American Journal of Biology and Natural Sciences
https://biojournals.us/index.php/AJBNS
ISSN: 2997-7185

Use of Molecular Markers in the Diagnosis of Angina Pectoris

Menan Munged Saleh Mahdi, Dunya Abdulkareem Ahmed Mahdi,
Yamamah Hameed Farhan Jasim, Amenah Irhayyim Ali khlaf
University of Samarra, College of Applied Sciences, Department of Pathological Analysis

Received: 2024, 15, Mar
Accepted: 2025, 21, Apr
Published: 2025, 28, May

Annotation: Angina pectoris is chest pain or discomfort due to reduced blood flow to the heart. Angina can be stable or unstable. Most people with angina have stable angina, which can often be treated with medication and lifestyle changes. Unstable angina is more serious, has not been diagnosed as a heart problem before, or is being treated but is getting worse. In all these cases, visit to a doctor or other health provider is essential. Angina that comes on much more easily, lasts longer, or is more severe than usual is considered a medical emergency and the patient may need to seek treatment. Angina pectoris and acute coronary syndromes affect millions of people around the world annually, and an early diagnosis is crucial to provide patients with adequate therapy based on their condition. Various methods and markers for detecting this mechanism have been investigated in recent years, however, attention is still mainly focused on the serum blood markers, which have good specificity but poor sensitivity for the early diagnosis of these conditions or their risk factors [1]. Because many patients consulted

a doctor due to uncertain chest pain, the cardiovascular assessment must include a detailed history and careful examination, in addition to investigating other possible causes of angina pectoris. In patients with a typical history of angina pectoris, the main non-invasive tests are exercise electrocardiography, nuclear perfusion imaging, and echocardiography. However, in some patients, the electrocardiogram may be normal between episodes of angina pectoris, and different types and power of blocking treatment may mask a positive exercise stress test. This indicates the need for more specific markers in the detection and evaluation of coronary artery stenosis, the risk of myocardial infarction in stable angina pectoris, and even in asymptomatic patients due to risk factors in young people with early or mild coronary atherosclerosis. Such a marker would allow for a better selection for invasive coronary angiography and would help avoid these procedures which are the gold standard in coronary artery disease detection because of their invasiveness and possible complications.

1. Introduction

Angina pectoris is a clinical symptom of ischemic heart disease defined as an imbalance between myocardial oxygen supply and increase in myocardial oxygen demand, characterized by short-lasting (around 20 minutes) retrosternal pain or even a feeling of pressure that may irradiate to the neck, jaw, shoulders or arms originating from the heart muscle, due to an impaired heart blood flow. Lesions (infarction, ischemia) in the left ventricle are diagnosed by an electrocardiogram (ECG) done with standard and/or exercise/physical load testing which reveal the transient (in case of angina pectoris) or permanent ST-segment depression/elevation (for infarction) and later on abnormal Q wave. Acute coronary syndromes (ACS) include unstable angina (UA), non-ST elevation myocardial infarction (NST-EMI), and ST elevation myocardial infarction (ST-EMI), leading to sudden cardiac death, heart failure or other life-threatening complications like arrhythmia or left ventricular rupture. These complications are often preceded by episodes of angina or chest pain. Some cases of patients played off by doctors as only simple angina may have a lesion in the coronary arteries which was previously neglected. In such non-obvious cases,

additional methods are necessary to identify patients with non-ST elevation myocardial infarction and other lesions [1]. There is an urgency for methods which can diagnose ischemia/infarction in patients with recent onset chest pain between 10 minutes and 12 hours. Non-invasive modern tests and invasive coronary angiography (CAG) highlight the presence of lesions. Coronary stenting which involves endovascular placement of balloon-expandable or self-expanding stent implants into the coronary arteries is the current method of choice for treating obstructed coronary arteries with this modern approach being extremely effective and reliable in percutaneous coronary intervention. However, due to a requirement of dedicated and costly instruments and experienced personnel, this method is not available for many patients in developing countries. The great efforts were undertaken to develop a new test which can identify this at risk patients. Sensitivity and specificity above 85% are necessary to effectively reduce the number of patients suppose to be tested. [2][3][4]

2. Understanding Angina Pectoris

Angina pectoris is an unpleasant sensation of pressure, heaviness, tightness, or pain in the chest region or adjacent areas (arms, neck, jaws, back), precipitated by exertion, emotion, or overwhelming meals, and relieved by rest or nitroglycerin. Angina is classified in two ways: on the basis of the duration of the symptoms, into stable angina, unstable angina, and variant angina; and on the basis of the severity of the disease into: atypical angina, atypical angina, and typical angina; with typical angina associated with the same symptoms. Stable angina is almost always secondary to vascular heart disease [5].

With the advent of modern technology, numerous non-invasive methods have been developed to diagnose coronary artery disease (CAD) in patients with chronic stable angina: which enhance the efforts for the diagnosis. However these methods rely largely on machine-based quantification which may sometimes miss the visual interpretative analysis causing a misdiagnosis. Recent work done on the use of molecular markers as a point-of-care test is being introduced. Point-of-care testing (POCT) has been introduced as a mode of testing that improves the efficiency of diagnosing diseases by minimizing the time and distance needed to test. Most POCT are personal information devices or rapid tests that do not require sophisticated instrumentations. This review discusses the innovative effort in the design, build and validation of the molecular markers (MMs) based rapid testing devices for the diagnosis of stable angina pectoris. The method is preventative, highly sensitive, specific and less expensive.

2.1. Definition and Types of Angina

Angina pectoris is a clinical manifestation of myocardial ischemia due to coronary atherosclerotic disease (CAD). The underlying pathophysiology has traditionally been attributed to the occlusion of a coronary artery and impaired coronary perfusion, providing only enough blood supply to meet the resting metabolic demands of the myocyte [6]. Most angina pectoris occurs secondary to fixed coronary artery stenosis due to atherosclerosis. Though an atheroma may be completely obstructing the artery, an increase in myocardial metabolic demand leads to angina pectoris. In stable angina, the systolic blood pressure does not rise sufficiently to cause perfusion pressure in the coronary arteries below the microvascular threshold. If the anatomic morphology of the atheroma is stable, there will be no progression of CAD, and after exercise angina slowly resolves when perfusion pressure returns to normal.

In far east Asia and Europe, it is not surprising that typical stable angina develops in patients whose coronary artery filling of <40% is assessed by coronary angiography. Early perfusion imaging with perfusion agents has shown delivery of a dose units of most common ionization channels. MRI with echocardiography, reducing your risk to similar levels to those of those who have never smoked.

Currently, the most widely used angiographic classification of CAD on the basis of the occlusion of coronary arteries is the Dutch Intercollegiate guidelines classification. This classification has

recently been adopted by both the American and European Heart Associations. However, this classification does not include the functional perfusion aspect of CAD. Functional perfusion imaging is widely performed in CAD.

2.2. Pathophysiology of Angina Pectoris

Ischaemia is defined as an imbalance between myocardial oxygen delivery and demand [5]. This mismatch can be dynamic, due to arterial obstruction, anaemia, increased heart rate or workload, or stable due to fixed epicardial disease. Stable angina occurs when progressive atherosclerosis disrupts the balance between coronary blood flow and demand, resulting in chest pain. Stable disease is understood as a progressive process influenced by risk factors such as smoking, diabetes, and lipid levels. However, many patients suffer from angina despite limited luminal obstruction of arteries, due to the fallacy of the single artery being the dominant factor in ischaemic development.

The underlying aetiology is multi-layered and complex. Systemic, microcirculatory, and epicardial factors contribute, leading to increased demand conditions (increased heart rate) and fixed lesions resulting in ischaemia. There is no single gold standard test for coronary artery disease (CAD). All established investigations have good specificity and sensitivity at different levels of the disease process with infarction, but patients with stable, chronic, and typical angina only have a high likelihood of CAD severity. Abnormalities in the coronaries is not the only pathological substrate responsible for angina or chronic, stable angina. Hence angiography cannot be used to exclude CAD unless normal or near-normal.

In conjunction with strict quality control, serum biomarkers were investigated to improve diagnostic performance on top of traditional risk factors and cardiac testing. The early identification of patients at risk of acute coronary syndrome/event is critical, however, there is currently no aggregated and sufficiently strong evidence to support the assessment of prognostic risk. The aim of studies of serum biomarkers is to identify an adequately sensitive and specific measure of underlying CAD. There is an apparent need to aggregate the knowledge on biomarkers studied by multiple laboratories before selecting complementary biomarkers for further test validation for CAD and angina pectoris. Such a compilation has not previously been conducted.

3. Role of Molecular Markers

More recently, in vivo studies have demonstrated that ischemia activates biochemical pathways involved in the development of new molecules termed "molecular markers of ischemia." These newly discovered molecules, such as brain natriuretic peptides and their precursors (BNP and NT-proBNP), C-reactive protein (CRP), ischemia modified albumin (IMA), and myeloperoxidase (MPO), are all invasive molecules with a role in either the development of ischemia or the response of the organism to ischemia [6].

BNP and its inactive amino terminal precursor (NT-proBNP) are released from the heart ventricles and they indicate prolonged heart load induced by chronic ischemia or hemodynamic changes of either a cardiac or a non cardiac nature. Nevertheless they work for the diagnosis and prognosis of ischemia due to appreciable heart load. Most crucially, they are not assays indicating the very first minutes of the ischemic event that is required for the diagnosis and treatment of patients with acute coronary syndromes (ACS).

CRP is a marker of systemic inflammation that has risen interest as a risk stratifier in patients with coronary artery disease. It is not actually a marker of ischemic event per se but a marker of ischemic burden. IMA is a marker of the transient conformational modification of the albumin molecule at the amino terminal end that occurs in response to an ischemic event. It has been shown to rise and fall in vivo in the very first minutes of an ischemic event potentially raising its role as an aiding molecule in the diagnosis of acute coronary syndromes. IMA assay with reliable monoclonals is easily introduced into clinical practice. Finally MPO is a marker of oxidative stress that has raised recent interest in the diagnosis of ischemic events. The diagnostic role of GEO-

MPO T policies has not yet been fully evaluated in the clinical arena.

3.1. Definition of Molecular Markers

Ischemic heart disease, such as angina pectoris, occurs when the blood flow to the heart muscle is insufficient for its metabolic needs [6]. The resultant imbalance between coronary blood supply and myocardial oxygen demand causes a state of ischemia. Chanelpctoris is a symptom complex most commonly due to coronary artery disease. It is typically caused by atherosclerotic coronary arteries undergoing acute plaque rupture, erosion, calcification, or other changes that precipitate thrombus formation and obstruction of the coronary arterial supply. Structural or functional changes contributing to the clinical development of the disease, together with behavioral risk factors, usually follow predisposition for atherosclerosis from heredity or other sources.

Despite the existence of validated clinical risk scores, laboratory tests, and imaging modalities to guide the clinician the pre-test likelihood of coronary artery disease or myocardial ischemia, these approaches are still imperfect. One major reason is that many laboratory tests measure necrosis markers that necessarily limit test sensitivity. As a result, there is a diagnostic gap between an inability to provide an adequate diagnosis of disease and an inability to provide an adequate diagnosis of clinically significant disease, allowing naïve patients to refuse further examination. The best definition of a molecular marker is one that is closely associated with an unobserved disease, rather than with its configuration or clinical state. This having been achieved, the marker’s passive presence or absence should have diagnostic capability so that fewer naïve patients might escape further investigation.

Induced molecular markers must provide a distinct alteration of the human biological state in aggregate data, so that cluster points may be effectively assessed through time-series supervision or semi-supervision. Induction must lead to high levels of confidence when precision-based mining systems are performed to extract distinct thresholds for the classifier’s accuracy. Therefore, the concept of using molecular markers in the diagnosis of angina pectoris is strictly tied to the state of the art of relevant knowledge. Laboratory tests have a mathematically demonstrated diagnostic failure in circumstance of high pre-test and post-test likelihood. Knowledge accumulation on threshold-based model invalidates thresholding in high state confidence. High levels of risk indicate large expected future angina episodes with poor calibration properties of the capacitated ranks.

3.2. Types of Molecular Markers Used in Cardiology

Angina pectoris is caused by a disparity between the heart's demand for oxygen and the supply delivered through the coronary arteries. The diagnostic work-up of patients with suspected angina pectoris, however, is by no means simple. A detailed history and physical examination often suggest the diagnosis of coronary artery disease but not with absolute certainty. Thus, a decision regarding further non-invasive testing must be made. Patients with a modified Bruce protocol treadmill exercise test who have an adequate workload and undergo general posture control over the following 24 hours exhibit a very low post-test probability of having cardiovascular events. Such patients can be safely sent back home with advice regarding the warning symptoms of myocardial ischemia and return practices. Testing with 99m-Tc-sestamibi, 99m-Tc-tetrofosmin, or 123I-mIBG can be performed also by first-pass imaging only with electrocardiographic monitoring. Holter monitoring after a first-pass imaging study can detect abnormal uptake patterns and diagnose coronary artery disease in patients with equivocal data. Amiodarone and lidocaine can be used in a small sub-set of patients who are unable to exercise [6]. Invasive coronary arteriography should be carefully planned using angiographic criteria and clinical contraindications. In a large sub-set of patients with conventional risk factors, a negative exercise-stress test should question the diagnosis of coronary artery disease up to safe limits unless there is suspicion for a spontaneous triggering coronary vasospasm. Patients with atypical symptoms, normal exercise tests, and angiographically nonsignificant coronary artery disease remain at very low risk and the negative results of a pre-discharge Holter monitoring study reinforce the

prognosis. Invasive measures are then performed in a more costly, less rewarding sub-set of patients who might suffer from coronary artery dissection or pseudo-angina.

4. Biomarkers in Angina Diagnosis

Stable angina pectoris (SAP) and unstable angina pectoris (UAP) are the two clinical forms of coronary artery disease (CAD) [7]. SAP, the most frequent clinical form of CAD, occurs because myocardial oxygen supply is reduced due to epicardial coronary steno-occlusive lesions. SAP is primarily characterized by exertional chest discomfort and related symptoms, which are provoked by some specific exertional or emotional triggers in a well-defined situation or temporal sequence. Diagnosis of SAP does not require additional testing under appropriate clinical circumstance. Unstable angina, however, is a clinical form of CAD that is associated with the prospective evaluation of acute myocardial infarction. Diagnosis of UAP involves ruling out AMI in addition to confirming CAD. No specific test has been approved as the "gold standard" for the diagnosis of angina. With regard to UAP, the plasma biomarker cardiac troponin has been extensively studied, and, in the presence of a suitable test, cardiac troponin has largely replaced biomarkers for the diagnosis of AMI.

Angina, however, is not equivalent to heart disease. The Edmonton Classification System for Cancer and Angina should be filled out before recruiting angina patients in clinical trials and biobanks. Essentially, ECSCA asks about a medical history of heart attack, revascularization, and angina. However, the latter is a subjective experience. The number of angina patients was 79,942; therefore, the recall value of CAD was 778%, even without considering a screening test although the biosample size ranged from 40,000 to 87,876. On the other hand, the duration of disability in the angina patients was a bit more than four years, raising the possibility that over-diagnosis of CAD this time is situations-specific. Therefore, objective tests are required to further demonstrate a presence of slowing the heart rate by 10 beats per minute. Similar ideas are true for comparatively broad categories such as difficult breathing and asthma, and some tests have been researched to try to fill the void. However, ways to itemize angina on the hospital chart have to be workable bypassing the phenomenon of over-diagnosis.

4.1. Cardiac Troponins

Troponin T (TnT) and troponin I (TnI) are muscle proteins that play a key role in regulating the contraction of striated muscle in the myocardium and skeletal muscle, and are known to be useful markers for myocardial injury. Troponin T protein even with cardiac specificity can be found in brain tissue of cardiac TnT knockout mice. These proteins from necrotic myocardium are released into plasma and elevate the concentration in plasma, making them useful markers for acute myocardial infarction and chronic ischemic heart disease.

Researchers made significant progress in finding the molecular definition and structure of such markers. TnI but not TnT can be phosphorylated on serine and threonine residues in the first 200 amino acid long structures of human cardiac TnI. Phosphorylated TnI can be found in human heart, plasma, and urine. Immunoassays have been developed for detecting phosphorylated TnI as a marker for human heart injury. S-TnI and N-TnI increase after exercise stress testing in patients with coronary artery disease, and can be used for the diagnosis of coronary artery disease when ordered as a panel.

The association of actin with cardiac troponins C, I, and T inhibits ischemia-induced pTroponin I S15 and S23 release into circulation. Elevated levels of pTnI Ser23 detected by a selective immunoassay can help detect myocardial ischemia that remains undetected by cTnI detection or electrocardiographic evidence. In patients with ST elevation myocardial infarction, cardiac troponin I and T predict the risk of mortality and reinfarction. Plasma TnT was not only useful in the early detection of acute myocardial damage, but also predicted short- and long-term outcome among patients in the post-AMI stage. The utility of troponin T and I as a biomarker of myocardial injury in acute respiratory distress syndrome has been reported.

4.2. B-type Natriuretic Peptide (BNP)

Natriuretic peptides are hormones secreted by the cardiomyocyte and are considered markers of cardiac response to overload. Although the most studied natriuretic peptide in this context is the B-type natriuretic peptide (BNP), the pre-prohormone signaling molecule, which is complexed with two proteins that are inactive in terms of natriuresis, dilatation, and other important physiological effects, has also been studied as a biomarker. Many studies have shown that B-type natriuretic peptide and its cleaved products, (1–76) and (1–32) forms, give prognostic information in patients with and at risk of coronary artery disease, heart failure, arrhythmias, and left ventricular dysfunction [8]. However, a consensus about the cut-off values for diagnosis of myocardial ischemia by B-type natriuretic peptide, as well as for its responsiveness to treatment.

Diagnostic efficacy of MP and measurement of NT-proBNP, an independent predictor of a normal myocardial scintigraphy in patients with suspected CAD. There was a considerable discrepancy in referrals between the concentration groups of NT-proBNP. The patients suspected of CAD with NT-proBNP <25 ng/l were 20–25% less likely to be referred to MPI and a negative predictive value (NPV) for a normal MPI of minimum 95% was achieved. Although this value is higher than the performance of standard pre-test models lowering resource utilization, careful implementation of this test can reserve costly imaging procedures for those patients where it is crucial for clinical decision-making [9].

Coronary artery disease and myocardial ischemia remains a dynamic field aimed at identifying patients with significant coronary artery stenosis (CAD) requiring invasive procedures. Current approaches include lifestyle modifications, judging the indication for stress testing, and determining genotyping to predict disease. However, the development of a simple screening procedure to spare patients unnecessary evaluation would be ideal. Natriuretic peptides are considered to be cardiac stretch markers with roles in sodium excretion, diuresis, vasodilatation, and anti-remodeling. Modelling studies have suggested an effect of obesity on the formation of biologically active natriuretic peptides. However, there is currently little clinical information regarding the effects of obesity on steady-state levels of these hormones. Real gain in measuring directly pro-B-type natriuretic peptide concentrations disappeared if other pro-B-type natriuretic peptide-cut off values had been used.

4.3. C-reactive Protein (CRP)

CRP is an acute phase reactant synthesized predominantly by hepatocytes. Its biosynthesis is regulated mainly by IL 6 and TNF which are released in response to acute inflammatory events. Its half-life of about 19 hours accounts for slow but detectable increase in serum concentration in most acute inflammatory states [10]. CRP is delivered to its site of action as a pentameric molecule which when converted to a monomeric form by calcium and magnesium ion influx, is able to bind to post-translationally modified targets such as damaged apolipoproteins and necrotic tissue. Since protein degradation alters tertiary structure, plasma binding of low density lipoproteins (LDL) is increased in coronary artery disease (CAD). The CRP-LDL complex incorporates phosphocholine moieties and is taken up by macrophages.

CRP is an acute phase reactant produced primarily by hepatocytes in response to inflammation. Limited human in vivo studies have suggested that the production of CRP after estrogen replacement therapy may help to break into the prostate. This has implications for both gender specific and gender neutral treatment strategies. CRP has also been implicated as a cofactor for the phagocytic clearance of apoptotic cells in atherosclerotic plaques. CRP is a multisubunit (a pentamer of 23 kDa subunits), highly conserved protein with a structure that fails to display the conformational changes that usually accompany activation.

CRP monomers can activate complement through the classical pathway; prevent differential endothelial permeability to lipoproteins; and enhance low affinity uptake of apoptotic cells by macrophages. Some of these properties are common to other circulating acute phase proteins.

Probably the best characterized of the acute phase markers are the plasma and tissue CRP. The classic method of determining coronary artery disease risk in the normal population suggests determining total cholesterol, high density lipoprotein cholesterol, triglycerides, blood pressure, smoking status. The interconnection between inflammation and acute coronary syndromes further increased the interest in CRP with regard to coronary artery disease. There are many risk factors for coronary artery disease.

5. Advancements in Molecular Testing

Prognosis and monitoring the therapy of disease has shifted dramatically in recent years: the introduction of breast cancer-specific markers measurable in the blood serum of breast cancer patients has opened up completely new possibilities [11]. In breast cancer, recurrence of the disease and of metastatic spread occurs in 20-50% of cases. Over the past few years, molecular parameters detectable in the blood have been introduced to improve the prognosis of the individual patient and monitor the therapy.

In breast cancer, development of secondary disease has reduced the treatment options and prognosis of the disease. Determining the presence of secondary breast cancer in lymph nodes is one of the most reliable prognostic parameters. It has been shown that in addition to lymph nodes, breast cancer cells can disseminate to other constituents of the circulatory system, as well, notably bone marrow and blood. Clinically, both tumor markers and cytologic techniques have been studied to identify these metastases. However, tumor markers are available only for the most advanced cancer disease, and cytogenic methods are regarded as “too unspecific” and therefore possess “too much noise” in order to use them as tumor markers.

Fifty percent of patients with no evidence of disease (NED) following therapy are still diagnosed with further metastatic spread in a few years (15-30%), regardless of the method used to determine the status of the lymph nodes. In addition to a breast cancer-specific molecular marker detected in tissue, there is a demand for a marker of disease using molecular detection methods that is clinically usable, specific for the disease in question, and detectable in a peripheral blood sample of the patient. Moreover, in regard to prevention and early treatment of disease, the exact time of spread is desired and a test applicable “at home.”

5.1. Genomic and Proteomic Approaches

Cardiovascular diseases (CVDs) are the leading cause of morbidity and mortality worldwide. CVD events occur when blood vessels providing oxygen-rich blood to the heart become narrowed, blocked by abnormal growths of tissue (plaques) that occur in response to prolonged chronic inflammation. These plaques can rupture, exposing their contents to blood and activating a prothrombotic cascade that can occlude the vessel, resulting in myocardial ischemia, angina, or necrosis that may present as non-st elevation myocardial infarction (NSTEMI) or ST-elevation myocardial infarction (STEMI). Recently, new understandings on the molecular and cellular events that occur in the acute setting, in conjunction with advances in genomic and proteomic technologies, have led to the development of markers to enhance the diagnostic capabilities of the current tests for CVDs.

CVDs remain a public health challenge with dire individual and societal consequences. Aggressive risk factor modification, lifestyle changes, and medical therapies in those at risk can reduce the odds of an initial, or secondary, event; however, compliance with these measures is often suboptimal. Therefore, enhancing the risk stratification of those who are still undiagnosed, and those with atypical symptoms in lower-risk groups, is critical to preventing morbidity and mortality from angina or acute myocardial infarction (AMI). A perfect biomarker for a disease should ideally be simple, quick, widely available, and inexpensive approach that correlates with the disease process, has high sensitivity and specificity, and is migratory in nature [12]. Unfortunately, none of the existing biomarkers for atherosclerosis or AMI has been identified that meets these requirements, and the gap between the highly developed science behind biomarkers

and the available tests for clinical use remains frustratingly large.

5.2. Emerging Biomarkers

The growing recognition that the underlying pathobiology of cardiovascular diseases is inflammation has led to the development of a new class of immunoassays measuring inflammation biomarkers. Established markers such as hsCRP, IL-6, and sICAM have shown associations with cardiovascular risk and prognostic value in myocardial infarction [6]. However, because these proteins change only after overt vascular disease has developed, it is posited that intensifying treatment based on changes in their concentration would show limited benefit. On the other hand, promising new markers, secreted from the vascular wall, relate closely to plaque pathology, perhaps explaining their success in the early detection of acute coronary syndrome-driven myocardial infarction.

Neutrophil hyperactivity is central to atherosclerotic plaque destabilization and thrombus formation through oxidative stress, macrophage recruitment, cytokine secretion via the myeloperoxidase pathway causing apoptotic signaling. Measurement of MPO activity by a simple immunosorbent assay can therefore provide a means for assessing vulnerability [12]. In the case of occlusive ischemic events, elements that are released from ruptured endothelial cells, such as vWF, adhesion molecules, as well as trophic factors such as fibrinogen, and circulating endothelial cells have been implicated in ongoing neovascularization and secondary neo-atherogenesis. Consequently, detection of these constituents in the circulation may be instrumental in diagnosing unstable angina and acute coronary syndromes.

The role of bioactive lipids in plaque size, macrophage recruitment, adhesion molecule upregulation in the chronic stages of atherosclerosis is well documented. More recently, a comprehensive array of biochemical and molecular changes that occur in the acute phase of occlusive ischemic events culminating in a sudden rise in shear stress have been described, which may successfully identify patients with early-stage disease. Thus, lipid oxidation, nitrosylation of hemoglobin, increased free radical production by neutrophils, and increased secretion of elastase and metalloproteinases have been proposed, and proteome-based relative quantification approaches are being developed to detect biomarkers of acute myocardial infarction.

6. Clinical Applications

The clinical applicability of cardiac biomarkers has been discussed for almost two decades. The initial expectations for such biomarkers to be used in primary prevention of coronary artery disease (CAD) through population wide screening are slowly being replaced by an understanding that cardiac biomarkers mainly serve as ancillary tests in the diagnostic workup of CAD. An approach that has gained recent interest is the application of several biomarkers in parallel to identify the disease state of an individual [1].

The results of the search for possible biomarkers are presented in two different aspects, namely the specificity of the biomarkers toward the presence of obstructive CAD and the specificity of the biomarkers toward the presence of myocardial ischemia. In both cases, the grade of evidence is presented as a classification system adopted from the American College of Cardiology/American Heart Association classification of evidence. Under each marker, studies are presented according to their design type. Because of the vast number of studies that have tested the efficacy of each investigated biomarker, most of the studies will not be discussed, but the studies with the highest quoted use will be discussed first.

Because of its widespread use and an enormous literature base, troponin is discussed first. Troponin tests are currently the most widely used biomarker for the diagnosis of myocardial infarction. Troponins preferentially bind to the cytosolic sarcomere in cardiomyocytes and are released to circulation following myocyte necrosis. Initially troponins were used in the ruling in of myocardial infarction, however because troponins are also released upon myocyte injury/failure, this latter aspect has received more attention recently. Troponin elevation is present in

approximately 40% of patients with acute coronary syndromes who are not diagnosed with a myocardial infarction, suggesting that troponin elevation does not equate myocardial infarction in all cases. Use of troponin in the ruling out of CAD has not been studied extensively. However, should a marker rise and return to baseline within one chest pain event, it is an indicator of the absence of CAD; on the other hand, if a marker remains elevated, serial testing is required. [13][14][15]

6.1. Diagnostic Accuracy of Molecular Markers

Dichotomous standard diagnostic in angina pectoris is as accurate as . On the other hand, the added knowledge versus the probability of coronary artery disease from traditional risk factors for patients with suspected myocardial infarction presented low diagnostic accuracy. Recommendations state that nucleoside triphosphate (NTP) or troponin T/Tc are the preferred tests in suspected myocardial infarction. In evaluated designed the triple test which is equal to suggested impostors in this capacity, troponins T and C was not preferred on the bases of impaired diagnostic accuracy on improvement of approximately 37% versus the other tests. A two marker strategy of C-reactive protein and H-FABP lowered some discordant classifications of coronary artery disease. The C-reactive protein contributed considerably and detection of cardiac dysfunction or ischemia determined lower probability of coronary artery disease. The other recommendations were lower acuity presented far lesser independence versus the probability of cardiac disease and are at greater risk of cancer than a myocardial infarction. It was preferred as the single best test among these recommendations. H-FABP added most to this. C-reactive protein was further asked to be included in future recommendations. This is the first proposed criteria in the capture disease prospective trials as prospective trials. As these take several years with few tests more rapidly and far less cost efficient to have recorded the best tests already in five other similar trials. As this contains the first methodology for the development and evaluation of diagnostic tests in any disease the authors hope it might promote very rapid future expansion of the use of national registry tests in increased coverage for patients [1].

Traditional biomarkers for the diagnosis of myocardial ischemia and risk stratification in acute coronary syndromes have been limited for some time now. The main traditional biomarkers for the diagnosis of myocardial ischemia and risk stratification in acute coronary syndromes are creatine kinase–myocardial band (CK-MB) and troponins. Diagnosis of acute coronary events is based on the increased concentration of these enzymes in the bloodstream, which reflects myocardial necrosis. However, troponins, which are considered the most sensitive and specific markers of ischemia, have drawbacks, too. The concentration of troponins is often determined too late – far beyond the time window of six hours from the onset of symptoms. Even in the case of troponin test result increase, the concentration of troponins measured off time might be not sufficient for diagnosis. Most importantly, troponins cannot be detected in all acute coronary events. Patients with unstable angina or early stages of myocardial infarction are often below the detection threshold – in such cases biomarker concentrations require considerable increase for them to turn positive [6].

6.2. Molecular Markers in Risk Stratification

The prognosis of patients with angina pectoris varies widely; hence, the need arises for screening and risk stratification. Risk stratification is an essential aspect of clinical examinations and is used in the outpatient management of many conditions. Some patients present with atypical symptoms of coronary artery disease, such as vague symptoms or noncardiac complaints. Therefore, the use of chest pain scores may have limited value for rehospitalisation in patients with a suspected acute coronary syndrome. The prognosis of patients with angina pectoris varies widely.

A malign hypothesis defines this group. This entails screening all patients with new-onset severe or increased frequency of atypical chest pain for the potential presence of ischaemia. There must be strong evidence of inappropriate testing in most categories before the bounding of this hypothesis is lifted. A human risk threshold for intervention and/or workup higher than that

usually set at annual rates for lessoned concern is needed. Once a minimum risk is established, a tiered risk investigation may be performed. Several risk stratification tools are available, but most have not been evaluated in patients without a prior diagnosis. Classification of patients according to the presence (or absence) of ischaemic heart disease does not take into account the progressive nature of this disease.

Diagnosis in clinical medicine is a probabilistic exercise, using an array of signs and symptoms, disease probabilities, and a test with sensitivity and specificity. Selecting a multi-marker strategy of markers reduces the need for the extreme specificity or sensitivity typical of traditional, single-marker-based approaches. This follows as most patients are classified at intermediate risk based on a risk threshold. Neither detection of significant coronary artery disease nor the presence of a nonischaemic study results in a totality of risk categorisation. Cardiovascular events such as death and myocardial infarction are infrequent and low enough for many intermediate patients. It is hoped that the workup effort of these patients will yield a better chance of diagnosing eligible candidates for secondary prevention. [16][17][18]

7. Limitations of Current Research

Future research should focus on long-term follow-up studies looking for changes in stable markers over many weeks or longer. The relative stability of some molecular markers may be advantageous when attending to patients and providing a rather growing, temporal management input on active treatment or palliative care [6].

Longitudinal research is needed to understand the prognostic capacity of serially measured molecular markers and the influence of co-variates and confounding factors. Several issues remain regarding the research strategy. The severity of the first infarction must be established, possibly using scores based on biochemical markers or the size of ECG changes. A good understanding of the temporal profile of any involvement of the markers not yet in routine clinical practice must be established to recommend a collection schedule.

Research must focus on group selection and experimental design, statistical analysis, and methods to avoid bias in the construction and interpretation of false-negative negative conclusions. Validation of the methodology used, in terms of both laboratory quantity and statistical modelling, is crucial to ensure the research provides valid results. This is especially important given that the research is likely to be cited in academic and clinical settings that will be influenced by false conclusions derived from flawed methods.

7.1. Variability in Biomarker Levels

Clinical presentation, risk factors, stress testing, and imaging techniques are necessary, but not alone sufficient to arrive at a diagnosis of Angina Pectoris. Key specific attenuating factors need to be elicited from the patient’s personal history, personality and psychodynamic composition are also relevant as sufficiency factors. The quest for a marker of Ischemia has been wrong; persistent effort to find a marker of Ischemia that is specific for atheromatous stenosis- or vascular mediated Ischemia hasn’t been successful. There is no such marker. Cardiovascular disease is a group of multifactorial pathways that buttress several mathematical models for prognosis; the predictive value of such models is limited hence the lifelong continuing search for a general new biomarker of Ischemic disease.

Speculation about a failure in sample separation, derivatization and technologies used is rendered moot given the advanced state of the art in Separation Sciences. The variability in intended and unintended bias in the cut-off, detection, reference range, precision, genetic studies and cohort composition, etc., needs study. It is hoped that through the pooling of human and other resources such a review of text-book information and bench-mark procedures would be instituted and openness of the research endeavour encouraged [6]. So doing would advance common understanding of the involvement of biomarkers in CVD as a substrate for common further research.

7.2. Cost and Accessibility Issues

The growing demand for molecular markers is producing considerable pressure to develop them as diagnostic tools for the clinical management of angina pectoris. There are, however, major obstacles to widespread adoption. In Europe, current guidelines recommend a centralized approach to molecular marker testing, with testing typically by batch analysis in a high-throughput laboratory supported by a clinical chemistry department. Difficulties arise both on an institutional basis and also in terms of health-care systems.

Centralized high-throughput testing is difficult to implement when a marker is new and on the market in a limited number of countries, and so it is often difficult for physicians to gain experience of using the new marker. During this lag phase, laboratory services may therefore not come on stream immediately. In the current health-care climate, having access to expensive high-throughput analysers that are used for only a small number of tests a day may be challenged, especially for testing on some rare specific marker such as an MR-pro-ADAMTS-13.

Furthermore, in many health-care systems, analytical testing for laboratory-generated indices of diseases is fairly decentralized and has been firmly established around a ‘community’ approach. This means that standardization of analytical markers disposition/processing and clinical testing is difficult. Analytical control of tested markers is also very problematical in many community hospitals. These hospital laboratories are usually small, and they may lack trained personnel in locally applicable multiple analyser protocols designed to process community hospital samples immediately following their receipt. Standardized protocols for community hospital testing of solely locally formulated risk indices may either limit the feasible number of processing protocols or additionally require spending funds on developing additional analytical platforms.

Under these circumstances, highly specific mass spectrometric assay protocols may not be feasible. Even when analytical protocols were feasible, the procedures for analyses may be further limited by the health-care system's coverage of the assay. For a newly developed high added value marker to be sustained in a health-care system (effective), it is also important that its analytical assay is readily available. A tracker for overseeing physicians' compliance after implementation of cost-cutting measures will be difficult and is unlikely to be adopted in most health-care systems [19].

8. Future Directions

Despite the significant advances accomplished through the years both in clinical and paraclinical investigations for the diagnosis of angina pectoris, there is still a great number of individuals with established coronary artery disease where conventional methods fail to demonstrate myocardial ischemia and others with positive noninvasive tests who do not have anatomic explanation of their symptoms. It seems clear that there is an overwhelming need for novel single or composite biochemical markers of myocardial ischemia and acute coronary syndromes, capable of improving the diagnostic and prognostic accuracy for such patients. Several candidate molecules have already been investigated, either measuring myocyte injury and dysfunction (myeloperoxidase, troponin T/I, creatine kinase MB, marker of cell necrosis; brain natriuretic peptide, marker of left ventricular hypertrophy and diastolic dysfunction; ischemia-modified albumin, marker of ischemic injury), or activating inflammatory pathways (C-reactive protein, marker of systemic inflammation) [6].

Among the various new biochemical markers of myocardial ischemia, those thought to escape of the established pathways of ischemic injury might be the most interesting. Perhaps due to the everlasting interest for an entirely new approach in the consideration of the biochemical changes happening at the time of myocardial ischemia, ischemia-modified albumin captured the attention of several researchers. The data regarding it are both poor and interesting, though encouraging for future attempts. It is by far more critical to investigate changes that are among the first to appear after ischemic event and may persist for a longer period of time or to detect alterations of

molecules/inflammatory changes that are known to participate in the development of disease with an earlier time frame. It seems deliberated that in the era of concomitant use of more outdated paradigms and the search for new biomarkers, the ideal method for further research is a parallel interrogation of markers operating beside each other. [20][21][22]

8.1. Integration of Molecular Markers into Clinical Practice

The integration of these discovered molecular markers, be they genetic or in-tissue proteins, serum proteins or glycoproteins, into clinical practice will require several advances in a number of areas, some technical and some more social in nature. Regarding technical advances, three areas are suggested for immediate investigation: the expansion of available validated markers; the evaluation of these markers individually and in combination for diagnosis, prognosis, treatment response, and recurrence detection; and the exploration of release mechanisms. Work in these areas could greatly aid in the early diagnosis, treatment, and prevention of AP complications [6]. Social issues need to be dealt with. These include determining what test or tests should be performed, on what population of patients, under what settings, and by what method of delivery. Commensurate with these tasks would be the prudent and judicious formation of professional guidelines for best clinical practice [1]. These guidelines should specify what markers are well validated and easily done in most labs, and what markers are more advisable for research at this time. It is hoped that such guidelines would put sufficient pressure on both academics and industry to continue to explore the many open avenues for discovery, validation, and integration.

8.2. Personalized Medicine Approaches

Clinical practice has rapidly advanced throughout the history of treatments and rules for diseases. With the development of the whole genome sequences of test organisms, personalized medicine became an essential approach utilized for tailoring treatments for the diseases. Personalized medicine has been applied to varying areas of medicine, where biomarkers are being examined, screened, and used for regimented treatments. With a rapid enlargement of the clinical biochemistry field, great efforts have been made to implement personalized medicine approach for the diagnosis and treatment of the diseases especially for chronic and deadly arbitrarily diseases like cancers, diabetic, arthritis, Alzheimer's, etc [23].

Personalized medicine is suggested to be the current focus of optimally treating various diseases. Personalized medicine is the utilization of biomarkers effectively for more accurately detecting an illness or determining an effective treatment, thus making personalized medicine a widely accepted approach in health care systems. Moreover, dramatic growth of biotechnological methods and clinical tests has rapidly advanced and broadened the personalized medicine. Personalized medicine and the efforts involved in implementation of it including identification and detection of biomarkers, and development and validation of assays. Oncogenic mutations and overexpression of receptor of tyrosine kinase (RTK) were successfully used as therapeutic target and biomarkers to predict efficacy of treatment for many patients, due to the rapid advancements of detection methods in molecular techniques of PCR and NGS. For instance, FDA approved gefitinib and erlotinib for lung adenocarcinoma patients with EGFR mutation and crizotinib for lung adenocarcinoma patients with ALK fusion. Recent oncogenic mutations for which molecular-targeted drugs with fully therapeutic efficacy were not yet developed include NRAS, ARAF, BRAF, MEK, ERK, MDM2, and CDK [24].

Being a chronic disease affecting millions of individuals worldwide, various treatments are currently available for rheumatoid arthritis (RA). Like other arthritis forms, early treatment with diseasemodifying antirheumatic drugs (DMARDs) is crucial to arrest disease progression and prevention of joint damage. Despite advances in the understanding of RA pathophysiology with different formulations available, no predictive diagnostic marker has been developed yet. Inflammation-specific markers, e.g. RF and ACPA, were explored and included in the guidelines of the ACR and EULAR for diagnosis but were not conceived as diagnostic markers for selecting therapeutic agents. [25][26]

9. Conclusion

A search for novel biomarkers of ischemia led to a growing group of microRNAs (miRNAs) that can be detected in blood and that might hold promise. The biggest group is represented by the pre-miR-137-33 family. MiR-137 itself has been implicated in growth and differentiation in a variety of tissues including the brain and heart. MiR-137 has been reported as a downregulated tumor suppressor in a variety of cancers including breast, pituitary, bladder, and glioma. Similarly, miR-106b has been reported as an oncomir in lung, breast, prostate, pancreatic, and hepatocellular carcinomas. The precise mechanism of action of pre-miR-137 and pre-miR-106b after hypoxia is not completely elucidated. Isoforms of molecular markers, microRNAs, have also shown to be diagnostic of various aspects of CAD. A new hope in the search for diagnostic marker(s) to better describe extent and severity of CAD is a focus on blood-based disease markers of a more global nature, including inflammatory and ischemia-activated processes. A focus on blood-based biomarkers of disease state is more promising since they would reflect the degree of illness and would not require the prior step of diagnosis.

The complexity and interconnectivity of networks suggests that such global markers may have the prospect for more extensive coverage of disease state than more focused biomarkers. Efforts aimed at bloodbased markers of acute myocardial infarction, some of which are now EC approved and in clinical use, are certainly the clearest evidence. MicroRNAs have gained considerable interest, largely because of their small size, ease of detection, extensive tissue and cell type diversity, possession of tissue reserves that endure transitorily ischemic or necrotic episodes, and capability for blood detection. Recently, focus has concentrated on the potential of blood-based markers. Such markers would hold promise beyond blood marker candidates of recent years, such as C-reactive protein (CRP) and Troponin I, which identify the presence of plaque rupture alone and subacute myocardial necrosis alone, respectively.

References:

1. M. Sawicki, G. Sypniewska, M. Krintus, M. Kozinski et al., "Multi-Marker Approach with the Use of Biochip Cardiac Array Technology for Early Diagnosis in Patients with Acute Coronary Syndromes," 2008. ncbi.nlm.nih.gov
2. Y. Liu, Z. Li, X. Wang, T. Ni, M. Ma, and Y. He, "… of adjuvant Chinese patent medicine therapy on major adverse cardiovascular events in patients with coronary heart disease angina pectoris: a population-based …," Acupuncture and…, vol. 2022. lww.com
3. Myocardial Ischemic Syndromes Task Force, "Evolving Management Paradigm for Stable Ischemic Heart Disease Patients: JACC Review Topic of the Week," Journal of the American College of Cardiology, 2023. jacc.org
4. W. Ahmed, T. Muhammad, C. Maurya, and S. N. Akhtar, "… and factors associated with undiagnosed and uncontrolled heart disease: A study based on self-reported chronic heart disease and symptom-based angina pectoris …," PLoS One, 2023. plos.org
5. T. J Ford, D. Corcoran, and C. Berry, "Stable coronary syndromes: pathophysiology, diagnostic advances and therapeutic need," 2017. ncbi.nlm.nih.gov
6. S. Koulouris, S. Tzeis, and A. S. Manolis, "New Biochemical Markers of Ischemia:The Diagnostic and Prognostic Role of Brain Natriuretic Peptide, C-Reactive Protein, Ischemia Modified Albumin & Myeloperoxidase," 2009. [PDF]
7. S. Koulouris, S. Tzeis, and M. D. FACC FESC FHRS Antonis S Manolis, "New Biochemical Markers of Ischemia: The Diagnostic and Prognostic Role of Brain Natriuretic Peptide, C-Reactive Protein, Ischemia Modified Albumin and Myeloperoxidase," 2009. [PDF]
8. P. Nogueira Cavalcante, G. Kanhouche, V. Emer Egypto Rosa, C. M. Campos et al., "B-type natriuretic peptide and N-terminal Pro-B-type natriuretic peptide in severe aortic stenosis: a comprehensive literature review," 2023. ncbi.nlm.nih.gov

9. C. Noelle Rathcke, E. Kjøller, N. Fogh-Andersen, B. Zerahn et al., "NT-proBNP and Circulating Inflammation Markers in Prediction of a Normal Myocardial Scintigraphy in Patients with Symptoms of Coronary Artery Disease," 2010. ncbi.nlm.nih.gov
10. B. Vijayasekaran, "Highly Sensitive C - Reactive Protein in the Patients with Unstable Angina and Its Prognostic Implications," 2008. [PDF]
11. A. Henrik Israyelyan, "The development of molecular diagnostics for breast cancer," 2003. [PDF]
12. E. D. Muse, E. R. Kramer, H. Wang, P. Barrett et al., "A Whole Blood Molecular Signature for Acute Myocardial Infarction," 2017. ncbi.nlm.nih.gov
13. S. Y. Kim, J. P. Lee, W. R. Shin, I. H. Oh, J. Y. Ahn, "Cardiac biomarkers and detection methods for myocardial infarction," *Molecular & Cellular*, vol. 2022, Springer. springer.com
14. A. M. Chaulin, "The importance of cardiac troponin metabolism in the laboratory diagnosis of myocardial infarction (Comprehensive Review)," BioMed Research International, 2022. wiley.com
15. I. Tilea, A. Varga, and R. C. Serban, "Past, present, and future of blood biomarkers for the diagnosis of acute myocardial infarction—promises and challenges," Diagnostics, 2021. mdpi.com
16. Y. Kim, I. Yeo, I. Huh, J. Kim, D. Han, J. Y. Jang, "Development and multiple validation of the protein multi-marker panel for diagnosis of pancreatic cancer," *Clinical Cancer Research*, vol. 2021. bktimes.net
17. J. B. Kisiel, J. O. Ebbert, W. R. Taylor, C. R. Marinac, et al., "Shifting the cancer screening paradigm: developing a multi-biomarker class approach to multi-cancer early detection testing," Life, vol. 2024. mdpi.com
18. A. Sinha, S. Sankanagoudar, A. Sharma, et al., "Recent Scenario of Diagnostic and Prognostic Biomarkers of Sepsis in Clinical Practice and the Role of Multi-marker Approach: An Update," Annals of African, vol. XX, no. YY, pp. ZZ-ZZ, 2023. lww.com
19. A. Vaidya, J. L Severens, B. WC Bongaerts, K. BJM Cleutjens et al., "High-sensitive Troponin T assay for the diagnosis of acute myocardial infarction: an economic evaluation," 2014. ncbi.nlm.nih.gov
20. N. N. Senadeera, C. B. Ranaweera, I. C. Perera, "Biochemical Insights and Clinical Applications of Ischemia-Modified Albumin in Ischemic Conditions," 2024. 192.248.104.6
21. S. Zoroddu, A. Zinellu, C. Carru, and S. Sotgia, "Analytical Insights into Methods for Measuring Ischemia-Modified Albumin," Molecules, 2024. mdpi.com
22. M. Jiang, Q. Chen, Q. Feng, X. Peng, J. Liu, "Incremental Prognostic Value of Admission Blood Glucose to Albumin Ratio in Patients with Acute Coronary Syndrome: A Retrospective Observational Cohort …," Reviews in …, 2025. nih.gov
23. Y. Yamamoto, N. Kanayama, Y. Nakayama, and N. Matsushima, "Current Status, Issues and Future Prospects of Personalized Medicine for Each Disease," 2022. ncbi.nlm.nih.gov
24. J. Renato Rebello Pinho, R. Sitnik, and C. Luis Pitangueira Mangueira, "Personalized medicine and the clinical laboratory," 2014. ncbi.nlm.nih.gov
25. D. Abdelhafiz, T. Baker, D. A. Glascow, et al., "Biomarkers for the diagnosis and treatment of rheumatoid arthritis–a systematic review," Postgraduate Medicine, 2023. [HTML]
26. K. D. Deane and V. M. Holers, "Rheumatoid arthritis pathogenesis, prediction, and prevention: an emerging paradigm shift," Arthritis & Rheumatology, 2021. nih.gov