revolutionized biomarker research in the past 10 years. In contrast to decades of research on single biomarkers, a shift from individual markers to multi-marker panels for cardiovascular risk prediction is occurring. Integration of multiple complementary platforms, such as micro-RNA, transcriptome, proteome, metabolome, and lipidome analysis is expected to advance cardiovascular biomarker discovery efforts. Novel methodologies for biomarker discovery in atherosclerosis pose enormous opportunities but also challenges for the cardiovascular community. Explorative and quantitative high-throughput mass spectrometry-based metabolomics and lipidomics have provided the atherosclerosis field with significant new advances in defining the metabolic networks and dysregulation underlying atherosclerosis progression. The risk-predicting performance of newly discovered biomarkers has only been assessed in small populations of patients, mainly in cross-sectional studies. None of the biomarkers are yet implemented in clinical practice [26].

14. Conclusion

Coronary heart disease, cerebrovascular heart disease, peripheral artery disease, and rheumatic heart disease are all classified as cardiovascular diseases (CVDs). CVDs are the leading cause of death and disability globally. With an estimated annual cost of 863 billion dollars in Europe and 238 billion in America, they are also a significant burden in terms of projecting health costs in future years. Lately, advanced methods have been applied for early diagnosis and prognostication during treatment of CHD, such as echocardiography, nuclear scintigraphy, transesophageal echocardiography, magnetic resonance imaging, and invasive approaches. Nevertheless, all of these methods require advanced technologies and are either associated with radiation exposure or be expensive, complicated, and time-consuming. Therefore, efforts to develop cost-effective methods such as electrocardiogram monitoring, recordings of oxygen saturation or other markers in blood or urine, and portable devices are increasing. In parallel, there is a growing interest to develop blood or urine tests for early detection of CVDs, which is becoming increasingly feasible given the advances in proteomics, metabolomics, genomics, transcriptomics, and epigenomics . It is already well established that several abnormalities in circulating markers precede the onset of cardiovascular frastructure and function changes that ultimately lead to cardiovascular syndromes (e.g., CVDs). In addition to use as an easy to use point-of-care test as a personal health monitoring tool, an increasing number of biomarkers for risk stratification of CVDs may diplicate costs and labor for monitoring multiple procedures. Very recently, however, studies reported that two easily accessible and economic tests of 24-hour urinary sodium level and uric acid level were useful for evaluating cardiovascular risk (CVR). The former was tested in a large cohort of young individuals aged 18 to 56 showing a stronger normalized CVR prediction performance than 10 index tests. Therefore, it would be of great interest to explore both traditional and novel urinary markers in CVD research as there is a large untapped pool of urine-based small molecules or metabolites for the development of biomarker tests with better predictive abilities.

References:

1. J. Luis Romero-Cabrera, J. Ankeny, A. Fernández-Montero, S. N. Kales et al., "A Systematic Review and Meta-Analysis of Advanced Biomarkers for Predicting Incident Cardiovascular Disease among Asymptomatic Middle-Aged Adults," 2022. ncbi.nlm.nih.gov
2. R. V. John, T. Devasiya, N. V.R., S. Adigal et al., "Cardiovascular biomarkers in body fluids: progress and prospects in optical sensors," 2022. ncbi.nlm.nih.gov
3. L. Feng, S. Nian, S. Zhang, W. Xu et al., "The associations between serum biomarkers and stenosis of the coronary arteries," 2016. ncbi.nlm.nih.gov
4. A. K. Pancholia, N. Kumar Kabra, and R. Gupta, "Laboratory evaluation of lipid parameters in clinical practice," 2024. ncbi.nlm.nih.gov
5. T. Ilva, "Sensitive Troponin Assays: Diagnostic and Prognostic Use in Cardiology," 2010. [PDF]
6. R. Maalouf and S. Bailey, "A review on B-type natriuretic peptide monitoring: assays and biosensors," 2016. ncbi.nlm.nih.gov
7. E. A Andreadis and C. Geladari, "Brain Natriuretic Peptide: Structure, Action and Role in the Diagnosis and Prognosis of Heart Failure," 2015. [PDF]
8. V. Gupta Neelima Singh Sanjeev K. Singh Nivedita Singh Arun Raiza Harshverdhan Singh Int J Cur Biomed Phar Res. Ajay K. Singh, "Role of immunoturbidimetric plasma fibrin D-dimer test in patients with coronary artery disease as well as ischemic heart disease in emergency medicine," 2011. [PDF]
9. R. Halaby, C. J. Popma, A. Cohen, G. Chi et al., "d-Dimer elevation and adverse outcomes," 2015. [PDF]
10. N. GH. AbdulKarim, M. A. Taher, and A. N. Almarayati, "The Role of Some Inflammatory Markers (IL-6 and CRP) in the Pathogenesis of Acute Coronary Syndrome in Iraqi CCU for Heart Diseases," 2017. [PDF]
11. S. Hesameddin Abbasi and M. Ali Boroumand, "Expanded Network of Inflammatory Markers of Atherogenesis: Where Are We Now?," 2010. ncbi.nlm.nih.gov
12. M. V. Wainstein, M. Mossmann, G. N. Araujo, S. C. Gonçalves et al., "Elevated serum interleukin-6 is predictive of coronary artery disease in intermediate risk overweight patients referred for coronary angiography," 2017. ncbi.nlm.nih.gov
13. R. H Heinisch, C. R Zanetti, F. Comin, J. L Fernandes et al., "Serial Changes in Plasma Levels of Cytokines in Patients with Coronary Artery Disease," 2005. ncbi.nlm.nih.gov
14. S. Y. Lee, A. M. Zaske, T. Novellino, D. Danila et al., "Probing the mechanical properties of TNF-α stimulated endothelial cell with atomic force microscopy," 2011. ncbi.nlm.nih.gov
15. R. Lee, M. Margaritis, K. M. Channon, and C. Antoniades, "Evaluating Oxidative Stress in Human Cardiovascular Disease: Methodological Aspects and Considerations," 2012. ncbi.nlm.nih.gov
16. M. Rasool, A. Malik, T. Tahir Butt, M. Abdul Basit Ashraf et al., "Implications of advanced oxidation protein products (AOPPs), advanced glycation end products (AGEs) and other biomarkers in the development of cardiovascular diseases," 2019. ncbi.nlm.nih.gov
17. J. Sundström, "Myocardial biomarkers for prediction of cardiovascular disease," 2009. [PDF]
18. N. Veljkovic, B. Zaric, I. Djuric, M. Obradovic et al., "Genetic Markers for Coronary Artery Disease," 2018. ncbi.nlm.nih.gov

19. D. Shiffman, J. P. Kane, J. Z. Louie, A. R. Arellano et al., "Analysis of 17,576 Potentially Functional SNPs in Three Case–Control Studies of Myocardial Infarction," 2008. ncbi.nlm.nih.gov

20. A. Angelakopoulou, T. Shah, R. Sofat, S. Shah et al., "Comparative analysis of genome-wide association studies signals for lipids, diabetes, and coronary heart disease: Cardiovascular Biomarker Genetics Collaboration.," 2011. [PDF]

21. X. Jia, H. Ju, L. Yang, and Y. Tian, "A novel multiplex polymerase chain reaction assay for profile analyses of gene expression in peripheral blood," 2012. ncbi.nlm.nih.gov

22. B. A. Stanley, R. L. Gundry, R. J. Cotter, and J. E. Van Eyk, "Heart Disease, Clinical Proteomics and Mass Spectrometry," 2004. ncbi.nlm.nih.gov

23. C. C Patterson, S. Blankenberg, Y. Ben-Shlomo, L. Heslop et al., "Troponin and BNP are markers for subsequent non-ischaemic congestive heart failure: the Caerphilly Prospective Study (CaPS)," 2018. ncbi.nlm.nih.gov

24. G. Hulsegge, A. M. W. Spijkerman, Y. T. van der Schouw, S. J. L. Bakker et al., "Trajectories of Metabolic Risk Factors and Biochemical Markers prior to the Onset of Cardiovascular Disease – The Doetinchem Cohort Study," 2016. ncbi.nlm.nih.gov

25. I. E Hoefer, S. Steffens, M. Ala-Korpela, M. Bäck et al., "Novel methodologies for biomarker discovery in atherosclerosis," 2015. [PDF]

26. J. Wang, G. J. Tan, L. N. Han, Y. Y. Bai et al., "Novel biomarkers for cardiovascular risk prediction," 2017. ncbi.nlm.nih.gov

27. T. C. van Holten, L. F. Waanders, P. G. de Groot, J. Vissers et al., "Circulating Biomarkers for Predicting Cardiovascular Disease Risk; a Systematic Review and Comprehensive Overview of Meta-Analyses," 2013. ncbi.nlm.nih.gov