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*Review Article*

# An integrated approach to coronary heart disease diagnosis and clinical management

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**Abstract:** The major issue in coronary heart disease (CHD) diagnosis and management is that symptoms onset in an advanced state of disease. Despite the availability of several clinical risk scores, the prediction of cardiovascular events is lacking, and many patients at risk are not well stratified according to the canonical risk factors alone. Therefore, adequate risk assessment remains the most challenging issue. Recently, the integration of imaging data with biochemical markers in a radiogenomic framework has been proposed in many fields of medicine as well as in cardiology. Multimodal imaging and advanced processing techniques can provide both direct (e.g., remodeling index, calcium score, total plaque volume, plaque burden) and indirect (e.g., myocardial perfusion index, coronary flow reserve) imaging features of CHD. Furthermore, the identification of novel non-invasive biochemical markers, mainly focused on plasma and/or serum samples, has increased the specificity of findings, reflecting several pathophysiological pathways of atherosclerosis, the principal actor in CHD. In this context, a multifaced approach, derived from the strengths of all these modalities, appears promising for finer risk stratification and treatment strategies, facilitating the decision-making and clinical management of patients. This review underlines the role of different imaging modalities in the quantification of coronary atherosclerosis and describes novel blood-based markers that could improve diagnosis and have a better predictive value in CHD.

**Keywords:** Atherosclerosis, coronary heart disease, imaging, biomarkers

## Introduction

Cardiovascular diseases (CVDs) are the primary cause of mortality worldwide with 17.3 million deaths per year, and an estimation of 23.6 million in 2030, placing it as a relevant issue for the public health system [1, 2]. Coronary heart disease (CHD) is the largest contributor of CVDs and mortality rate is due in prevalence to atherosclerosis, a chronic inflammatory condition of the arterial wall. Unfortunately, myocardial infarction (MI) is still a first common manifestation of CHD and, in about 50% of patients, angina pectoris is the first symptom of the pathology [1]. For this reason, an accurate and prompt diagnosis in CHD patients could improve prognosis and/or quality of life and allow timely and adequate therapeutic treatments (percutaneous or surgical myocardial revascularization, pharmacological therapy). Furthermore, efforts should be focused on primary prevention or early detection of subjects suffering from coronary atherosclerosis, in order to implement therapeutic strategies, so reducing morbidity, health expenditure, and mortality [3-6]. The risk of clinical manifestations of CHD is currently estimated according to multifactorial integrated prediction models developed on the basis of population studies that have allowed to evaluate the likelihood of cardiac events, even though some of them have a poor predictive value [7-13]. Imaging techniques have deeply increased early detection of CHD, although the invasive approach restricts their feasibility mainly to symptomatic patients. Among them, for their higher spatial resolution, intravascular

ultrasound (IVUS), X-ray angiography (XRA), and computed tomography coronary angiography (CTCA) provide a direct evaluation and quantification of coronary artery alteration, while cardiac magnetic resonance (CMR) and nuclear medicine techniques (single photon computed tomography (SPECT), and positron emission tomography (PET)) provide indirect information of CHD, estimating myocardial perfusion and metabolism abnormalities that are consequent to coronary artery disease [14]. In addition, serum/plasma biomarkers can be mini-invasively extracted also in asymptomatic patients in order to analyze at different levels (e.g., cellular, biochemical, epigenetic and/or transcriptional) atherosclerosis and CHD development.

This review underlines the role of different imaging modalities in the setting of coronary atherosclerosis and describes novel blood-based markers that could improve diagnosis and have a better predictive value in CHD.

## Imaging markers for direct CHD diagnosis

Several imaging techniques have been developed and used extensively over the last years in order to exclude and/or detect CHD with the aim to guide optimal patient management.

IVUS employs a miniature ultrasound probe placed at the tip of a coronary catheter; the signals received according to the different acoustic impedance are reconstructed into a real-time tomographic gray-scale image [15]. In coronary arteries, two borders are well defined: the blood-intimal border and the external elastic membrane (EEM). Some morphologic features of atherosclerotic coronary plaques can be invasively assessed by this tool such as soft, fibrous, calcified, necrotic and lipid components. The reliability of ultrasound imaging in predicting the composition of atherosclerotic plaque components has been demonstrated in histological comparative studies [16]. Indeed, IVUS allows to clearly depict the vascular wall and accurately calculate the remodeling index.

Many clinical trials have disclosed significant improvements in patient outcomes and reduced complications [17, 18]. Meanwhile, plenty of clinical pharmacological trials employs IVUS to demonstrate its beneficial effects because this technique offers great value for the precise quantification of atherosclerosis progression or regression [19].

Differently, XRA is a procedure that uses a special dye (contrast medium) and x-rays to selectively highlight the arterial lumen (lumenogram), allowing the identification of caliber alterations, like stenoses/occlusions or aneurysms, and, at the same time, to treat these conditions. This technique is a reliable reference standard for the estimation of the real coronary lumen and intracoronary pressure measurement for the detection of functionally relevant stenoses and fractional flow reserve (FFR) calculation. However, XRA is an invasive technique like IVUS, but unlike it, it is not convincing with regard to prognostic value [20]. Moreover, conversely to other imaging techniques for direct CHD diagnosis, XRA does not allow the evaluation of vascular wall, and CHD is largely not a disease of the lumen itself but an abnormality of the vessel wall [21].

CTCA has overcome several of previous technique drawbacks, allowing an accurate quantitative evaluation of the arterial lumen, vessel wall and the extension, severity and composition of the atherosclerotic plaque [14, 22]. A first level of evidence that can be extracted by CTCA is represented by the detection of coronary calcium deposits and the assessment of lumen stenosis [23]. Calcium burden is usually quantified using the 'Agatston score', an aggregate score across entire vessel territories rather than across specific atherosclerotic plaques, and mainly related to risk stratification of asymptomatic patients [24], than of symptomatic ones [25]. The amount of calcium correlates roughly to the total amount of coronary atherosclerosis but the correlation with the degree of luminal narrowing is poor. Even with severe calcifications, luminal stenosis is not necessarily present and a 'zero' calcium score cannot be used to rule out coronary artery stenosis in symptomatic individuals, especially in young subjects and in individuals with acute symptoms [24, 26]. This evidence can be described by the remodeling index, a CTCA parameter used to define the main grow direction of plaque toward the inner or outer layers of the vascular wall and not evaluable by XRA technique [27]. Moreover, atherosclerotic plaques are often composed by both calcific and non-calcific components (mixed plaque), a feature highly predictive for CHD complications and easily quantifiable (e.g., total plaque volume, calcific plaque volume, non-calcific plaque vol-

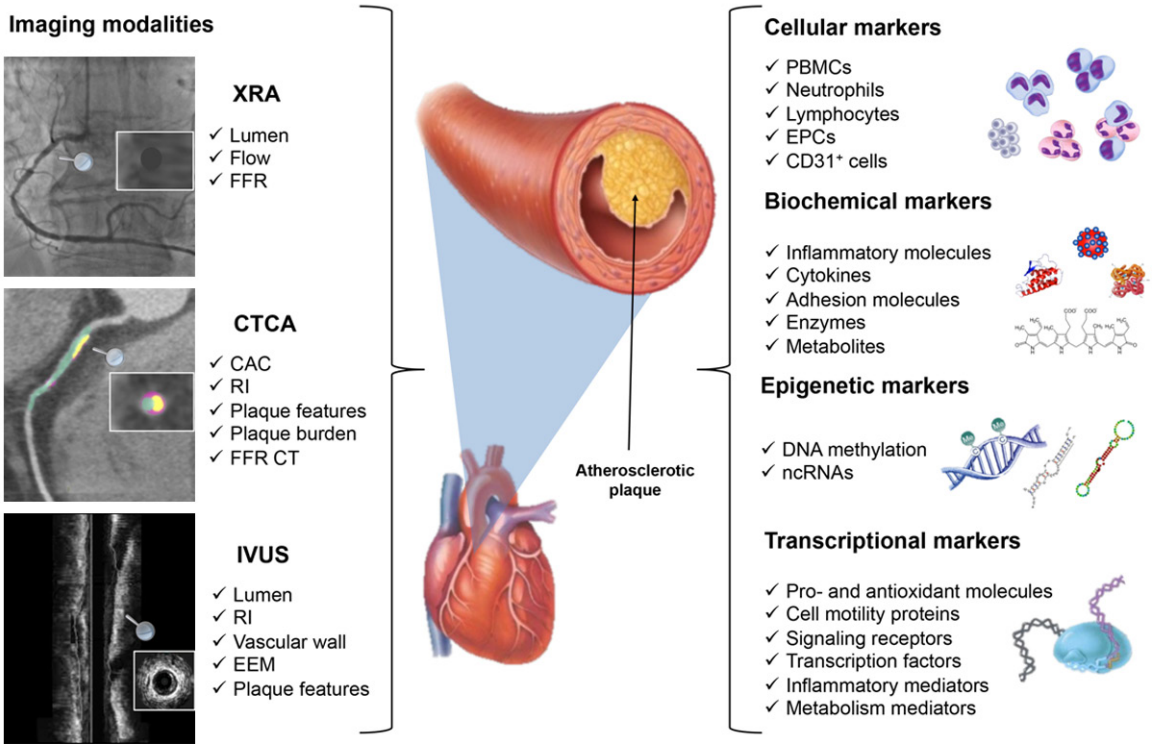


**Figure 1.** Integrated diagnostic approach in coronary heart disease. The figure shows the imaging modalities for direct assessment of coronaries and the diversity of circulating biomarkers that could be associated with the diagnosis, outcome prediction and risk assessment in coronary heart disease (CHD). The left panel represents X-ray angiography (XRA), coronary computed tomography angiography (CTCA) and intravascular ultrasound (IVUS) in coronary imaging and respective parameters. For XRA: luminal narrowing, blood flow and fractional flow reserve (FFR); for CTCA: coronary artery calcium (CAC), remodeling index (RI), plaque features, plaque burden and FFR CT; for IVUS: lumen, RI, vessel structure, external elastic membrane (EEM) and plaque features. The right panel depicts markers that can be analyzed in serum/plasma or whole blood and reflect coronary artery alterations: cellular: peripheral blood mononuclear cells (PBMCs), neutrophils, lymphocytes, endothelial progenitors cells (EPCs), CD31+ cells; biochemical markers: inflammatory pathways, cytokines, adhesion molecules, enzymes and metabolites; epigenetic markers: DNA methylation levels and differential expression of circulating non-coding RNAs (ncRNAs); and transcriptional markers: genes coding for pro-and antioxidant molecules, cell motility proteins, signaling receptors, transcription factors, inflammatory and metabolic mediators.

ume) by advanced CTCA scanners, like dual energy CT or spectral CT [28, 29]. These state-of-art computed tomographies, thanks to improved spatial and temporal resolution, better tissue characterization, and lowering of dose and iodine load, have a wide range of applications also in patients with high or irregular heart rate, encompassing from myocardial perfusion, coronary blood flow velocity and pressure and non-invasive FFR assessment by the application of the computational fluid dynamics and mathematical models [30]. In conclusion, among the imaging techniques for direct CHD diagnosis, CTCA is the unique that allows a "simultaneous" analysis of the entire coronary tree at a low dose of contrast medium load, proposing this tool as suboptimal for early diagnosis and CHD risk assessment (**Figure 1**).

### Imaging markers for indirect CHD diagnosis

CMR provides excellent soft tissue contrast, allowing functional, perfusional, morphological and anatomical evaluations, without exposing the patient to ionizing radiation, like both XRA and CTCA [14]. Recently, CMR has been applying also to direct anatomical coronary visualization, although it is still limited in the visualization of distal coronary segments due to inferior spatial resolution, compared to CTCA [31]. Several applications for the assessment and follow-up of patients with CHD include the estimation of myocardial viability thanks to $T_1$ mapping, $T_2$ mapping and the monitoring of left ventricle remodeling after acute myocardial infarct through diffusor tensor imaging besides first pass perfusion CMR and delayed contrast enhancement [32].

Nuclear medicine imaging by SPECT and PET, instead, provide, through the injection of a radiotracer, functional and perfusional analysis, the evaluation of ischemia and blood flow quantification [33, 34]. Myocardial perfusion imaging triggered by ECG monitoring allows also consideration of wall motion and thickening; moreover, changes in blood flow are considered positive for suspected CHD. Cardiac PET is useful for perfusion imaging, functional evaluation and assessment of myocardial viability. For perfusion imaging, commonly used PET radiotracers are $^{82}$Rubidium, $^{13}$Nitrogen-ammonia and $^{15}$Oxygen-water. $^{18}$F-FDG is used for diagnosis of myocardial viability and it is considered the most sensitive modality for predicting left ventricular functional recovery post-coronary revascularization. In particular, the integration of perfusion and viability, detected by PET, allows the identification of myocardial stunning (normal perfusion but reduced metabolism) and myocardial hibernation (reduced perfusion but preserved metabolism) [35].

## *In vitro* biomarkers for CHD diagnosis and risk prediction

Several studies focused on the introduction of multiplex biomarker assays analyzing different circulating molecules and their integration with current clinical scores in multivariable prediction model for a better diagnosis and risk stratification [36, 37]. Concerning *in vitro* biomarkers, many molecules can be extracted from serum and/or plasma of asymptomatic subjects and CHD patients that could reflect at different levels (e.g., cellular, biochemical, epigenetic and/or transcriptional) atherosclerosis and coronary alterations (**Figure 1**).

### *Cellular markers*

Circulating cells have been proposed as blood-derived biomarkers in CHD in the pathogenesis of CHD, secreting a wide plethora of specific biomarkers [38]. Several studies showed an increased number of monocytes in the blood of patients with atherosclerosis and in subjects with cardiovascular risk factors [38, 39]. The multiple roles of monocytes in the atherogenic process are attributed to the existence of subpopulations characterized by the expression of different surface markers with consequent functional changes and response to stimuli as well as alterations in gene expression [40-42]. Circulating neutrophil count and neutrophil/lymphocyte ratio are also emerging markers of the presence and severity of CHD, correlating with plaque vulnerability [43, 44]. In peripheral blood mononuclear cell (PBMCs) of CHD patients, were found a set of 190 genes differentially expressed compared to healthy controls [45]. Another heterogeneous class of cells involved in CVDs is represented by the endothelial progenitor cells (EPCs). Several studies have reported a variation of cell number and a functional impairment in patients with CHD strictly dependent on the presence of cardiovascular risk factors and associated with the severity of coronary lesions and clinical outcome [46-48]. Finally, Kim et al. performed a flow cytometry analysis of CD31(+) cells from the peripheral blood of CHD patients showing a significant correlation between CD31(+) cell count and the number of atherosclerotic vessels [49] (**Table 1**).

### *Biochemical biomarkers*

Inflammatory biomarkers appear to have an important prognostic value in patients with CVDs and may be useful in the diagnosis of apparently healthy subjects without known CHD who cannot be assessed with conventional risk factors. In the last years, several biomarkers have been identified, although not for everyone there are studies that show a possible correlation with instrumental imaging parameters.

In particular, the group of biomarkers of interest includes: transforming growth factor beta (TGF-β1) [50]; cellular adhesion molecules (CAMs) [51, 52]; monocyte chemoattractant protein-1 (MCP-1) [53-55]; stromal cell-derived factor-1α (SDF-1α) [56-58]; lectin-like oxidized low density lipoprotein receptor 1 (LOX-1) [59-61]; pentraxin 3 (PTX3) [62-64]; bilirubin [65-67] and haemoglobin A1c (HbA1c) [68, 69], whose serum and/or plasmatic levels were associated with the presence and severity of CHD.

In addition, among emerging biomarkers, we selected: serum amyloid A (SAA) proteins [70, 71]; fibrinogen [72, 73]; myeloperoxidase (MPO) [74-77]; paraoxonase-1 (PON1) [78]; matrix metalloproteinases (MMPs) [79, 80]; proprotein convertase subtilisin/kexin type 9 (PCSK9) [81-83]; lipoprotein-associated phospholipase A2 (Lp-PLA2) [84, 85]; retinol-binding protein-4 (RBP4) [86, 87]; angiopoietin-like 4 (ANGPTL4) [88] and sphingolipids [89-91] that could be

**Table 1.** Cellular markers associated to CHD diagnosis and prognosis

| Biomarker | Source | Regulation | Clinical role | Ref. |
|---|---|---|---|---|
| CD14++CD16-CCR2+; CD14++CD16+CCR2+; CD14+CD16++CCR2- monocytes | PBMCs | Reduced expression of CD14 and CD14+CD16++CCR2- sub-population in CHD | Diagnostic | [40] |
| CD4+CD28null T cells | PBMCs | CD4+CD28null population from RF and ACS groups had higher expression levels of cytotoxic molecules | Predictive for treatment | [41] |
| CD3+/CD31+ T cells | PBMCs | Increased levels in ACS patients | Predictive after PCI | [42] |
| Neutrophil/Lymphocyte ratio | Leucocytes | Increased N/L ratio associated with severity of CHD and plaque vulnerability | Diagnostic/Predictive of long term outcome | [43, 44] |
| EPCs | PBMCs | Number reduction and functional impairment in CHD patients; cell count dependent on the number of RF; associated with the severity of coronary lesions and less sub-stent plaque burden | Diagnostic/Predictive of future CV events and PCI follow-up | [46-48] |
| CD31(+) cells | Blood | Increased CD31(+) cells in UA patients; significant correlations between cell count and the number of atherosclerotic coronaries | Diagnostic/Predictive of UA | [49] |

Abbreviations: CHD = Coronary heart disease; RF = risk factor; ACS = Acute coronary syndrome; PBMC = Peripheral blood mononuclear cell; PCI = Percutaneous coronary intervention; UA = Unstable angina.

potential biomarkers for the assessment of CHD severity. Indeed, these emerging biochemical markers could potentially find a useful application for the diagnosis and prognosis assessment in CHD patients. Furthermore, several works focused on the introduction of multiplex biomarker assay analyzing different circulating molecules and their integration in new grading score for a better diagnosis and risk stratification [55, 92-96] (**Table 2**).

*Epigenetic markers*

Epigenetic modifications are involved in a variety of pathological conditions as well as in CVDs and atherosclerosis [4-6, 97-101]. Epigenetic mechanisms include DNA methylation, histone modifications, and regulation by non-coding RNA (ncRNA) [102, 103]. Several studies evaluated the methylation status of genomic DNA from blood cells in CHD and ACS patients and in subjects with cardiovascular risk factors, observing a different methylation pattern, as well as a methylation signature predictive of increased risk of cardiac events, ischemic heart disease, stroke, and mortality [104-108].

Furthermore altered specific DNA methylation has been reported in several promoters, particularly in genes related to inflammation [109, 110], coagulation [111], hypertension [112], glucose [113, 114] and lipid metabolism [115, 116].

In addition, regulatory T (Treg) cells have also been shown to play a protective role in atherosclerosis avoiding plaque rupture. DNA demethylation of the transcription factor Forkhead box P3 (FOXP3) gene was found to be essential for the maintenance of the suppressive properties of this cellular subtype. Indeed, an increase of FOXP3 methylation was observed in cultures of PBMCs obtained from ACS patients compared to controls [117] (**Table 3**).

MicroRNAs (MiRNAs) are short noncoding single-strand RNAs with the function of inhibiting gene expression through mRNA degradation or translational repression [118]. MiRNAs are stable in several bodily fluids, so several studies focused on the identification of these molecules in plasma specimens as well as in blood cells with the aim to correlate their expression with CHD [119, 120].

**Table 2.** Biochemical markers associated to CHD diagnosis and prognosis

| Biomarker | Source | Regulation | Clinical role | Ref. |
|---|---|---|---|---|
| TGF-β1 | Serum | High levels in AMI patients compared to SA and UA | Diagnostic/Risk stratification | [50] |
| sVCAM-1/sICAM-1 | Serum | Higher levels in UA patients | Predictive of ACS | [51, 52] |
| MCP-1 | Plasma | Positive correlation with the extent of coronary atherosclerosis in UA patients detected by RXA; high concentrations in ACS patients | Diagnostic/Predictive of increased risk for death or AMI | [53-55] |
| SDF-1α | Plasma | Reduction in STEMI patients compared to SA or to NSTEMI; association with disease severity | Diagnostic/Predictive of negative outcome | [56-58] |
| LOX-1 | Serum | Increased concentrations in CHD patients in association with disease severity | Diagnostic/Predictive of increased risk of CHD | [59-61] |
| PTX3 | Plasma | Biomarker of plaque vulnerability | Diagnostic | [62-64] |
| Bilirubin | Serum | Lower levels in patients with critical stenosis and non-calcified/mixed plaques detected by CTCA | Diagnostic/Predictive of risk stratification, CHD onset and long term-mortality | [65-67, 162] |
| HbA1c | Plasma | High levels associated to CAC and CHD severity in non diabetic patients | Diagnostic/Predictive | [68, 69] |
| SAA | Plasma | Elevated levels in CHD patients | Predictive of disease risk and worse prognosis | [70,71] |
| MPO | Plasma | Marker of plaque instability and disease severity | Diagnostic | [74-77] |
| PON1 | Plasma | Reduced activity in CHD patients | Diagnostic/Predictive | [78] |
| MMP-9/MMP-8 | Plasma/Serum | Biomarkers of plaque instability | Diagnostic | [79, 80] |
| PCSK9 | Serum | High concentrations are associated with CAC and disease severity | Diagnostic/Predictive of CV events | [81-83] |
| Lp-PLA2 | Plasma | High levels associated with CHD severity | Diagnostic/Predictive of CHD and mortality | [84, 85] |
| RBP4 | Serum | High levels associated with coronary lesion severity in stable CHD and ACS | Diagnostic/Predictive of CV events | [86, 87] |
| ANGPTL4 | Plasma | High levels in patients at risk | Predictive of CV event occurrence | [88] |
| Sphingolipids | Plasma | Positively related to CHD and subclinical atherosclerosis | Predictive of CV events | [89-91] |
| hs-TnT/hs-CRP | Serum | Associated with CHD burden and change in plaque composition detected by CCTA | Diagnostic | [154] |
| IL-10 | Serum | Reduced levels in patients with ACS | Diagnostic/Predictive of long-term adverse outcomes | [155, 156] |
| IL-8 | Serum | High levels in patients in CHD patients | Predictive of long-term outcome | [157] |
| IL-6 | Plasma/Serum | High concentration in patients with multivessel atherosclerosis and calcified plaque assessed by CCTA | Diagnostic | [158] |
| Adiponectin | Serum | Low levels associated with lipid-rich, non-calcified plaques and multivessel CHD by CTCA | Diagnostic | [159-161] |

Abbreviations: ACS = Acute coronary syndrome; CAC = Coronary Artery Calcium; CHD = Coronary heart disease; CTCA = Coronary Computed Tomography Angiography; SA = stable angina; STEMI = ST-elevation myocardial infarction; NSTEMI = non-ST-elevation myocardial infarction; AMI = acute myocardial infarction; UA = unstable angina.

Circulating levels of specific endothelial cell, smooth muscle cell and inflammation associated miRNAs, were significantly reduced in patients with stable CHD, while cardiac-specific miRNAs were upregulated [121-123].

Several studies also showed the important role of miRNA signature in vulnerable CHD, particularly for their ability to discriminate patients with UA from those with SA, suggesting that these circulating molecules could be useful to identify patients at risk for ACS and predict the clinical outcome [124-127].

Serum levels of miR-31 were higher in CHD patients with in stent restenosis compared to patients without stent restenosis and miR-214 downregulation was linked to disease severity

**Table 3.** Epigenetic markers associated to CHD diagnosis and prognosis

| Biomarker | Source | Regulation | Clinical role | Ref. |
|---|---|---|---|---|
| Global DNA methylation | Lymphocytes | Hypermethylation in patients related to hyperhomocysteinemia | Diagnostic | [104] |
| LINE-1 | Leucocytes | Lower methylation in CHD patients | Diagnostic/Predictive of increased risk of acute events and mortality | [105] |
| Alu/Sat2 | Leucocytes | High methylation in CHD patients with cardiovascular risk factor | Diagnostic | [106] |
| Global DNA methylation | Leucocytes | Hypermethylated regions in patients with hyperhomocysteinemia | Diagnostic | [107] |
| Global DNA methylation | Leucocytes | Identification of 47 CpG islands associated with ACS | Diagnostic | [108] |
| PLA2G7 | Leucocytes | High promoter methylation in CHD patients | Diagnostic/Predictive of CHD risk gender and age specific | [109] |
| PTX3 | Leucocytes | Lower promoter methylation in CHD group | Diagnostic | [110] |
| Factor VII | PBMCs | Promoter hypomethylation in CHD patients | Diagnostic | [111] |
| HSD11B2 | PBMCs | Promoter methylation associated with hypertension | Diagnostic | [112] |
| INS/GNASAS | Leucocytes | Locus hypermethylation in AMI patients | Predictive of risk of AMI in women | [113] |
| GCK | Leucocytes | Hypomethylation in CHD patients compared to controls | Predictive of risk of CHD onset | [114] |
| ABCA1 | Leucocytes | Higher methylation in CHD patients associated with low HDL; association with aging and CHD in men | Diagnostic | [115, 116] |
| FOXP3 | Regulatory T cells | Increased methylation in ACS patients | Diagnostic | [117] |
| Microarray | Plasma/Serum | Downregulation: miR-17-92 cluster, -126, -145, -155; Upregulation: miR-133, -208a associated with CHD severity | Diagnostic | [121-123] |
| Microarray | PBMCs | Upregulation of miR-134, -135a, -147, -198, -370 in UA compared to SA patients | Diagnostic/Predictive of acute events | [124] |
| Microarray | Plasma | Upregulation of miR-1, -126, and -483-5p in SA patients vs. controls; upregulation miR-1, -126, and -133a in UA patients vs. controls | Diagnostic | [125] |
| Microarray | Plasma | Overexpression of miR-106b/25 cluster, miR-17/92a cluster, miR-21/590-5p family, miR-126* and miR-451 in patients with vulnerable CHD | Diagnostic | [126] |
| mir-197/mir-223 | Serum | Elevated levels in CHD patients | Predictive of cardiovascular death | [127] |
| miR-31 | Serum | Higher levels in CHD patients with restenosis compared to patients without restenosis and in healthy controls | Diagnostic | [128] |
| miR-214 | Plasma | Circulating levels related to the severity of coronary stenosis | Diagnostic | [129, 130] |
| Realtime PCR | Plasma | Upregulation of miR-122 and miR-370 in hyperlipidemic CHD patients; association of increased levels of miR-122 and miR-370 with disease severity | Diagnostic | [131] |
| Microarray | Plasma | Low levels of miR-145, miR-155 and let-7c levels in CHD patients compared to controls | Diagnostic | [132] |
| Realtime PCR | Plasma | High levels of miR-17-5p are associated with CHD severity | Diagnostic | [133] |
| Microarray | Platetets | Upregulation of miRNA340* and miRNA624 in patients with CHD | Diagnostic | [134] |
| Realtime PCR | Plasma/PBMCs | Downregulation of miR-155 and miR-146a/b during ACEI/ARB treatment | Predictive markers of CHD risk and treatment efficacy | [135, 136] |
| EPCs | PBMCs | Upregulation of miR-221, miR-222 and miR-92a; increased EPC number and decreased miR-221/222 levels after atorvastatin therapy | Predictive markers of treatment efficacy | [137, 138] |

Abbreviations: ACEI = Angiotensin converting-enzyme inhibitor; ARB = Angiotensin II receptor blocker; CHD = Coronary heart disease; PBMC = Peripheral blood mononuclear cells; AMI = acute myocardial infarction; SA = stable angina; UA = unstable angina; EPC = endothelial progenitor cell.

[128-130]. In addition, other studies also showed that miRNA alterations are associated with disease severity [131, 132].

Furthermore, miR-17-5p overexpression was reported to be an independent factor associated with the severity of atherosclerosis [133].

Platelets play an important role, both during plaque rupture and in the formation of atherosclerotic plaque. Indeed, miRNA expression profiles of platelets from patients with premature CHD revealed an upregulation of miR-340* and miR-624* in patients compared to healthy controls [134].

**Table 4.** Transcriptional markers associated to CHD diagnosis and prognosis

| Biomarker | Source | Regulation | Clinical role | Ref. |
|---|---|---|---|---|
| Microrray | PBMCs | Upregulation of cytokine and EGR family genes; EGR1 levels able to discriminate ischemic and non-ischemic CHD patients | Diagnostic | [140] |
| Whole-genome microarray | PBMCs | Differential expression of 14 genes related to coronary stenosis | Diagnostic | [141] |
| Array | PBMCs | 160 differently expressed genes in patients; expression signature correlated with the severity of CHD and gene expression in vascular tissues | Diagnostic | [142] |
| Myocardin/GATA4/Nkx2.5 | PBMCs | Higher transcriptional levels in patients in relation to disease severity | Diagnostic | [143] |
| Nrf2 | PBMCs | Lower gene expression in patients than control subjects | Diagnostic | [144] |
| Homer1/IL-1β/TNF-α | Leucocytes | Higher mRNA levels in CHD patients compared to controls | Diagnostic | [145] |
| MT-COI | Monocytes | Low mRNA levels in patients at risk | Predictive of CV events | [146] |
| MSH2/XRCC1/ATM | PBMCs | Upregulation in diabetic CHD patients | Diagnostic | [147] |
| Microarray | Leucocytes | Downregulation of COX7C, ATP5I NDUFA1 and CASP3 | Predictive markers after cardiac rehabilitation | [148] |
| Microarray | Monocytes | Downregulation of ABCA1, ABCG1 and RGS1, upregulation of ADRB2 and FOLR3 | Diagnostic | [149] |
| GES score | Whole blood | GES score is associated with plaque volume and phenotype by IVUS and atherosclerotic plaque burden and stenosis by CTCA | Diagnostic | [152, 153] |

Abbreviations: EGR = Early growth response; CHD = Coronary heart disease; PBMCs = Peripheral blood mononuclear cell.

The pattern of miRNA expression is also influenced by therapeutic treatments, suggesting important implications for patient management. In whole blood samples of CHD patients and controls, 11 miRNAs were significantly down-regulated in CHD group. Particularly, miR-155, which is known to target the AT1 receptor, was found to be associated with ACEI/ARB use [135]. Furthermore, miR-146a/b were higher in PBMCs of CHD patients but under angiotensin II receptor blocker inhibitors and statin treatments, their expression levels decreased [136]. Interestingly, also lipid lowering therapy with statins is able to influence miRNA expression in CHD [137, 138] (**Table 3**).

*Transcriptional markers*

Genome-wide gene expression profiling is a promising strategy for the identification of novel disease biomarkers [139]. Several studies on gene expression profiling of blood cells identified a differential transcriptional signature in CHD patients and healthy subjects. Particularly, the major alterations were discovered in genes codifying for pro- and antioxidant molecules, cell motility proteins, signaling receptors, transcription factors, inflammatory molecules and mediators. Interestingly, the expression pattern was found to correlate with the severity of CHD and gene expression in vascular tissues, indicating a mirroring between circulating cells and changes in the atherosclerotic vessel wall [140-146]. Transcriptional deregulations were also found in genes involved in DNA repair, as reported in a recent study by Ahmadi el al. [147].

Gene expression changes may reflect not only the presence and activity of disease but also environmental modifier effects, as well as treatment response. Indeed, in a study by Taurino et

al. 365 differentially expressed genes were found in patients with CHD vs. healthy controls (175 genes were upregulated, and 190 genes were downregulated). Furthermore, in a group of patients was analyzed whole-blood gene expression before and after a cardiac rehabilitation following surgical coronary revascularization. In patients underwent rehabilitation, were found 645 genes differentially expressed at the beginning and the end of the program, with 196 genes upregulated and 449 genes downregulated. The expression levels of genes involved in oxidative phosphorylation and mitochondrial dysfunction were higher in CHD patients compared to control subjects. Completion of the rehabilitation treatment was characterized by a downregulation of the genic signature of oxidative stress and mitochondrial impairment [148]. Furthermore, a whole genome expression analysis identified six genes with a differential expression in monocytes of patients versus controls; ABCA1, ABCG1, and RGS1 were downregulated in patients, whereas ADRB2 and FOLR3 were upregulated compared to matched controls and this expression pattern was influenced by aspirin and statin therapy [149] (**Table 4**).

### Integration of biomarkers and direct imaging features in CHD

While several studies have tested circulating biomarkers in imaging-detected CHD (only recently by CTCA), some authors have tried to correlate imaging features to serum/plasma biomarkers, in order to increase diagnostic power and risk stratification of patients with coronary alterations.

In a multicenter study a gene expression score (GES) based on age, sex and the expression levels of 23 genes in peripheral blood cells was developed to assess the likelihood of CHD in non diabetic patients [150]. A subsequent study showed that GES could be a more accurate predictor of obstructive CHD compared to clinical estimation scores [151].

In the Atlanta study, Joshi et al. correlated the validated GES score and coronary plaque composition by IVUS with radiofrequency backscatter analysis (IVUS/VH). GES was significantly associated with plaque volume, necrotic core composition, and dense calcium. Data suggested that this composite gene expression score is not only predictive of obstructive coronary artery disease, as previously reported, but also predictive of larger atherosclerotic plaque burden with a more vulnerable phenotype [152]. GES was also significantly associated with plaque burden and luminal stenosis assessed by CTCA. Indeed, in a study on 610 patients, Voros et al. reported a significant association between GES score, plaque burden expressed by coronary artery calcium and stenosis severity reported as score index. Particularly, GES significantly correlated with maximum luminal stenosis and segment stenosis score index. A low score had a sensitivity of 0.90 and a high score a specificity of 0.87 for stenosis ≥ 70% [153].

C-reactive protein (CRP) is the most extensively studied systemic marker of inflammation. A recent study by Seifarth et al. has shown that plasmatic levels of high-sensitivity C-reactive protein (hs-CRP) and high-sensitivity troponin T (hs-TnT) are weakly associated with a significant increase in CHD burden and change in plaque composition detected by CTCA using a semiquantitative crosssection-based score in a 2-year follow-up [154].

Several studies showed also the involvement of interleukins in plaque instability and their possible predictive value of cardiovascular events in long-term outcome [155-157]. Indeed, in a recent study Harada K. et al. evaluated the association between inflammatory markers and coronary artery plaque parameters assessed by CTCA on 220 subjects with suspected CHD showing that circulating levels of hs-CRP and IL-6 were significantly higher in CHD patients. Particularly, plasma IL-6 concentration was significantly associated with 4-9 segment plaques compared to patients without or with 1-3 segments. On the other hand, plasma hs-CRP level was associated with the presence of calcified plaque, independently of traditional cardiovascular risk factors [158].

MCP-1 plays a key role in the recruitment of monocytes to sites of inflammation, promoting the initiation of the fatty streak, plaque instability, as well as remodeling after MI. A positive correlation between circulating MCP-1 levels and the extent of coronary atherosclerosis, expressed by an angiographic severity score, was found in patients with UA. These findings suggest that circulating MCP-1 concentration likely reflects the coronary wall damage [53].

Adiponectin is a protein hormone playing a protective role of vascular walls from atherosclerosis. Several studies showed a significant inverse correlation between multivessel CHD and lipid-rich non-calcified plaques assessed by CTCA and serum levels of adiponectin independently by other significant risk factors [159-161]. Although bilirubin has long been considered a waste product, it is currently recognized as an endogenous antioxidant molecule, involved in the attenuation of lipid peroxidation and playing an anti-atherogenic role. In a study on 1151 patients the association between total serum bilirubin levels and presence, severity and plaque composition evaluated by CTCA was determined. Data showed that subjects with primarily non-calcified plaques and mixed plaques had lower bilirubin levels compared to patients with calcified plaques and normal subjects. Furthermore, serum total bilirubin levels were found to be lower in patients with any coronary plaque [162].

## Conclusions and future perspectives

The need to improve diagnosis and risk prediction has prompted the search for novel markers in cardiovascular medicine. Literature data suggest that CTCA could substantially reduce the number of invasive procedures, increasing the safety of patients, and allows a more precise planning of potential treatment options. Furthermore, strong evidence has also emerged on the usefulness of coronary calcium score assessed by CTCA. In association with imaging improvements, novel high-throughput platforms investigating proteomic, metabolomic, epigenomic, and transcriptomics profiles together with genome-wide association studies may generate "multimarker CHD scores" with a higher predictive power than the use of a single biomarker. Surrogate biomarkers of coronary atherosclerosis and advanced imaging techniques could represent important cornerstones to characterize sub-clinical and clinical atherosclerosis with a consequent facilitation in the decision-making and clinical management of patients.

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## Disclosure of conflict of interest

None.

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