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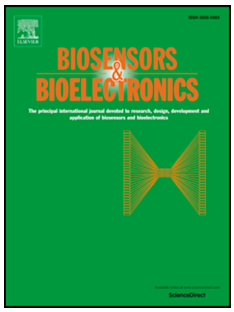

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A Review of Biosensor Technologies for Blood Biomarkers Toward Monitoring Cardiovascular Diseases at the Point of Care

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ABSTRACT

Cardiovascular diseases (CVDs) cause significant mortality globally. Notably, CVDs disproportionately negatively impact underserved populations, such as those that are economically disadvantaged and often located in remote regions. Devices to measure cardiac biomarkers have traditionally been focused on large instruments in a central laboratory but the development of affordable, portable devices that measure multiple cardiac biomarkers at the point-of-care (POC) are needed to improve clinical outcomes for patients, especially in underserved populations. Considering the enormity of the global CVD problem, complexity of CVDs, and the large candidate pool of biomarkers, it is of great interest to evaluate and compare biomarker performance and identify potential multiplexed panels that can be used in combination with affordable and robust biosensors at the POC toward improved patient care. This review focuses on describing the known and emerging CVD biosensing technologies for analysis of cardiac biomarkers from blood. Initially, the global burden of CVDs and the standard of care for the primary CVD categories, namely heart failure (HF) and acute coronary syndrome (ACS) including myocardial infarction (MI) are discussed. The latest United States, Canadian and European society guidelines recommended standalone, emerging, and add-on cardiac biomarkers, as well as their combinations are then described for the prognosis, diagnosis, and risk stratification of CVDs. Finally, both commercial *in vitro* biosensing devices and recent state-of-art techniques for detection of the cardiac biomarkers are reviewed that leverage single and multiplexed panels of cardiac biomarkers with a view toward affordable, compact devices with excellent performance for POC diagnosis and monitoring.

KEYWORDS: Biosensors, point-of-care monitoring, commercial diagnostic devices, cardiovascular disease, cardiac biomarkers, multiplexed biomarker panel

1. Introduction

Cardiovascular diseases (CVDs) are the leading global cause of death, accounting for 17.6 million deaths in 2016, including 840,678 deaths in the United States (Benjamin et al., 2019). The American Heart Association (AHA) projected that by 2035, 45.1% of the US population would have some form of CVD, and the total costs of CVDs are expected to reach $1.1 trillion (RTI International, 2016).

CVDs are known to disproportionately negatively impact underserved populations, such as those in economically disadvantaged regions, and these underserved populations often correspond to regions with large minority populations. The 2019 AHA statistics show that the presence of ideal cardiovascular health varies by race and ethnicity, where African Americans and Hispanics tend to have fewer metrics at ideal levels than other groups (Benjamin et al., 2019). Furthermore, African Americans are reported to have a higher CVD mortality than whites (Carnethon et al., 2017). These disparities have been largely attributed to healthcare system factors including access to care, differential treatment, and referral patterns; as well as environmental factors such as socioeconomic status (Leigh et al., 2016). To improve cardiovascular health for all populations, including these most vulnerable underserved groups, technology-enabled healthcare systems that are easy to access, affordable, and deliverable across rural and urban communities at the point-of-care (POC) including clinics, ambulances, and even home settings is highly desired. Toward that end, the development of affordable, accurate, and timely POC CVD biosensors for diagnosis, prognosis, and/or monitoring targeting optimal biomarkers and multiplexed panels of biomarkers is critical.

A primary class of CVDs, known as acute coronary syndrome (ACS), can be divided into three categories that ultimately dictate the biosensors that need to be developed and biomarkers that need to be monitored including: ST-elevated myocardial infarction (STEMI), non-ST-elevated myocardial infarction (NSTEMI), and unstable angina. In STEMI, the ST segment on an electrocardiogram (ECG) appears elevated when the coronary artery is completely blocked so that a large proportion of the heart muscle being supplied by that artery begins to die. This is the most severe form of ACS. Unstable angina is characterized as ACS in which a blood clot is not yet large enough, or does not persist long enough, to produce any evidence of heart muscle damage but symptoms are often produced without yet causing the heart muscle to die. Similarly, NSTEMI is characterized as a partial blockage in the coronary artery that leads to laboratory evidence of heart muscle damage but does not result in the ST segment of the ECG being elevated. People with NSTEMI or unstable angina have a risk of progressing to STEMI. Both STEMI and NSTEMI are subtypes of a broader term, myocardial infarction (MI). According to data from the National Health and Nutrition Examination Survey, between 2011 and 2014 the overall prevalence for MI is 3% in US adults ≥20 years of age (Benjamin et al., 2018). Moreover, the mortality rate for ACS/MI, based on an AHA calculation, is estimated that ~14% for people who experience an MI (Benjamin et al., 2018). AHA and ACC (American College of Cardiology) recommends the administration of therapy to eligible patients within 12 hours of the symptom onset and emphasizes that the gains from reperfusion are greatest in the first few hours of symptom onset and rapidly decline afterwards (Amsterdam et al., 2014). Thus, the early diagnosis of ACS/MI can lead to life-saving and life-extending interventions, where rapid treatment usually requires a less invasive intervention procedure and also leads to a much lower

mortality (Amsterdam et al., 2014; Lambert et al., 2010; O'Gara et al., 2013). For the current ACS/MI standard of care, timely detection of biomarkers from blood is important for deciding the need for hospitalization and assisting in the selection of an optimal treatment for patients presenting with chest pain (Amsterdam et al., 2014).

Heart failure (HF), another type of CVD that could benefit from POC biosensing, is defined as a condition in which the heart cannot fill or pump blood properly due to any structural or functional cardiac abnormality (Yancy et al., 2013). Similar to ACS/MI, the prevalence and mortality of HF is problematic (Benjamin et al., 2019, 2018). It has been shown that the number of American adults ≥20 years of age who had HF increased from ~5.7 million between 2009 and 2012 to ~6.2 million between 2013 and 2016 (Benjamin et al., 2019). Moreover, HF is responsible for 9% of the deaths attributable to CVD in the United States (Benjamin et al., 2018). In particular, both ACS and chronic coronary artery disease (CAD) can result in myocardial cell necrosis and fibrosis, which often restricts heart pumping ability and eventually can lead to HF (Mendis et al., 2011). In addition to CAD, high blood pressure and diabetes are also prevalent causes of the disease (Yancy et al., 2013). HF can be categorized as acute heart failure and chronic heart failure. Chronic HF refers to those patients with an established diagnosis of heart failure and can either remain in a compensated stable state, have recurrent acute exacerbations, or have a slow progression of the disease state to an end stage heart failure state (Ponikowski et al., 2016). Many patients get diagnosed initially with an Acute HF exacerbation and can have a rapid or indolent onset (first occurrence) of symptoms and/or signs of HF. In this paper, the general term HF will be used, unless the studies/guidelines specify the results are related to acute HF or chronic HF. The timely diagnosis and, perhaps more important, risk stratification of patients with HF is used to determine admission or discharge, the proper pharmacological treatment and/or proper device therapy (Yancy et al., 2017). Timely diagnosis is also used to determine when a cardiac assist devices or heart transplant is required. Based on American College of Cardiology Foundation (ACCF) and AHA guidelines, diagnosis and risk stratification depends on a combination of medical history, physical examination, ECG and diagnosis of blood biomarkers (Yancy et al., 2013).

The standard of care for ACS/MI and HF requires the measurement of multiple cardiac biomarkers and their timely diagnosis or monitoring at the POC may provide for more effective treatment options. In the following sections, existing and emerging cardiac biomarkers for diagnosis, prognosis, and monitoring of ACS/MI and HF based on authoritative guidelines for clinical practice and recent clinical studies are first reviewed. Multi-marker panels and their clinical benefits are then discussed. The review then culminates in a discussion and evaluation of commercial CVD biosensors and recent research for detection and monitoring of these existing and emerging cardiac biomarkers and biomarker panels.

2. Cardiac biomarkers: Guidelines and Categories

The cardiac blood-based biomarkers that are recommended for ACS/MI and HF are based on recent clinical studies and authoritative guidelines for clinical practice from the American Heart Association (AHA), American College of Cardiology (ACC), European Society of Cardiology (ESC) and the Canadian Cardiovascular Society (CCS). In total, fourteen blood-based biomarkers are discussed. Corresponding to the guidelines, in Table 1 the ACS/MI and HF focused biomarkers are listed in terms of Class of Recommendation (COR) and Level of

Evidence (LOE) for prognosis, diagnosis, and risk stratification. Further, the fourteen biomarkers are divided in Table 1 into three general categories: (1) six well-established standalone cardiac biomarkers with reasonable specificity for CVDs; (2) three emerging biomarkers with relatively high specificity; and (3) five add-on biomarkers, which individually may not be as specific, but in combination with other biomarkers can achieve better sensitivity, accuracy, earlier detection, and/or specificity. These cardiac biomarkers are also listed in Figure 1 along with their recommended clinical cut-off concentrations in ng/mL. As noted in Figure 1, the range varies considerably depending on the biomarker used and other factors such as age and risk tier. In this review, sensitivity, specificity, accuracy, negative predictive value (NPV), and positive predictive value (PPV) are used to quantitatively show the clinical performance of biomarkers. In clinical studies, sensitivity refers to the percentage of people with the disease who are correctly identified by the test, specificity refers to the percentage of people without the disease who are correctly excluded by the test, and accuracy refers to the percentage that the positive/negative tests correctly indicate people with/without the disease. In addition, NPV is the percentage of patients with a negative test who do not have the disease, and PPV is the percentage of patients with a positive test who actually have the disease (Parikh et al., 2008).

2.1. Established cardiac-specific biomarkers

The six standalone, well-established and fairly specific, cardiac biomarkers for ACS/MI and HF defined in Figure 1 and Table 1, include cardiac troponin I (cTnI), cardiac troponin T (cTnT), high-sensitivity c-reactive protein (hs-CRP), B-type natriuretic peptide (BNP), N-terminal propeptide B-type natriuretic peptide (NT-proBNP), and mid-regional-fragment of pro-atrial-natriuretic-peptide (MR-proANP).

2.1.1 Established Biomarker Utility for ACS/MI:

All three guidelines have identified **cardiac troponin (cTn),** referring to cTnI or cTnT, as the preferred biomarker for ACS/MI and the evaluation of myocardial injury (Table 1). High-sensitivity cTn (hs-cTn) assays are recommended for routine clinical use (Jneid et al., 2017; O'Gara et al., 2013; Thygesen et al., 2018). cTn is the primary, most sensitive and specific, biomarker for STEMI (O'Gara et al., 2013) and NSTE-ACS (Amsterdam et al., 2014). cTn is a protein released in blood when the cardiomyocytes are injured and both cTnI and cTnT can be used to identify cardiac myocyte damage, which could be caused by ACS/MI and HF (O'Brien et al., 2011). The cTn levels rise within a few hours of symptom onset and typically remain elevated for several days, therefore it is recommended to be measured in all patients with symptoms consistent with ACS at presentation and 3–6 h after symptom onset (Amsterdam et al., 2014). ESC guidelines recommend measurement of hs-cTn in patients with suspected NSTE-ACS and that the results be obtained within 60 mins (Roffi et al., 2016). Early cTn measurement (within 6 hours of arrival) is recommended for all patients with acute NSTEMI (Jneid et al., 2017). Myocardial injury is defined as the detection of an elevated cTn value above the 99th percentile upper reference limit (URL), and the injury is considered acute if there is a rise and/or fall of cTn values (Thygesen et al., 2018). The 99th percentile value is assay dependent. For example, the cut-off of 0.027 ng/mL was used for a hs-cTnI assay (ARCHITECT i2000SR, Abbott) (Neumann et al., 2016); and the cut-off of 0.014 ng/mL was reported for hs-cTnT assay (Elecsys 2010, Roche) (Rubini Giménez et al., 2016). Rule-in and rule-out criteria at 0 h varies

based on the decision algorithms, but typically hs-cTnT less than 0.005-0.012 ng/mL or hs-cTnI less than 0.002-0.006 ng/mL at 0 h are used to rule-out MI while hs-cTn ≥ 0.052 ng/mL are used to rule-in MI (Twerenbold et al., 2017).

Beyond diagnosis, the presence and magnitude of elevation of cTn is also useful for short- and long-term prognosis in ACS (Amsterdam et al., 2014) and adds prognostic information to clinical and ECG variables for patients with NSTE-ACS for short- and long-term mortality (Roffi et al., 2016). While both hs-cTnI and hs-cTnT assays were reported to have comparable diagnostic accuracy, the latter provided greater prognostic accuracy (Roffi et al., 2016). Furthermore, for early risk stratification in ACS, serial cTn levels are recommended to be obtained at presentation and 3-6 h after symptom onset in all patients who present with symptoms consistent with ACS, in order to identify a rising and/or falling pattern of values (Amsterdam et al., 2014).

BNP and NT-pro-BNP are recommended as an ACS biomarker (Amsterdam et al., 2014), specifically as a prognostic marker for early risk stratification, where they may be considered to assess risk in patients with suspected ACS. ESC also confirmed that extensively validated natriuretic peptides (NPs) provide prognostic information on top of cardiac troponin for ACS (Roffi et al., 2016; Thygesen et al., 2012). BNP is secreted in response to myocyte stretch (Kragelund et al., 2005) and thus more applicable to HF. BNP is synthesized as a prohormone (proBNP), which is then split into a physiologically active C terminal fragment (i.e., BNP) and an inactive N terminal fragment (i.e, NT-proBNP), with the latter exhibiting a longer half-life (Weber and Hamm, 2006). In addition, **MR-proANP** has been recognized as a prognostic marker for ACS by ESC (Roffi et al., 2016; Thygesen et al., 2012). Due to its low receptor binding and protein interactions, MR-proANP is a stable and reliable NP biomarker (Savic-Radojevic et al., 2017) and although recognized as a prognostic marker for ACS, is mostly indicative of HF.

2.1.2 Established Biomarker Utility for HF:

According to the guidelines from AHA (Yancy et al., 2017, 2013), CCS (Ezekowitz et al., 2017), and ESC (Ponikowski et al., 2016), **cTn** has been recommended as a prognostic marker for acute HF. However, the AHA emphasized that cTn responds similarly for ACS and acute HF, thus elevations in cTn must be interpreted in the clinical context for prognosis of acute HF (Yancy et al., 2017). For patients with chronic HF, AHA recommended measurements of hs-cTn for risk stratification additive to NP (Yancy et al., 2017). In a clinical study, a cut-off of 0.018 ng/mL for hs-cTnT, a lower level than for MI, was reported to be prognostic in chronic HF (Aimo et al., 2018).

Measurement of baseline levels of **natriuretic peptides (NPs)** on admission to the hospital is recommended across the board to establish a diagnosis for and prognosis of HF. AHA guidelines (Yancy et al., 2017, 2013) recommend the measurement of BNP or NT-proBNP for (1) ambulatory patients with dyspnea to support clinical decision making regarding the diagnosis or exclusion of HF; and (2) to establish prognosis or evaluate disease severity in both acute and chronic HF. Moreover, measurement of NP levels is recommended for prognostic stratification in patients with an established diagnosis of HF, in view of optimizing medical therapy

(Ezekowitz et al., 2017). Further, the CCS guideline (Moe et al., 2015) suggests NP levels be used to implement strategies to prevent HF for individuals with risk factors for the development of HF.

Defining clinical cut-offs for HF is complicated because multiple factors affect NP levels (Chow et al., 2017). To rule-out acute HF, clinical cut-offs of < 0.1 ng/mL for BNP and < 0.3 ng/mL for NT-proBNP are recommended by AHA (Chow et al., 2017) and ESC (Ponikowski et al., 2016). To rule-in HF, BNP with a single clinical cut-off of > 0.4 ng/mL is recommended by AHA (Chow et al., 2017) and CCS (Ezekowitz et al., 2017). As for NT-proBNP, age-related cut-offs for HF are adopted by AHA (Chow et al., 2017) and CCS (Ezekowitz et al., 2017) to improve PPV, where 0.45, 0.9, and 1.8 ng/mL are recommended for <50 years, 50-75 years, and >75 years, respectively. In the non-acute setting, ESC (Ponikowski et al., 2016) guidelines recommend an upper limit for normal of 0.035 ng/mL for BNP and 0.125 ng/mL for NT-proBNP to rule-out HF. CCS (Ezekowitz et al., 2017) suggested BNP of 0.050 ng/mL and NT-pro-BNP of 0.125 ng/mL as clinical cut-offs for the screening of individuals at risk for the development of HF.

MR-proANP is recommended as a diagnostic marker for acute HF in the emergency department, as an alternative to BNP and NT-proBNP to help in the differentiation of acute HF from non-cardiac causes of acute dyspnea with the upper limit of normal as 0.12 ng/mL (Ponikowski et al., 2016). However, similar to BNP and NT-proBNP, MR-proANP levels may be affected by age, race, sex, body mass index, or comorbidities such as atrial fibrillation (Maisel et al., 2010). AHA (Chow et al., 2017) recognizes MR-proANP as one of the cardiac biomarkers that is predictive of new onset HF if multi-variably adjusted for these factors.

2.1.3 Established Biomarker Utility for Risk Prognostication in a Chronic Stage:

High-sensitivity C-reactive protein (hs-CRP) is the most intensively studied inflammatory biomarker associated with CVDs (Greenland et al., 2010), and it has utility for risk stratification in CVDs (Genest et al., 2009; Goff et al., 2014; Perk et al., 2012). CRP is a protein found in blood plasma, whose levels rise in response to inflammation. hs-CRP was crucial in both primary and secondary cardiovascular prophylaxis (Adukauskienė et al., 2016). Over 60 prospective cohort studies confirm that multiple inflammatory biomarkers are associated with future vascular risk in otherwise healthy populations (Ridker, 2014). Of these, hs-CRP is the standard for vascular risk prediction largely due to its ease of measurement and abundance of clinical data. According to Center for Disease Control guidelines, hs-CRP levels lower than 1 µg/mL, within 1 and 3 µg/mL, and within 3 and 10 µg/mL are classified as indicating low, intermediate and high coronary risk, respectively (Pearson et al., 2003). Guidelines from ACC/AHA (Goff et al., 2014; Greenland et al., 2010), ESC (Perk et al., 2012), and CCS (Genest et al., 2009) all recommend the measurement of hs-CRP as part of refined risk assessment in patients with an unusual or moderate CVD risk profile. However, hs-CRP levels are affected by sex and ethnicity (Cushman et al., 2009).

2.2. Emerging cardiac biomarkers

Emerging cardiac biomarkers of copeptin, soluble suppression of tumorigenicity 2 (sST2), and galectin-3 (Gal-3) have been recognized for their promising clinical value (Table 1). Specifically, copeptin may be useful for both ACS/MI diagnosis and HF prognosis while sST2 and Gal-3 are primarily indicated for their potential for prognosis and risk stratification for HF.

Copeptin has clinical value for the diagnosis of NSTE-ACS and MI when combined with cTn (Roffi et al., 2016). It is stoichiometrically co-secreted with vasopressin, however, it is significantly preferred over vasopressin due to its extended half-life in the blood making it easier to quantify (Morgenthaler et al., 2008). A clinical cut-off of 0.05 ng/mL was used in clinical studies (Möckel et al., 2015; Roffi et al., 2016). ESC guidelines (Roffi et al., 2016) emphasize that the additive value of copeptin to conventional cTn, non-hs, assays is substantial and thus the routine use of copeptin as an additional biomarker for the early rule-out of MI is recommended when only less sensitive cTn assays are available. When hs-cTn assays are available, copeptin may have some additive value in the early rule-out of MI (Roffi et al., 2016). However, the incremental value of copeptin over hs-cTn assays remains to be fully elucidated. Further, copeptin was recognized by AHA (Chow et al., 2017) as one of the cardiac biomarkers that aid in HF prognostication.

sST2 showed high specificity and sensitivity as a cardiac biomarker for acute (Rehman et al., 2008) and chronic HF (Bayés-Genís et al., 2017; Rehman et al., 2008). sST2 is overexpressed in specific pathologic conditions of myocardial stress or injury (Aleksova et al., 2019). Since the level of sST2 varies less as a function of age, sex, body mass index, or renal function, the AHA recommends the measurement of sST2 be considered for additive risk stratification among patients with HF (Chow et al., 2017; Yancy et al., 2017, 2013). The relative independence of sST2 from prevalent comorbidities represents its potential advantage for prognostication over the commonly used NPs (Chow et al., 2017). sST2 receptor is predictive of hospitalization (Ezekowitz et al., 2017; Moe et al., 2015), hence may provide additional prognostic value beyond NPs with suspected low week-to-week variations. The clinical cut-off of 35 ng/mL for HF was used in clinical studies (Chow et al., 2017; Gaggin et al., 2013) and a commercial IVD product (Boman et al., 2018).

Gal-3 is associated with fibrosis and inflammation, and has been implicated in the development, early risk assessment, management, and progression of HF (Dong et al., 2018; Ezekowitz et al., 2017). The ACCF/AHA recommends Gal-3 as an emerging marker of myocardial fibrosis for HF in ambulatory and acute settings with the benefit of additive risk stratification (Yancy et al., 2017, 2013), as it is predictive of hospitalization in patients with HF. Gal-3 concentrations of $\leq$ 17.8, 17.9-25.9, and >25.9 ng/mL indicate low, intermediate, and high risk categories, respectively (Chow et al., 2017; Mueller et al., 2016). It is recognized that sST2 might be superior compared to Gal-3 in a multivariable risk prediction model (Ezekowitz et al., 2017; Moe et al., 2015).

Despite their guideline recognition for use in multi-marker panels to assist in prognosis or risk stratification, no definite consensus has been reached on the clinical use of these emerging cardiac biomarkers, although data suggests that the predictive accuracy is significantly increased when the cardiac biomarkers are used together in a multi-biomarker panel (Chow et al., 2017).

2.3. Cardiac biomarkers that provide add-on value for CVDs

Add-on biomarkers are typically not recommended in the guidelines as standalone cardiac biomarkers either due to their low specificity or sensitivity as an independent biomarker or due to limited repetitive evidence in clinical studies. However, their value in multi-biomarker panels are demonstrated in clinical studies and in combination they may achieve better sensitivity and accuracy. Additionally, some markers may provide for earlier detection due to rapid release in the bloodstream or a longer half-life for late diagnosis (Kemp et al., 2004). Five biomarkers that provide these added features in multi-biomarker panels include: myoglobin (Myo), creatine kinase muscle/brain (CK-MB), myeloperoxidase (MPO), heart-type fatty acid-binding protein (h-FABP), and mid-regional-fragment of pro-adrenomedullin-peptide (MR-proADM).

Myo and **CK-MB** are both well-studied cardiac biomarkers for ACS, especially MI. Myo is a protein widely present in both the skeletal (low specificity) and cardiac muscle that rises rapidly when muscle injury occurs (MYTHILI and MALATHI, 2015), and reaches peak values 1-4 h after onset of STEMI (O'Brien et al., 2011). The clinical cut-off of Myo at 200 ng/mL led to a high NPV to rule-out acute MI (Melanson et al., 2004). CK-MB is well-known for its use with other biomarkers such as hs-cTn for diagnosis of acute MI due to its fast reaction time and good sensitivity (O'Brien et al., 2011). The clinical cut-off for serum CK-MB is 7.5 ng/mL (Melanson et al., 2004).

MPO provides prognostic value for ACS, especially in patients testing negative using a hs-cTnI assay in the emergency department (Searle et al., 2013). MPO is an inflammatory enzyme that promotes weakening of the fibrous cap and results in the formation of vulnerable atherosclerotic plaques (Fong et al., 2015). Current lab testing of MPO targets individuals at intermediate or high risk for developing CVD, for which the clinical cut-offs of <63, 63-71.9, ≥72 ng/mL suggest low, moderate, and high risks, respectively ("MPO-Practitioner-One-Pager-CHL-D006b," n.d.)

The add-on diagnostic value of **h-FABP** has been reported for early detection of ACS with a cut-off value of 6.2 ng/mL (Inoue et al., 2011; Reiter et al., 2013). h-FABP is highly sensitive to myocardial ischemic injury, and can be detected earlier than Myo, even as early as 1 h after acute MI (Ye et al., 2018). h-FABP is a protein that leaks out of myocardial tissue when myocardial ischemia occurs, and its plasma concentration reaches a peak value 8 h after onset of symptoms with a clearing time (i.e., returning to the reference range) within 36 h of onset (Reiter et al., 2013). The add-on value of h-FABP as an MI biomarker in the presence of skeletal muscle injury and renal insufficiency has been demonstrated despite its low specificity (Morrow et al., 2007).

MR-proADM is an add-on biomarker for predicting HF or cardiovascular death (Khan et al., 2007; Maisel et al., 2010; Tzikas et al., 2013). Adrenomedullin (ADM) is a vasodilatory peptide with potent hypotensive effects, and its concentration increase in plasma is associated with chronic HF (Maisel et al., 2010). MR-proADM is a fragment of the prohormone of ADM and is released in equimolar concentrations as ADM (Vila et al., 2009). However, due to its longer half-life compared to ADM, MR-proADM is more reliable for clinical use (Morgenthaler et al., 2005; Vila et al., 2009). The clinical cut-off of 2.96 ng/mL was reported for the prognosis of HF (Spinarova et al., 2018).

2.4. Multiplex panels of cardiac biomarkers and their clinical benefits

Multi-biomarker panels have been reported for the diagnosis, prognosis, and risk stratification of ACS/MI or HF. The multi-biomarker panel can be composed of combinations of all three types, as summarized in Table 2. Multi-biomarker panels using established CVD biomarkers include the combination of NT-proBNP and cTnT, which showed a higher sensitivity compared to either marker alone in predicting adverse events (MI included) in ACS (Cameron et al., 2007). The combination of MR-proANP with cTnI improved early rule-out of NSTE-ACS (Tajsic et al., 2018). The combination of BNP and MR-proANP showed improved performance in the diagnosis of acute HF compared to either marker alone (Maisel et al., 2010). MR-proANP has a slight, but significant, enhancement with BNP in the diagnostic performance for acute HF (Chow et al., 2017). BNP, cTnI and CRP form a triplex panel showing better prediction of major adverse cardiac events (MI and HF are included) after cardiac surgery versus each biomarker alone (Fellahi et al., 2009).

Multi-biomarker panels with the emerging cardiac biomarkers are also identified and summarized (Table 2). The additive value of copeptin with cTn has been demonstrated for early rule-out of MI (Roffi et al., 2016) and improved performance in diagnosis of MI compared to cTn alone (Keller et al., 2010; Möckel et al., 2015; Ray et al., 2012; Roffi et al., 2016). sST2 was paired with NT-proBNP or CRP to improve risk stratification for HF events. Further, Gal-3 has been duplexed with BNP or NT-proBNP to improve the prediction of mortality in HF patients.

The value of add-on cardiac biomarkers are widely reported in the literature for enhanced performance (Khan et al., 2007; Lassus et al., 2013)(McCann et al., 2008); (Viswanathan et al., 2010); (Pelsers et al., 2005; Reiter et al., 2013) (Sawicki et al., 2011); (Inoue et al., 2011); (Morrow et al., 2008); (O'Donoghue et al., 2016) (Table 2). However, Myo, CK-MB, and cTn (cTnI or cTnT) is the panel most often used in hospitals to assess acute MI among patients with symptoms, such as chest pain (Ye et al., 2018). Despite the prevalence of these panels, new multi-marker strategies with higher sensitivities and specificities for CVD including acute MI diagnosis at very early stages of myocardial injury are still greatly needed (Ye et al., 2018).

3. Commercial devices for cardiac blood biomarker detection

As described below and in Table 3, several commercial single biomarker products have been developed for the detection of cardiac blood biomarkers with many approved by the Food and Drug Administration (FDA) in the United States or that have received the Conformité Européenne (CE) mark in Europe. Sometimes these systems can measure multiple single biomarkers and there are also systems that combine multiple markers into a single cartridge or platform for a particular diagnosis like MI (Table 4).

3.1 The use of POC devices for CVD blood-based biomarker detection

POC tests that use handheld or benchtop devices, with simpler sample collection and preparation steps, can allow for more rapid results and improved patient management. Specifically, for common cardiac biomarker measurements, the turnaround time was reduced from 110 min using

centralized laboratory tests to 17 min using POC tests (Gaze, 2016). Clinical studies revealed that patients who received POC cardiac biomarker tests may also benefit from a reduced length of stay (Collinson et al., 2004; Goodacre et al., 2011) and higher chance of successful discharge home (Goodacre et al., 2011; Griffiths et al., 2015), due to a combination of rapid diagnosis and structured decision-making. For pre-hospital emergency care such as remote health centers, POC tests of multiple markers enabled more informed triaging of patients with acute medical presentations to a tertiary hospital and ruling out the need for hospital admissions (Spaeth et al., 2017).

Beyond simple treatment of CVD, in recent years the policy and guidelines have begun to shift toward CVD prevention through personalized treatment recommendations and cardiovascular risk reduction therapies (Arnett et al., 2019; Levine et al., 2019; Robinson and Ray, 2016). For this shift to occur, the POC measurement of not only the known biomarkers and multi-marker panels of CVD described above, but those that can be used to monitor risk factor such as hs-CRP, LDL-C and other lipid markers at the POC will be crucial for the quantitative CVD risk assessment. In particular, POC testing has been identified as a promising means to promote personalized CVD prevention by the National Heart, Lung, and Blood Institute Working Group (King et al., 2016). The use of POC tests for CVD risk assessment would enable clinicians to obtain immediate test results to facilitate clinician-patient discussion and decision-making, and relieve the burden of follow-up visits for patients. A recent study evaluated the effects of pre-visit POC tests and quantitative CVD risk assessment immediately before a routinely scheduled primary care provider (PCP) visit (Karmali et al., 2017). Results suggested that the implementation of pre-visit POC tests are a feasible intervention, deemed highly acceptable by participants, and may promote guideline-recommended statin initiation in primary prevention.

Beyond use in cardiovascular clinical care, POC tests can be used to expand quantitative data collection to broader populations, including more underserved or underrepresented groups, and therefore improve the generalizability of study results (King et al., 2016).

One of the main hurdles preventing the adoption of POC tests is the high cost per assay (Gaze, 2016; Plüddemann et al., 2012), despite reports that overall charges to patients may decrease with the implementation of POC tests due to reduced costs associated with boarding, pharmacy, and medical procedures. (Apple et al., 2006). Other challenges are related to sensitivity, robustness, ease of use, integration into clinical workflow (King et al., 2016), and the interpretation of test results obtained from capillary blood samples using finger-prick tests compared to venous blood samples (Gaze, 2016). Ideally, as first identified by the World Health Organization as the ASSURED criteria (Peeling et al., 2006) and later modified to REASSURED (Land et al., 2019), POC technologies should be or have; Real-time connectivity, Ease of specimen collection, Affordable by those at risk, Sensitive with very few false-negatives, Specific with very few false-positives, User-friendly tests that are simple to perform and require minimal training, Rapid, to enable treatment at first visit, and Robust, for example not requiring refrigerated storage, Equipment-free, and Delivered to those who need it.

3.2 Devices for detection of standalone biomarkers with separate assays or cartridges

Commercial *in vitro* diagnostic (IVD) products for the measurement of individual cardiac biomarkers (Table 3a), can generally be separated into 4 categories namely; (a) handheld devices, (b) benchtop devices, (c) larger clinical lab systems, and (d) qualitative tests read by eye without the need of a reader. Note, there are also commercial assay kits available that have been approved for the detection of cardiac biomarkers in clinical settings. However, these products are not included in the table since they are assay kits that rely on manual steps and then are put in a standard microtiter plate, e.g., the ST2 Presage assay kit. The primary CVD biomarker measurement approach involves taking patient venous samples and testing them in central laboratories in a clinical setting using large systems. These include the 9 listed as type (c) lab-system units or sometimes the 12 listed as (b) benchtop systems in Table 3a. The central lab-based products possess powerful analytical capabilities, but the systems typically require larger sample sizes, prolonged turnaround times, skilled users, multiple often cumbersome steps, and are expensive. The benchtop systems typically provide shorter turn-around time and, although not as complex or expensive, are still at the size requiring a clinical bench top environment, are typically run by trained users, and often still have cumbersome steps, for example, that require pipetting of the sample. Thus, this also limits their role as a POC device outside the hospital or advanced clinic, especially in underserved and remote locations. However, handheld POC platforms as well as a few turn-key bench top systems provide shorter turnaround time and a smaller footprint, with the flexibility to be used outside centralized labs.

Among the commercial IVD systems for clinical testing of single cardiac biomarkers, 15 devices have the potential to be used for POC testing and can be classified as either a handheld (3 type (a) units in Table 3a) or a benchtop (12 type (b) units in Table 3a) devices. The three handheld devices can individually monitor only 6 of the aforementioned 14 CVD biomarkers (and only three of those are FDA approved namely cTnI, BNP, and CK-MB). Of the six total, 4 of them are from the 6 guideline recommended CVD biomarkers (i.e. excluded are hs-CRP or MR-proANP), and 2 of them are from the 5 add-on CVD biomarkers (i.e. excluded are h-FABP, MPO, and MR-proADM) while none of the 3 emerging biomarkers (Gal-3, copeptin, and sST2) can be monitored with the commercially available hand-held devices. Further, the benchtop devices add 5 more of the 14 biomarkers (hs-CRP, h-FABP, MPO, Gal-3 and sST2) and most of these devices either are FDA approved and/or have the CE mark. The CVD biomarkers that are only measurable using the lab-systems include MR-proANP (established), copeptin (emerging), or MR-proADM (add-on) and they are limited to one lab-based system namely the Thermo Scientific B·R·A·H·M·S. Due to the guideline recognition of MR-proANP as an independent established biomarker as well as the emerging copeptin and the good add-on value of MR-proADM in a multi-biomarker panel, more commercial devices, especially POC devices, would be beneficial for their adoption in clinical use.

The performance of the 15 devices that have some potential for use at the POC for a single biomarker are summarized in Table 3b. All of the 15 devices are based on immunoassays, which use antibodies as the recognition element. Fluorescence, chemiluminescence, electrochemistry, colorimetry, and turbidimetry, are the transduction modalities used in these devices. In addition, all of the devices provide results within 30 mins, indicating their ability to provide a relatively rapid result for diagnosis and risk stratification of ACS/MI and HF. Most of these devices are compatible with whole blood sample, except Mini VIDAS, DZ-Lite c270, and SMART/CUBE. These three devices use plasma or serum as the specimen, indicating a preprocessing step to

extract it from whole blood is needed. Among the devices compatible with whole blood samples, a chromatographic method is often used to achieve separation of blood from serum/plasma. The measurable concentration ranges of the 15 devices for BNP, NT-proBNP, hs-CRP, Myo, CK-MB, h-FABP, MPO, Gal-3, and sST2, cover their respective clinical cut-off values shown in Figure 1 and Table 3b. In particular, the devices that are able to measure cTnI and cTnT can measure as low as 0.01 ng/mL, which is adequate for covering the cut-off values for diagnosis of MI. However, these devices are not sensitive enough to measure the cTn levels for early rule-out of MI (e.g. hs-cTnT <0.005 ng/mL or hs-cTnI <0.002 ng/mL). This may indicate that devices that can detect lower concentration of cTn are still needed. In addition, the current handheld *in vitro* diagnostic products developed for CVDs (i.e. i-STAT, Minicare I-20, and Cobas h232) target only a limited set of single cardiac biomarkers (cTn, BNP/NT-proBNP, Myo and CK-MB). Their accessibility also remains hindered by the high cost of the disposable cartridge, which can be a few hundred US dollars each. Further, 12 of these 15 devices are benchtop devices, and so, although they have the potential for POC clinics or emergency departments, typically with trained personnel, they are not adequate for home or ambulatory care. Therefore, the development of multiplexed, affordable, and hand-held platforms for CVDs that could be used at the POC, particularly in the home or ambulance, are needed.

3.3 Devices for multi-marker panels and their potential for POC

Multi-biomarker panels combine the characteristics of different cardiac biomarkers to achieve better sensitivity, specificity, accuracy, and/or ability for early detection compared with individual biomarkers alone. Therefore, multi-marker panels could lead to improved decision-making and disease management at different stages. To perform a multi-biomarker panel, traditional diagnostic workflows typically use single analyte assays and run several tests (i.e. separate biomarker cartridges for POC systems) for each biomarker. However, this approach may require more patient sample, higher reagent consumption, longer turnaround time, and higher assay cost. Therefore, a biosensor that detects multiple biomarkers simultaneously is highly desired.

Commercial devices adopting multi-marker strategies are summarized in Table 4. They are divided into two primary types, namely; (1) qualitative tests without a reader and (2) those with a reader and quantifiable readout. Some devices have both readout mechanisms but all the devices equipped with readout systems are benchtop devices.

As shown in Table 4, of the 15 commercial systems, cTnI is included in all multi-marker panels and CK-MB and/or Myo are very often included in the panels (13 out of 15 systems). Further, the triplex panel of cTnI, CK-MB, and Myo, targeting the rapid diagnosis of acute MI (AMI), is the most frequently used multi-marker panel for these devices. Of the two panels that do not include CK-MB or Myo, CardioDetect, is a duplex biomarker panel that takes advantage of the early concentration rises of h-FABP and the accuracy of cTnI to achieve early detection of AMI. Further, the Triage Cardio2 Panel is the second device that uses cTnI along with BNP for ACS/MI or HF diagnosis. There are two commercial products that adopt a four-plex panel with three biomarkers in common (cTnI, CK-MB, and Myo), where Nano-Check AMI 4-in-1 adds NT-proBNP for diagnosis of acute MI and Randox Biochip Array Technology adds h-FABP for the diagnosis and risk stratification of MI and ACS.

Only 3 devices in Table 4 are not exclusively indicated for AMI namely, the Randox Biochip Cardiac Array for ACS and Triage Cardio2 and Cardio3 Panel for ACS/HF/MI. Although limited devices are available for HF, POC devices for HF are needed to help confirm a clinical diagnosis of HF in patients presenting in community clinics or urgent care settings and for use as a rapid test to rule out patients from being hospitalized. Moreover, POC cardiac biomarker devices could provide a tool for the periodic monitoring and assessment of CVD risk factors, help the patient or health care provider in clinical prognostication, and help to address healthcare challenges, particularly among underserved and/or remote populations.

All the commercial multiplex devices summarized in Table 4 use immunoassays, with antibodies as the recognition element and either fluorescence, chemiluminescence, or colorimetry as the transduction modality. The most common technology is a chromatographic assay based on antibody capture and gold particles to produce a colorimetric change. Specifically, in this platform the biomarker is captured by a test line on a paper strip and antibody functionalized colloidal gold is bound to the test line and is displayed as a reddish line. Using this technique, either multiple test lines are designed in one test strip for each biomarker or multiple paper strips are assembled in one cartridge for a multiplexed detection. The Triage Cardiac Panel also puts multiple test zones for each biomarker in a microcapillary, where target biomarkers react with reagents and the amount of biomarker affects fluorescence intensity. Notably, the lower end of the detection range of cTnI achieved with the Triage Cardiac Panel series are lower than that of Nano-Check AMI series. This may be related to more sensitive assays typically achieved using fluorescence as the transduction element rather than using colorimetry. The Randox Biochip Cardiac Array uses a solid biochip with multiple reaction vessels to produce a chemiluminescent immunoassay. The Fluoro-Check AMI 3 in 1 uses a time resolved fluorescence method to achieve multiplexed detection. Most of the devices in Table 4 are compatible with whole blood samples, except the Randox Biochip Cardiac Array, Nano-Check AMI 2 IN 1 (ND-CD201S and ND-CD202P), and Nano-Check AMI 3 IN 1 (ND-CD301S). These four devices use plasma or serum as the specimen, requiring a preprocessing step to extract plasma or serum from whole blood. Among the devices compatible with a whole blood sample, either the chromatographic method or microcapillaries in a cartridge are used.

All of the devices achieve reasonable time responses of 15-20 minutes and use sample volumes between 75 and 800 microliters. The measurable concentration ranges of the quantitative devices cover the respective clinical cut-off values for Myo, CK-MB, BNP, NT-proBNP, and h-FABP shown in Figure 1. However, similar to the single biomarker commercial devices, although the quantitative devices are able to measure cTnI as low as 0.01 ng/mL, the detection ranges do not cover the levels for early rule-out of MI (0.002 ng/mL).

Of the 15 devices only the Triage Cardiac Panel has both FDA and CE Mark certification for quantitative detection of biomarkers for AMI. There are 2 others that have FDA approval for quantitative detection (Nano-check AMI 2 IN 1 and Nano-Check AMI 3 IN 1) and 1 for qualitative detection (LifeSign 2-in-1). There are four others that have only CE mark approval (RapidCard and Inchroma II for AMI and Triage Cardio2 and Cardio3 for ACS/MI/HF).

Overall, not only are several of these commercial multi-marker panels lacking certification, there is a gap between the commercially available multi-marker products and the panels recommended

in clinical studies, namely, none of these commercial devices use MR-proANP, hs-CRP, MPO, MR-proADM, copeptin, Gal-3, or sST2, in the multi-marker panels. More sensitive cTn assays are needed to measure low levels of cTn for HF prognosis, and even lower levels for early rule-out of MI. The development of a multiplexed, affordable, hand-held platform for ACS/MI that could be used at the POC, for example in an ambulance, are needed. Moreover, there is a great need and lack of affordable, hand-held, POC devices multi-marker IVD products for monitoring HF, especially in the home, for use in long-term CVD prognostication and risk stratification.

4. Recent research platforms for cardiac biomarker detection

As highlighted in previous sections, there is a need for lower limit of detection (LOD) biosensors (e.g. for cTn) and lower cost and POC-compatible multiplexed tests. Described in this section are the published research articles within the last 5 years tackling cardiac POC biosensing technologies. We focus on biosensing mechanisms that have shown demonstrated performance beyond initial testing in buffer (i.e., those with validation in complex biofluids, such as human serum, plasma, or patient samples). The biosensing technology employed, key performance metrics, and unique features towards POC applications are summarized in Table 5. Note, in this section and Table 5, LODs reported may be calculated using different methods.

4.1 Cardiac biomarker detection research toward overcoming commercial system limitations

Similar to commercial devices, most of the research platforms developed for cardiac marker detection that fit our selection criteria have still focused on guideline recommended markers such as cTn (Ceylan et al., 2018; Sinha et al., 2019; Su et al., 2019; Xu et al., 2020; Zhang et al., 2018; Zong et al., 2017), BNP (Ceylan et al., 2018; Li et al., 2019), NT-proBNP (Sinha et al., 2019; Su et al., 2019) and CRP (Ballard et al., 2020; Boonyasit et al., 2019; Ouyang and Di Carlo, 2019; Sinha et al., 2019), as well as widely used add-on markers such as Myo (Zhang et al., 2018) and CK-MB (Xu et al., 2020; Zhang et al., 2018). In comparison, other emerging and add-on markers received less attention. A few biosensors have been developed for copeptin (Li et al., 2020), Gal-3 (Primo et al., 2018), sST2 (You et al., 2017) and MPO (Wen et al., 2018), but are rarely reported for MR-proADM and MR-proANP. Research platforms targeting single emerging markers have been reported toward proof-of-concept for platforms with simplified assay workflows as an alternative to the conventional multi-step assays conducted in centralized laboratory settings (Primo et al., 2018; Wen et al., 2018).

Compared to commercial devices, where the transduction element is mainly focused on colorimetric and fluorescence based readouts, the research platforms show more variety, including the use of capacitive, impedance, and current gain as well as using other optical approaches such as localized surface plasmon resonance (LSPR) and surface-enhanced Raman spectroscopy (SERS) to improve the detection limit. Among all targeted cardiac biomarkers, cTn with its lower pg/mL clinical cutoffs, has been a primary focus since it is one of the most challenging to detect, especially for platforms with label-free readout and short incubation times. In the work of Ceylan et al., the detection of cTn in clinical samples is comparable or even lower than the commercial systems (0.01 ng/mL) using a label-free electrical readout (e.g., capacitance)

approach (Ceylan et al., 2018). The work of Sinha et al. achieved 0.001 ng/mL lower detection limit in buffer, using a field-effect-transistor based sensing modality with current gain as the readout (Sinha et al., 2019). This platform demonstrated a recovery rate of 96-107% for spiked Troponin I in clinical samples with a 5 min assay time without the need for sample dilution. The work of Zhang et al. used nanoparticles and Raman to reach lower detection limits on a paper-based lateral flow assay and were able to achieve a comparable (0.01 ng/mL) detection limit (Zhang et al., 2018). Another cTnI detection platform employing a plasmonic gold nano-island sensing array demonstrated a 130-fold signal enhancement using near-infrared enhanced fluorescence as a readout, and further demonstrated clinical performance with a sensitivity of 100% and specificity of 95.54% for MI diagnosis, which outperformed standard chemiluminescence commercial immunoassays (Xu et al., 2020). Su et al. also used metallic nanoparticles for near field coupling enhanced Raman and reported a 1 fg/mL lower detection limit in buffer (Su et al., 2019). This platform was validated using plasma, and achieved acceptable agreement with results obtained using the dot immunogold filtration assay. In addition, a chemiluminescence approach was reported for the detection of cTnI using a paper-based vertical flow assay, achieving a 0.5 pg/mL detection limit in buffer (Li et al., 2020). Another chemiluminescence platform targeting cTnT achieved a detection limit of 3 fg/mL in buffer (Zong et al., 2017). This platform used DNAzyme and antibody conjugated silver nanoparticles as a reporter. Even though the assay time is relatively long, i.e., 65 min, the ultrasensitive performance makes the system very competitive.

With the advent of sophisticated computational approaches, another means of enhancing the signal beyond using a more sensitive transduction element is to use machine learning in a multi-spot imaging platform as reported by Ballard et al. (Ballard et al., 2020). In this research, a paper-based multi-spot, vertical flow assay equipped with a hand-held colorimetric reader and machine learning framework for detection of the full range of CRP was used to improve the readout capability, sensing linearity, and to avoid the hook effect for high concentrations of antigen. It also was able to uniquely measure the low concentration region implicated for CVD risk stratification. It is envisioned that a similar computational imaging system could be used with multiple spots of various analytes spatially separated in an array. Furthermore, a unique swarm biosensing platform that detects analyte based on the change in the plasmonic signal from thousands of individual single nanoparticles (Ouyang and Di Carlo, 2019) is another means of enhancing quantitative accuracy. This particular platform demonstrated the successful differentiation of the 3-tier clinical cutoffs for CRP in serum using hue as the readout.

Beyond trying to improve sensing capability compared to commercial devices using enhanced signal transduction, computational, and unique swarm sensing approaches, the literature research has explored the use of alternative recognition elements, such as aptamers (Sinha et al., 2019) and chemical-based recognition, e.g., calcium-dependent binding affinity of CRP for phosphocholine (Boonyasit et al., 2019). Rather than widely adopted antibodies, aptamers reduce reagent cost, provide better functional stability, and increase shelf life, especially at ambient conditions (i.e. without the need for refrigeration). While the use of aptamers was demonstrated for a multiplex panel, they do pose concerns with cross-reactivity.

4.2 Research platforms for multiplex cardiac biomarker detection

For multi-marker detection, most of the research platforms surveyed achieved triplex or higher multiplexing. The detection of a 6-plex panel composed of two cardiac markers (e.g., cTnI, BNP) and additional markers (e.g. fibrinogen, TNF-α, SAA, IL-6) was demonstrated using a circular interdigitated capacitive array (Ceylan et al., 2018). Using a hand-held reader with a sensor embedded in a cartridge, this platform achieved 10 pg/mL and 50 pg/mL LOD for cTnI and BNP, respectively, with a 30 min assay time. Sinha et al. demonstrated a diverse panel of 4 markers for CVD risk assessment, which used a field-effect-transistor based system with a wide detection range spanning from pg/mL for cTnI and NT-proBNP, to µg/mL for CRP, and further mg/mL for fibrinogen (Sinha et al., 2019). This platform had a rapid assay time (5 min) and a portable system to automate the detection process. Zhang et al. achieved 3-plex detection of the commonly used AMI panel (cTnI, Myo, and CK-MB) using a paper-based lateral flow assay (Zhang et al., 2018). Using Raman dye encoded core-shell SERS nanotags, multiplex detection was demonstrated on a single test line, which shortened the assay time compared to their previous systems that required individual test lines for each biomarker. Another triplex device targeting AMI employed a more novel panel of markers, (cTnI, h-FABP, and copeptin) (Li et al., 2020). This paper-based assay employed a dual-signal amplification strategy using Co(II)-antibody-luminol-AuNPs, and achieved 20-fold signal enhancement compared to that of HRP-based amplification. It achieved a wide detection range, i.e., pg/mL to µg/mL for cTnI and h-FABP, and pg/mL to mg/mL for copeptin. A duplex platform targeting BNP and sST2 was developed to aid the prognosis of HF at the POC (You et al., 2017). This paper-based lateral flow assay used dual-color core-shell NPs to enhance fluorescence readout, and successfully detected a minimum concentration of 29.92 ng/mL for ST2 and 17.46 pg/mL for BNP in clinical samples.

The most popular multiplexing strategy has been spatial-based approaches, where different spots or lines are functionalized with individual capture probes in 1D or 2D array formats. However, for higher degrees of multiplexing, simultaneous detection of multiple markers within an area but separated instead by the transduction element (i.e. surface enhanced Raman peaks with different Raman reporters or swarm sensing with different sized and shaped particles) could provide a smaller footprint for the device, require less sample volume, and achieve a faster detection time. Such spectral encoding is a commonly used approach, but it tends to increase reagent cost, and typically relies on high performance multi-channel optical sensing systems for accurate classification. Instead, one promising strategy to achieve higher multiplexing while maintaining low cost is to use shape-coded particle based assays, which enables multiplex detection with simpler readout, e.g., either amplified signal in nanoliter droplets with bright field and a single fluorescent channel (Destgeer et al., 2020), or precipitation based colorimetric assays with color readout within the visible spectrum (Roh et al., 2020).

Compared to the multiplexed commercial devices in Table 4 that primarily rely on benchtop readers for quantitative readout, the multiplex research platforms achieved either similar benchtop portable systems but that automate the detection process (Sinha et al., 2019) or hand-held readers (Ceylan et al., 2018) including smartphone based portable readers (Ballard et al., 2020; You et al., 2017). The multiplexed research platforms also demonstrated more diverse sensing modalities and labeling (or label-free) strategies, and led to the successful detection over a wider concentration range, which provided flexibility in choosing biomarker combinations and targeted CVD types. Some of the research platforms demonstrated a higher degree of

multiplexing, i.e., 4-6 markers, compared to 3-4 markers in commercial products, which is promising for future development of higher-level multiplexed commercial platforms.

4.3 General challenges of research biosensor platforms for cardiac biomarker detection

Although many of the new recognition approaches for the biosensors being researched (Table 5) show comparable or considerably enhanced detection limits and demonstrated their sensing capabilities in complex biofluids, potential reasons limiting their adaptation for commercial POC applications may include the difficulty of miniaturizing the accompanied readers and reducing the overall cost. Further, some require the dilution (typically 10-fold) of the sample before detection, which adds another step to the workflow, and the performance could be affected by the dilution factor. Many of the research platforms still rely on the manual handling of the sample and require additional lab equipment in-between steps. The simplification and integration of sample preparation steps would be key towards the development of POC devices. Although some research approaches that perform multiplexed sensing use minimally-instrumented readers and low cost sample handling with paper-based assays show promising results (Jiang et al., 2019; Joung et al., 2019), several reported research platforms require the use of a conventional lab-based microscope or other large-scale equipment for measurement. These comprehensive systems typically provide an excellent signal to noise ratio but the performance when miniaturized where typically less sophisticated readers are implemented for POC testing could compromise the enhanced signals observed. Further, commercial multiplexed devices have demonstrated a consistently shorter assay time (12-20 min), where certain research platforms still struggle to meet this requirement for POC use. Also, the reliability and reproducibility of research platforms, specifically with patient samples, needs more comprehensive testing. The cardiac marker choices used for research platforms still remain too focused on the guideline-recommended and widely adopted marker panels in the commercial sector and, although the research shows strides especially in detection limits, these approaches should be further expanded and applied to the less investigated emerging cardiac biomarkers and multi-marker panels in a clinically relevant setting to assess whether they provide improved diagnostic or prognostic performance.

5. Summary and Future Perspectives

The measurement of cardiac biomarkers in blood is an important part of the standard of care, particularly for ACS/MI and HF. Based on guidelines for clinical practice and clinical studies, cTn, NPs, and CRP are recommended cardiac biomarkers and fairly specific to ACS/MI or HF. Specifically, cTnI and cTnT are the preferred biomarker for ACS/MI and the evaluation of myocardial injury, and also recommended as prognostic markers for acute HF. BNP and NT-proBNP are recommended cardiac biomarkers to establish a diagnosis and prognosis in HF, as well as prognostic markers for early risk stratification of ACS. MR-proANP is recognized as a prognostic marker for ACS and as a diagnostic marker for acute HF. hs-CRP is an inflammatory biomarker and used for risk stratification in CVDs. Emerging biomarkers include copeptin, which may be useful for both ACS/MI diagnosis and HF prognosis, and sST2 and Gal-3, which are promising for prognosis and risk stratification of HF. Biomarkers like CK-MB and Myo are also proven add-on biomarkers for clinical decision making, particularly for MI.

Besides the above protein biomarkers described in the guidelines, it should be noted that several other types of cardiac biomarkers are being studied including; microRNAs such as miR-1, miR-499 and miR-133 that are implicated in the diagnosis of acute MI (Chen et al., 2019) and HF (Vegter et al., 2016). Several fatty acids, such as F2 isoprostanes and trans-18:1 are also reported as potential biomarkers for ACS and CVD risk stratification (Osman et al., 2019). Exosomes derived from different immune cells or cardiomyocytes are also emerging as clinical cardiac biomarkers due to their *in vitro* stability (Wu et al., 2019).

Many factors contribute to the evaluation of a cardiac marker for the development of *in vitro* diagnostic tests. Sensitivity, accuracy and specificity are undeniably the most important criteria, and the cardiac troponins and BNP/NT-proBNP remain the dominant cardiac markers in terms of these criteria for ACS/MI and HF diagnostics, respectively. However, although CK-MB and Myo have lower specificity, because of their relatively rapid change with muscle damage, sensitivity, and maturity as well-studied markers, they are proven add-on biomarkers for clinical decision making. Thus, many commercial cardiac biosensing products have been developed with both high specificity markers and add-on biomarkers in multi-marker formats. The rapid change of the these add-on markers are favorable for AMI but the longer half-life of NT-proBNP, NT-proANP, MR-proADM, and copeptin are also favorable for clinical use like HF since this provides a longer detection window and more reliable quantitative results. Further, multi-biomarker panels can be used to combine the characteristics of different cardiac biomarkers to achieve better sensitivity, specificity, accuracy, or earlier detection compared with individual biomarkers alone, therefore leading to improved decision-making and disease management at different stages.

There are commercial triplex biomarker panels focused on traditional biomarkers (e.g. cTn, Myo, and CK-MB) mostly for MI devices but the majority of commercial systems focus on single biomarker detection. These single marker POC systems are mostly bench-top with expensive cartridges and, although the quantitative commercial devices are able to measure cTnI as low as 0.01 ng/mL, the LODs are not sufficient for early rule-out of MI (0.002 ng/mL). The biosensors currently being investigated in the research literature are showing equivalent or lower LOD relative to the commercial systems mostly through different transduction mechanisms like SERS with nanoparticles but also using innovative computational imaging including deep learning or swarm sensing across a number of individual reaction sites or nanoparticle sensors. Further, some investigators are beginning to explore other recognition elements like aptamers for better stability and long term storage at ambient conditions. Overall, there is still work to be done particularly in the development of affordable and portable POC devices that can quantifiably and robustly detect emerging multi-marker panels that show improved clinical outcomes.

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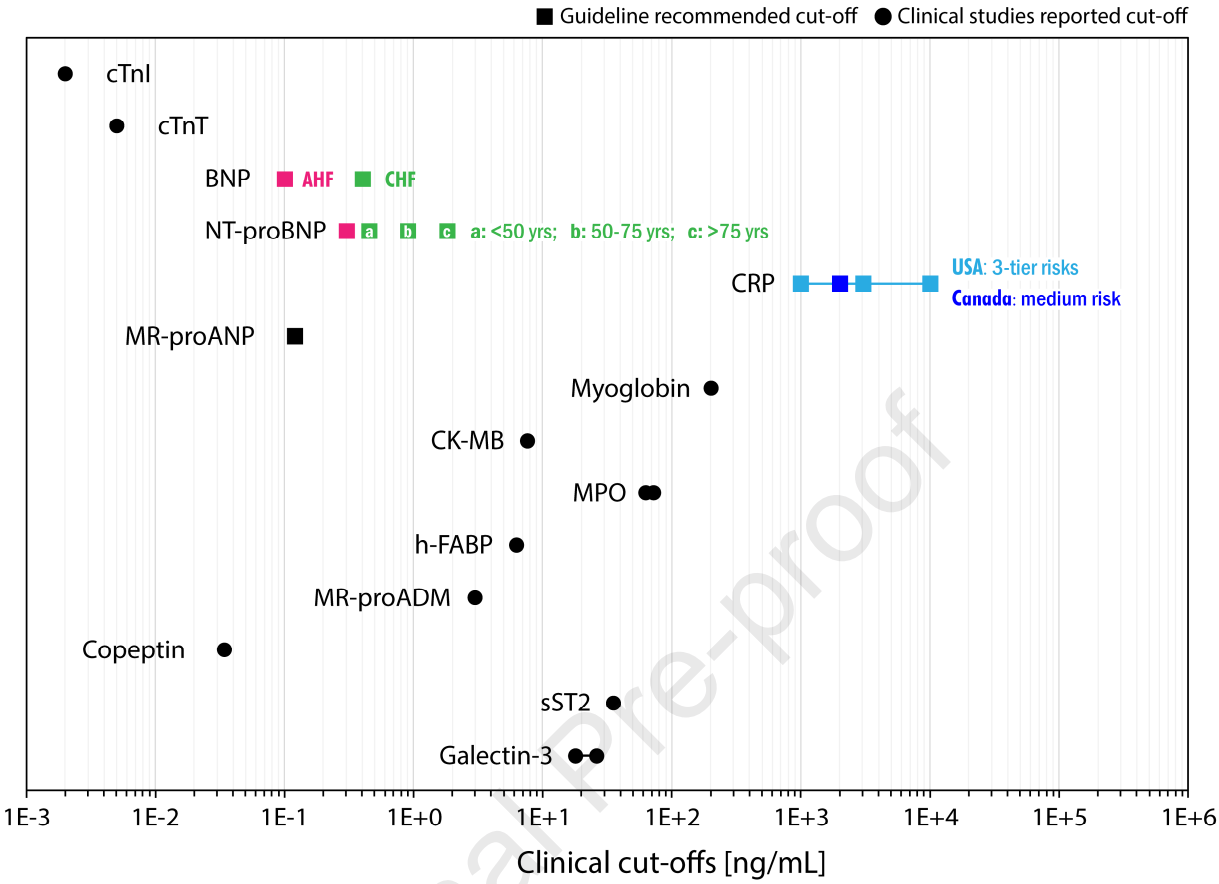

Figure 1. Clinical cut-offs of the fourteen cardiac biomarkers discussed in this review. Cardiac troponin I (cTnI), Cardiac troponin T (cTnT), B-type natriuretic peptide (BNP), N-terminal propeptide B-type natriuretic peptide (NT-proBNP), Mid-regional-fragment of pro-atrial-natriuretic-peptide (MR-proANP), High-sensitivity c-reactive protein(hs-CRP), Soluble suppressor of tumorgenicity 2 (sST2), Galectin-3 (Gal-3), Myoglobin (Myo), Creatine kinase muscle/brain (CK-MB), Myeloperoxidase (MPO), Heart-type fatty acid-binding protein (h-FABP), and Mid-regional-fragment of pro-adrenomedullin-peptide (MR-proADM), acute heart failure (AHF), chronic heart failure (CHF). The clinical cut-offs (marked as category a-c) are age-dependent, where 0.45, 0.9, and 1.8 ng/mL are recommended for <50 years, 50-75 years, and >75 years, respectively.

Table 1. A list of cardiac biomarkers discussed in this review.

♠ USA (AHA/ACC) ♣ Europe (ESC) ♦ Canada (CCS)

CARDIAC BIOMARKERS BY CATEGORY	BIOMARKER UTILITY		
	DIAGNOSTIC MARKER	PROGNOSTIC MARKER	RISK STRATIFICATION
Established Cardiac Specific Biomarkers			
Cardiac troponin I (**cTnI**) Cardiac troponin T (**cTnT**)	**MI** ♠ ♣		
	ACS ♠ (Class I, LOE A) ♣ (Class I, LOE A) ♦	**ACS** ♠ (Class I, LOE B)	**ACS** ♠ (Class I, LOE A)
		Acute HF ♠ (Class I, LOE A) ♣ (Class I, LOE C) ♦ (Strong Recommendation, High-Quality Evidence)	**Chronic HF (a)** ♠ (Class IIb, LOE B-NR) ♦
B-type natriuretic peptide (**BNP**) N-terminal propeptide BNP (**NT-proBNP**)		**ACS** ♣	**ACS** ♠ (Class IIb, LOE B)
	HF ♠ (Class I, LOE A) ♣ (Class I, LOE A) ♦ (Strong Recommendation, High-Quality Evidence)	**HF** ♠ (Class I, LOE A) ♦ (Strong R□comm□ndation; High-Quality Evidence)	**HF** ♠ (Class IIa, LOE B-R) ♦
High-sensitivity c-reactive protein (**hs-CRP**)			**CVDs** ♠ (Class IIa/b, LOE B) ♣ (Class IIb, LOE B) ♦ (Class IIa, Level B)
Mid-regional-fragment of pro-atrial-natriuretic-peptide (**MR-proANP**)		**ACS** ♣	
	Acute HF ♣ (Class I, LOE A)	**HF (a)** ♠	
Emerging Cardiac Biomarkers			
Soluble suppressor of tumorgenicity 2 (**sST2**)		**HF (a)** ♠ ♦	**HF (a)** ♠ (Class IIb, LOE B-NR)
Galectin-3 (**Gal-3**)		**HF (a)** ♠ (Class IIb, LOE B-NR)	**HF** ♦ **HF (a)** ♠ (Class IIb, LOE B-NR)
Copeptin	**MI (a)** ♣		
	ACS (a) ♣		
		Acute HF (a) ♠	
Add-on Cardiac Biomarkers			
Myoglobin (**Myo**)	**MI (a)**		
Creatine kinase muscle/brain (**CK-MB**)	**MI (a)** ♣		
Heart-type fatty acid-binding protein (**h-FABP**)	**MI (a)**	**MI (a)**	
	ACS (a)		
Myeloperoxidase (**MPO**)	**ACS (a)**		**ACS (a)**
		Chronic HF (a)	
Mid-regional-fragment of pro-adrenomedullin-peptide (**MR-proADM**)		**HF**	**Acute HF (a)**

MI: Myocardial Infarction; **ACS**: Acute Coronary Syndrome; **HF**: Heart Failure

(a) – Additive: Biomarkers are recommended in the guideline to be used together with other major markers, not by itself as a single marker, therefore providing "additive" effect. The "additive" value could be diagnostic/prognostic/risk stratification depending on the marker.

In an effort to align patient care with scientific evidence (Jacobs et al., 2013), the recommended classification system as defined by the American Heart Association (**AHA**), American College of Cardiology (**ACC**), European Society of Cardiology (**ESC**) and the Canadian Cardiovascular Society (**CCS**) is categorized by the class of the recommendation (**COR**) and level of evidence (**LOE**) when applied to diagnostic testing for patient care. AHA/ACC and ESC identify three classes of recommendation that range from Class I that has a high benefit to risk ratio down to Class III that indicate the risks outweigh the benefits. The CCS has a two-tier classification system namely Strong or Weak. Similarly, AHA/ACC and ESC have three levels of evidence with slight differences in definition, ranging from Level A-high quality evidence to Level C-consensus of opinion of the experts. CCS has four levels of evidence categories that range from High, Moderate, Low, and Very Low.

Table 2. Combinations of cardiac biomarkers for improved clinical performance.

CVD Type	Multi-Biomarker Panel	Clinical Improvement	Specific Add-on Values in Clinical Studies
ACS /MI	**cTnI + MR-proANP**	Early rule-out of NSTE-ACS	This panel increased the NPV from 97% (MR-proANP alone) and 98.3% (cTnI alone) to 100% in patients presenting within 2 hours from their symptom onset. (Tajsic et al., 2018)
	cTnT + NT-proBNP	Prognosis of adverse events in MI	This panel showed a higher sensitivity (78.6%) compared to either marker alone (50% for cTnT, and 69.0% for NT-proBNP) in predicting adverse events, e.g., death, MI and unstable angina etc, that occurred within 30 days in patients with suspected ACS. (Cameron et al., 2007)
	cTnT + copeptin	Diagnosis of acute MI	Compared to cTnT alone, this panel provided higher AUC (0.9 vs. 0.77), sensitivity (85.1% vs. 43%), and NPV (92.4% vs. 82.4%) for acute MI within 3 hours after chest pain onset. (Keller et al., 2010)
	cTnI or cTnT + copeptin	Diagnosis of MI Early rule-out of MI	The combination of normal levels of cTnI or cTnT together with low levels of copeptin at presentation showed NPV of up to 99% for MI, obviating the need for serial testing in selected patients (Möckel et al., 2015; Ray et al., 2012; Roffi et al., 2016). Furthermore, copeptin may provide additive value even over hs-cTnI/hs-cTnT in the early rule-out of MI. (Roffi et al., 2016)
	cTnT + Myo + CK-MB	Diagnosis of acute MI	Myo is added to the panel for its high sensitivity for acute MI in the early stages (Lindahl et al., 1995; Ye et al., 2018). CK-MB is added to provide additive value for the timing of the myocardial injury and the detection of early reinfarction. (Roffi et al., 2016)
	cTn + CK-MB	Diagnosis of MI	CK-MB provides additive value for the timing of the myocardial injury and the detection of early reinfarction due to its more rapid decline after MI as compared to cTnI/cTnT. (Roffi et al., 2016)
	cTnT + h-FABP	Early rule-out of acute MI	This panel provided a higher sensitivity (85%) compared to h-FABP alone (73%) or cTnT alone (55%), and improved NPV (91% combined v.s.74% h-FABP or 81% cTnT).(McCann et al., 2008)
	cTnI + h-FABP	Prognosis of death or MI	h-FABP showed additive value to cTnI in predicting adverse events (death or MI) in low- and intermediate-risk patients with suspected ACS. (Viswanathan et al., 2010)
	MPO + h-FABP	Diagnosis of ACS	Within the first 2 hours after the onset of chest pain, the specificity of this panel (84.2%) was much higher compared to MPO (<75%) or h-FABP(<70%) alone, and the sensitivity of the combination (69.2%) was higher than hs-TnT alone (<60%). (Inoue et al., 2011)
	cTnI + MPO	Diagnosis of ACS	This panel showed a higher sensitivity (56%) in diagnosis of ACS compared to cTnI alone (31%) in patients with unstable angina. (Sawicki et al., 2011)
	cTnI + BNP + MPO	Risk stratification after the onset of NSTE-ACS	This panel showed a better ability for risk stratification of recurrent ischemic events 30 days after the onset of NSTE-ACS. (Morrow et al., 2008)
HF	**BNP + MR-proANP**	Diagnosis of acute HF	This panel showed higher accuracy (76.6%) in the diagnosis of acute HF compared to BNP (73.6%) and MR-proANP (72.7%) each alone. (Maisel et al., 2010)
	NT-proBNP + sST2	Risk stratification in HF	This panel improved risk stratification and provided a better prediction of later mortality and HF events in long-term follow-up of stable ischemic heart disease. (Richards et al., 2015)
	NT-proBNP + Gal-3	Prognosis in acute HF	This panel improved ability in predicting short-term mortality in patients with acute HF. Specifically, within 60 days, the subjects with elevation in both Gal-3 and NT-proBNP showed higher rates of death than either of the two biomarkers alone. (van Kimmenade et al., 2006)
	CRP+ sST2	Risk stratification in acute HF	This panel improved risk stratification of patients with acute HF to predict 30-day and one-year mortality. (Lassus et al., 2013)
	NT-proBNP + MR-proADM	Prognosis of HF	This panel yielded a stronger prognostic value than individual biomarkers, for predicting death or HF after onset of acute MI. (Khan et al., 2007)
	CRP + MR-proADM	Risk stratification in acute HF	This panel improved risk stratification in patients with acute HF by predicting 30-day and one-year mortality. (Lassus et al., 2013)
	BNP + MPO	prognosis of chronic HF	This panel showed higher AUC (0.70) compared to BNP alone (0.66) for the prognosis of future events in patients with chronic HF. (Tang et al., 2007)
	cTnT +ST2 + MPO	Prognosis of death or HF	This panel showed good prognosis performance in predicting short-term (30 days) cardiovascular death or HF in patients with acute MI. (O'Donoghue et al., 2016)
Future CVDs	**cTnI + BNP + CRP**	Prognosis of major adverse cardiac events (MI/HF)	This panel showed better prediction of major adverse cardiac events after cardiac surgery when compared with each biomarker alone. (Fellahi et al., 2009)

AUC: Area under the curve, referring to the area under receiver operating characteristics (ROC). AUC can be interpreted as a probability of correct classification or prediction. **NPV**: Negative predictive value, referring to the percentage of patients with a negative test who do not have the disease. **PPV**: Positive predictive value, referring to the percentage of patients with a positive test who actually have the disease. **Sensitivity**: Referring to the percentage of persons with the disease who are correctly identified by the test. **Specificity**: Referring the percentage of persons without the disease who are correctly excluded by the test. **Accuracy**: Referring to the percentage that the positive/negative tests correctly

indicate people with/without the disease. The AUC, NPV, PPV, sensitivity, and specificity in the clinical studies shown in the table were with 95% confidence interval (CI).

Table 3a. FDA/CE **c**ertification of commercial IVD products for clinical testing of single cardiac biomarkers.

a. Handheld device; b. Benchtop device; c. Lab-system; d. Qualitative test without the need of a reader; ✓ Available products; ● FDA; ○ CE

Product Name	Company	Type	No. of single markers	Guideline recommended cardiac biomarkers						Cardiac biomarkers with add-on value					Emerging cardiac biomarkers		
				cTnI	cTnT	BNP	NT-proBNP	hs-CRP	MR-proANP	Myo	CK-MB	h-FABP	MPO	MR-proADM	Gal-3	Copeptin	sST2
Cobas h 232	Roche	a	4		✓○		✓			✓	✓						
i-STAT	Abbott		3	✓●○		✓●					✓●						
Minicare I-20	Philips		1	✓○													
PATHFAST	Mitsubishi Chemical	b	5	✓●○			✓●	✓●		✓●○	✓●○						
AFIAS	Boditech		5	✓○			✓○	✓○		✓○	✓○						
Ichroma II	Boditech		5	✓○			✓○	✓○		✓○	✓○						
Mini VIDAS	BioMerieux		5	✓●○			✓●○			✓	✓●				✓○		
DZ-Lite c270	Diazyme		5	✓○				✓●○		✓●○		✓○	✓○				
RAMP	Response Biomedical		4	✓●○			✓●			✓●○	✓●○						
AQT90 Flex	Radiometer		6	✓	✓		✓	✓		✓●	✓●						
QuickSens Ω100	8sens.biognostic		3	✓○				✓○				✓○					
Triage MeterPro	Quidel		3	✓○		✓○	✓○										
SMART/CUBE	EuroLyser		2	✓○				✓○									
Fluoro-Checker TRF	Nano-Ditech		2	✓			✓										
ASPECT-PLUS	Critical Diagnostics		1														✓○
Dimension Vista	Siemens	c	6	✓		✓	✓	✓		✓	✓						
ARCHITECT	Abbott		5	✓●○		✓●				✓●	✓●				✓●○		
Stratus CS Acute Care	Siemens		5	✓●○			✓●	✓●		✓●	✓●						
ADVIA Centaur	Siemens		5	✓●○		✓●○	✓			✓●○	✓●○						
RX series	Randox		4					✓		✓●	✓●	✓○					
Lumipulse G	Fujirebio		4	✓○		✓○				✓○	✓○						
Access 2	Beckman Coulter		3	✓●○						✓	✓						
B·R·A·H·M·S KRYPTOR compact PLUS	Thermo Scientific		3						✓					✓		✓○	
Cobas e analyzer	Roche		2		✓●		✓●○										
RapiCard InstaTest	Cortez Diagnostics	d	3	✓○						✓○	✓○						
Nano-Check	Nano-Ditech		3	✓●			✓○	✓○									
LifeSign	Princeton BioMeditech		3	✓						✓	✓						
CardioDetect	Renesa		2	✓								✓○					
Roche CARDIAC	Roche		1		✓												
Total No. of devices developed for the single marker				23	4	6	14	11	1	17	17	4	1	1	2	1	1

Both (a) handheld and (b) benchtop devices are portable systems with quantitative readout, while (c) are lab-systems that are typically bulky and not compatible with POC settings. Type (a) - (c) are equipped with readers developed by the vendor; while products in (d) are qualitative tests that are read by eye without the need of a reader.

Table 3b. Performance of the POC (handheld or benchtop) commercial IVD products for single cardiac biomarker detection.

Device Type	Commercial devices	Technology	Sample Volume (µL)	Sample Type	Assay Time (min)	Detection Range (ng/mL)										
						cTnI	cTnT	BNP	NT-proBNP	hs-CRP	Myo	CK-MB	h-FABP	MPO	Gal-3	sST2
Handheld	**Cobas h 232**	chromatographic based on gold particle	150	whole blood	8-12		0.04-2		0.06-9		30-700	1-40				
	i-STAT	electrochemical two site ELISA in microchannels	17-95	whole blood/ plasma	5-10	0.02-50		0.015-5				0.6-150				
	Minicare I-20	magnetic particle	30	whole blood/ plasma	10	0.018-6.1										
Benchtop	**PATHFAST**	chemiluminescence enzyme magnetic particle	100	whole blood/ plasma	<17	0.019-50			0.015-30	8×10^3-3×10^7	5-1000	1-250				
	AFIAS	fluorescent	10-50	whole blood/ plasma/ serum	3-15	0.01-15			0.01-30	5×10^3-2×10^5	2-500	3-100				
	Ichroma II	chromatographic fluorescent	-	whole blood/ plasma/ serum	3-12	0.1-50			0.01-30	2.5×10^3-3×10^5	5-500	3-100				
	Mini VIDAS	enzyme-linked fluorescent	150-250	plasma/ serum	17-30	0.01-30			0.02-25		5-1000	0.8-300			3.3-100	
	DZ-Lite c270	latex enhanced immunoturbidimetric	4-25	plasma/ serum	10	0.4-10.0				200-2×10^4	13.2-615.9		0.74-120	12-1000		
	RAMP	chromatographic fluorescent	75	whole blood	15-19	0.03-32			0.027-22		2.36-400	0.32-80				
	AQT90 Flex	time-resolved fluorimetric	-	whole blood/ plasma	11-19	0.01-25	0.01-25		0.012-35	5×10^3-5×10^5	20-900	1.5-300				
	QuickSens Ω100	chromatographic based on gold particle	120	whole blood/ plasma/ serum	15	*1				*500			0.6-75			
	Triage MeterPro	fluorescent in microcapillaries	175	whole blood/ plasma	15-20	0.01-10		0.005-5	0.02-35							
	SMART/CUBE	latex enhanced immunoturbidimetric	20-50	serum	3-10	1-10				250-2×10^4						
	Fluoro-Checker TRF	chromatographic fluorescent	80	whole blood/ plasma/ serum	15	0.03-30			0.02-12.8							
	ASPECT-PLUS	chromatographic fluorescent	35	plasma	20											12.5-257

The devices investigated in this table are the same as those included in the type a and b of Table 3a, company information can be found in Table 3a.
* Value indicates the concentration above which a positive test line appears.

Table 4. Performance of multiplex commercial IVD products for clinical testing of panels of cardiac biomarkers.

1. Qualitative rapid test without reader; 2. Quantitative readout with a reader

Commercial Devices	Company	Readout	Target CVDs	Certification	Technology	Sample Volume (µL)	Sample Type	Assay Time (min)	Multi-Biomarker Panel	Detection Range (ng/mL)
CardioDetect	Renesa	1/2	AMI	-	chromatographic assay	100-120	whole blood	15	cTnI h-FABP	-
Nano-Check AMI 2 IN 1, ND-CD201S	Nano-Ditech	1	AMI	FDA	chromatographic based on gold particle	200-250	plasma/serum	15	cTnI Myo	0.5 80
Nano-Check AMI 3 IN 1, ND-CD301S	Nano-Ditech	1	AMI	FDA	chromatographic based on gold particle	200-250	plasma/serum	15	cTnI CK-MB Myo	0.5 5 80
RapiCard InstaTest	Cortez Diagnostics	1	AMI	CE	chromatographic assay based on particle	500-800 (whole blood) or 300-500 (serum)	whole blood/serum	15	cTnI CK-MB Myo	1.5 7.0 100
Ichroma II Cardiac Triple	Boditech	2	AMI	CE	chromatographic fluorescent assay	75	whole blood/plasma/ serum	12	cTnI CK-MB Myo	0.01-15 3-100 5-500
Fluoro-Check AMI 3 in 1	Nano-Ditech	2	AMI	-	chromatographic based on time resolved fluorescence	80	whole blood/plasma/ serum	15	cTnI CK-MB Myo	0.03 - 30 2 - 200 10 - 500
Nano-Check AMI 2 IN 1, ND-CD202P	Nano-Ditech	1/2	AMI	-	chromatographic based on gold particle	100-120	plasma/serum	15	cTnI Myo	0.1 - 30 20 - 1,000
Nano-Check AMI 3 IN 1, ND-CD302P	Nano-Ditech	1/2	AMI	-	chromatographic based on gold particle	100-120	whole blood/plasma/ serum	15	cTnI CK-MB Myo	0.1 - 30 2 - 200 20 - 1,000
Nano-Check AMI 4 IN 1, ND-CD402P	Nano-Ditech	1/2	AMI	-	chromatographic based on gold particle	100-120	whole blood/plasma/ serum	15	cTnI NT-proBNP CK-MB Myo	0.1 - 30 0.03 - 15 2 - 200 20 - 1,000
LifeSign 2-in-1	Princeton BioMeditech	1/2	AMI	FDA (qualitative)	chromatographic	120	whole blood/plasma/ serum	15	cTnI Myo	1.5 50
LifeSign 3-in-1	Princeton BioMeditech	1/2	AMI	-	chromatographic	120	whole blood/plasma/ serum	15	cTnI CK-MB Myo	1.5 5 50
Triage Cardiac Panel	Quidel	2	AMI	FDA/CE	fluorescence in microcapillaries	-	whole blood/plasma	20	cTnI CK-MB Myo	0.05 - 30 1 - 80 5 - 500
Triage Cardio2 Panel	Quidel	2	ACS/MI/HF	CE	fluorescence in microcapillaries	-	whole blood/plasma	20	cTnI BNP	0.01 - 10 0.005-5
Triage Cardio3 Panel	Quidel	2	ACS/MI/HF	CE	fluorescence in microcapillaries	-	whole blood/plasma	20	cTnI BNP CK-MB	0.01 - 10 0.005-5 1 - 80
Randox Biochip Cardiac Array	Randox	2	ACS	-	chemiluminescent	-	serum	-	cTnI CK-MB Myo h-FABP	0.18-50 0.4-100 1.8-700 0.15-100

Table 5. Recent research platforms for the detection of cardiac biomarkers in complex biofluids.

Analytical performance of the platform was evaluated using: [1] spiked or clinical sample from patients/healthy donor; [2] buffer.

Target CVDs/ Reference	Labeling	Recognition Element	Platform	Readout	Complex Biofluids	Sample Volume (µl)	Assay Time (min)	Multiplex	Marker Panel	LOD	Detection Range	Cross-Reactivity	POC Feature or Highlights
N/A (Ceylan et al., 2018)	label-free	antibody	circular interdigitated capacitive array	capacitance	10× diluted clinical serum	2-5	30	6-plex spatial	[1]cTnI BNP TNF-α SAA IL-6 Fibrinogen	10 pg/mL 50 pg/mL 0.3 pg/mL 1 µg/mL 2.5 pg/mL 50 mg/dL	0.01-0.2 ng/mL 0.05-0.5 ng/mL 0.3-50 pg/mL 1-50 µg/mL 2.5-20 pg/mL 50-500 mg/dL	-	hand-held device with cartridge
CVD risks (Sinha et al., 2019)	label-free	aptamer	field-effect-transistor	current gain	clinical serum/plasma	4	5	4-plex, spatial	[2]cTnI NT-proBNP CRP Fibrinogen	0.394 pg/mL 0.832 pg/mL 0.14 mg/L 20.2 mg/dL	0.001-10 ng/mL 0.05-10 ng/mL 0.1-50 µg/mL 0.1-5 mg/mL	-	portable system to automate the detection process
AMI (Zhang et al., 2018)	encoded SERS nanotag	antibody	paper-based lateral flow assay	Raman intensity	clinical serum	100	17	3-plex single test line	[2]cTnI, Myo CK-MB	0.89 pg/mL 4.2 pg/mL 0.93 pg/mL	0.01-50 ng/mL 0.01-500 ng/mL 0.02-90 ng/mL	cTnI, Myo, CK-MB	multiplex on single test line
CRS (Su et al., 2019)	Ag-Au nanostars	antibody	Au-Ag-Au plasmonic array	plasmon near-field coupling enhanced Raman intensity	clinical plasma	20	~80	3-plex spatial	[2]cTnI NT-proBNP NGAL	0.76 fg/mL 0.53 fg/mL 0.41 fg/mL	1 fg/mL-1 µg/mL	cTnI, NGAL, NT-proBNP	low LODs
AMI (Li et al., 2020)	Co(II)-antibody-luminol-AuNPs	antibody	paper-based vertical flow assay	chemiluminescence	diluted clinical serum	2.5	~30	3-plex, spatial	[2]cTnI h-FABP Copeptin	0.3 pg/mL 0.06 pg/mL 0.4 pg/mL	0.5 pg/mL-1 µg/mL 0.1 pg/mL-1 µg/mL 1 pg/mL-1 mg/mL	cTnI, h-FABP, Copeptin	20-fold signal enhancement, wide detection range
MI (Xu et al., 2020)	fluorescence	antibody	plasmonic gold nano-island	near-infrared enhanced fluorescence	10× diluted clinical serum	10	150/ 60/30	2-plex spatial	[2]cTnI, CK-MB	10 pg/mL 0.272 ng/mL	0.01-1.2 ng/mL 0.25-64 ng/mL	cTnI, CK-MB	130-fold signal enhancement
HF (You et al., 2017)	dual-color core–shell NPs	antibody	paper-based lateral flow assay	fluorescence	10× diluted spiked/clinical serum	10	~20	2-plex, 2 test lines	[1]BNP ST2	17.46 pg/mL 29.92 ng/mL	0.05-1 ng/mL 10-250 ng/mL	cTnI, NT-proBNP CRP, Myo	household use, smartphone based portable reader
(Ballard et al., 2020)	AuNPs	antibody	paper-based vertical flow assay	colorimetric intensity	10× diluted clinical serum	5	12	spatial	[1]CRP	-	0-10 µg/mL	-	machine learning, hand-held reader
(Zong et al., 2017)	DNAzyme /antibody-AgNPs	antibody	glass-chip array	chemiluminescence brightness	clinical serum	8	65	-	[2]cTnT	84 ag/mL	0.003-270 pg/mL	CK-MB CRP	high throughput imaging (48 spots per chip)
(Li et al., 2019)	label-free	antibody	carbon-nanotube thin film	electrochemical impedance spectroscopy	clinical plasma	50	20	-	[1]BNP	16 pg/mL	0.016-4 ng/mL	-	wash-free, disposable test strip, miniature reader
(Boonyasit et al., 2019)	label-free	phosphocholine	paper-based screen-printed electrodes	electrochemical impedance spectroscopy	clinical plasma	30	~10	-	[2]CRP	1 ng/mL	0.005-500 µg/mL	HSA, IgG, fibrinogen	simple folding steps, low reagent cost
(Ouyang and Di Carlo, 2019)	AuNPs	antibody	single AuNPs immobilized on glass	hue	spiked serum	10	45	-	[2]CRP	1 ng/mL	0.001-10 µg/mL	-	differentiation of 3-tier clinical cut-offs in serum
(Primo et al., 2018)	label-free	antibody	thiol-modified Au substrate	surface plasmon resonance	3× diluted spiked serum	60	~60	-	[2]Gal-3	2 ng/mL	10-50 ng/mL	HSA, Hb, IgG, Tf	2-tier clinical cut-offs within linear range
(Wen et al., 2018)	label-free	antibody	CuPdPt NWs modified glassy carbon electrode	amperometric/ electrochemical	spiked serum	10	120	-	[2]MPO	33 fg/mL	100 fg/mL-50 ng/mL	-	wide detection range, low LOD

CRS: Cardiorenal syndrome; **HSA**: Human serum albumin; **Au**: gold; **Ag**: silver; **Co(II)**: Cobalt(II); **NPs**: nanoparticles; **NW**: nanowire; **SERS**: Surface-enhanced Raman spectroscopy; **BSA**: bovine serum albumin; **SAA**: serum amyloid A; **IL-6**: interleukin-6; **NGAL**: neutrophil gelatinase-associated lipocalin; **IgG**: Immunoglobulin G; **Hb**: hemoglobin; **Tf**: transferrin.

References

Adukauskienė, D., Čiginskienė, A., Adukauskaitė, A., Pentiokinienė, D., Šlapikas, R., Čeponienė, I., 2016. Clinical relevance of high sensitivity C-reactive protein in cardiology. Medicina (B. Aires). 52, 1–10. https://doi.org/10.1016/J.MEDICI.2015.12.001

Aimo, A., Januzzi, J.L., Vergaro, G., Ripoli, A., Latini, R., Masson, S., Magnoli, M., Anand, I.S., Cohn, J.N., Tavazzi, L., Tognoni, G., Gravning, J., Ueland, T., Nymo, S.H., Brunner-La Rocca, H.-P., Genis, A.B., Lupón, J., de Boer, R.A., Yoshihisa, A., Takeishi, Y., Egstrup, M., Gustafsson, I., Gaggin, H.K., Eggers, K.M., Huber, K., Tentzeris, I., Tang, W.H.W., Grodin, J., Passino, C., Emdin, M., 2018. Prognostic Value of High-Sensitivity Troponin T in Chronic Heart Failure. Circulation. https://doi.org/10.1161/circulationaha.117.031560

Aleksova, A., Paldino, A., Beltrami, A., Padoan, L., Iacoviello, M., Sinagra, G., Emdin, M., Maisel, A., 2019. Cardiac Biomarkers in the Emergency Department: The Role of Soluble ST2 (sST2) in Acute Heart Failure and Acute Coronary Syndrome—There is Meat on the Bone. J. Clin. Med. https://doi.org/10.3390/jcm8020270

Amsterdam, E.A., Wenger, N.K., Brindis, R.G., Casey, D.E., Ganiats, T.G., Holmes, D.R., Jaffe, A.S., Jneid, H., Kelly, R.F., Kontos, M.C., Levine, G.N., Liebson, P.R., Mukherjee, D., Peterson, E.D., Sabatine, M.S., Smalling, R.W., Zieman, S.J., 2014. 2014 AHA/ACC Guideline for the Management of Patients With Non–ST-Elevation Acute Coronary Syndromes. J. Am. Coll. Cardiol. 64, e139–e228. https://doi.org/10.1016/j.jacc.2014.09.017

Apple, F.S., Chung, A.Y., Kogut, M.E., Bubany, S., Murakami, M.M., 2006. Decreased patient charges following implementation of point-of-care cardiac troponin monitoring in acute coronary syndrome patients in a community hospital cardiology unit. Clin. Chim. Acta 370, 191–195. https://doi.org/10.1016/J.CCA.2006.02.011

Arnett, D.K., Blumenthal, R.S., Albert, M.A., Buroker, A.B., Goldberger, Z.D., Hahn, E.J., Himmelfarb, C.D., Khera, A., Lloyd-Jones, D., McEvoy, J.W., Michos, E.D., Miedema, M.D., Muñoz, D., Smith, S.C., Virani, S.S., Williams, K.A., Yeboah, J., Ziaeian, B., 2019. 2019 ACC/AHA Guideline on the Primary Prevention of Cardiovascular Disease. Circulation. https://doi.org/10.1161/CIR.0000000000000678

Ballard, Z.S., Joung, H.-A., Goncharov, A., Liang, J., Nugroho, K., Di Carlo, D., Garner, O.B., Ozcan, A., 2020. Deep learning-enabled point-of-care sensing using multiplexed paper-based sensors. npj Digit. Med. 3, 66. https://doi.org/10.1038/s41746-020-0274-y

Bayés-Genís, A., Núñez, J., Lupón, J., 2017. Soluble ST2 for Prognosis and Monitoring in Heart Failure: The New Gold Standard? J. Am. Coll. Cardiol. 70, 2389–2392. https://doi.org/10.1016/j.jacc.2017.09.031

Benjamin, E.J., Muntner, P., Alonso, A., Bittencourt, M.S., Callaway, C.W., Carson, A.P., Chamberlain, A.M., Chang, A.R., Cheng, S., Das, S.R., Delling, F.N., Djousse, L., Elkind, M.S.V., Ferguson, J.F., Fornage, M., Jordan, L.C., Khan, S.S., Kissela, B.M., Knutson, K.L., Kwan, T.W., Lackland, D.T., Lewis, T.T., Lichtman, J.H., Longenecker, C.T., Loop, M.S., Lutsey, P.L., Martin, S.S., Matsushita, K., Moran, A.E., Mussolino, M.E., O'Flaherty, M., Pandey, A., Perak, A.M., Rosamond, W.D., Roth, G.A., Sampson, U.K.A., Satou, G.M., Schroeder, E.B., Shah, S.H., Spartano, N.L., Stokes, A., Tirschwell, D.L., Tsao, C.W., Turakhia, M.P., VanWagner, L.B., Wilkins, J.T., Wong, S.S., Virani, S.S., Subcommittee, O. behalf of the A.H.A.C. on E. and P.S.C. and S.S., 2019. Heart Disease and Stroke Statistics—2019 Update: A Report From the American Heart Association. Circulation 139. https://doi.org/10.1161/CIR.0000000000000659

Benjamin, E.J., Virani, S.S., Callaway, C.W., Chamberlain, A.M., Chang, A.R., Cheng, S., Chiuve, S.E., Cushman, M., Delling, F.N., Deo, R., De Ferranti, S.D., Ferguson, J.F., Fornage, M., Gillespie, C., Isasi, C.R., Jiménez, M.C., Jordan, L.C., Judd, S.E., Lackland, D., Lichtman, J.H., Lisabeth, L., Liu, S., Longenecker, C.T., Lutsey, P.L., MacKey, J.S., Matchar, D.B., Matsushita, K., Mussolino, M.E., Nasir, K., O'Flaherty, M., Palaniappan, L.P., Pandey, A., Pandey, D.K., Reeves, M.J., Ritchey, M.D., Rodriguez, C.J., Roth, G.A., Rosamond, W.D., Sampson, U.K.A., Satou, G.M., Shah, S.H., Spartano, N.L., Tirschwell, D.L., Tsao, C.W., Voeks, J.H., Willey, J.Z., Wilkins, J.T., Wu, J.H.Y., Alger, H.M., Wong, S.S., Muntner, P., 2018. Heart disease and stroke statistics - 2018 update: A report from the American Heart Association. Circulation 137, E67–E492. https://doi.org/10.1161/CIR.0000000000000558

Boman, K., Thormark Fröst, F., Bergman, A.C.R., Olofsson, M., 2018. NTproBNP and ST2 as predictors for all-cause and cardiovascular mortality in elderly patients with symptoms suggestive for heart failure. Biomarkers 23, 373–379. https://doi.org/10.1080/1354750X.2018.1431692

Boonyasit, Y., Chailapakul, O., Laiwattanapaisal, W., 2019. A folding affinity paper-based electrochemical impedance device for cardiovascular risk assessment. Biosens. Bioelectron. 130, 389–396. https://doi.org/10.1016/j.bios.2018.09.031

Cameron, S.J., Sokoll, L.J., Laterza, O.F., Shah, S., Green, G.B., 2007. A multi-marker approach for the prediction of adverse events in patients with acute coronary syndromes. Clin. Chim. Acta. https://doi.org/10.1016/j.cca.2006.08.019

Carnethon, M.R., Pu, J., Howard, G., Albert, M.A., Anderson, C.A.M., Bertoni, A.G., Mujahid, M.S., Palaniappan, L., Taylor, H.A., Willis, M., Yancy, C.W., 2017. Cardiovascular Health in African Americans: A Scientific Statement From the American Heart Association. Circulation 136. https://doi.org/10.1161/CIR.0000000000000534

Ceylan, O., Mishra, G.K., Yazici, M., Qureshi, A., Niazi, J.H., Gurbuz, Y., 2018. A Hand-Held Point-of-Care Biosensor Device for Detection of Multiple Cancer and Cardiac Disease Biomarkers Using Interdigitated Capacitive Arrays. IEEE Trans. Biomed. Circuits Syst. 12, 1440–1449. https://doi.org/10.1109/TBCAS.2018.2870297

Chen, Y., Tao, Y., Zhang, L., Xu, W., Zhou, X., 2019. Diagnostic and prognostic value of biomarkers in acute myocardial infarction. Postgrad. Med. J. postgradmedj-2019-136409. https://doi.org/10.1136/postgradmedj-2019-136409

Chow, S.L., Maisel, A.S., Anand, I., Bozkurt, B., de Boer, R.A., Felker, G.M., Fonarow, G.C., Greenberg, B., Januzzi, J.L., Kiernan, M.S., Liu, P.P., Wang, T.J., Yancy, C.W., Zile, M.R., 2017. Role of Biomarkers for the Prevention, Assessment, and Management of Heart Failure: A Scientific Statement From the American Heart Association. Circulation 135. https://doi.org/10.1161/CIR.0000000000000490

Collinson, P., John, C., Lynch, S., Rao, A., Canepa-Anson, R., Carson, E., Cramp, D., 2004. A prospective randomized controlled trial of point-of-care testing on the coronary care unit. Ann. Clin. Biochem. 41, 397–404. https://doi.org/10.1258/0004563041731547

Cushman, M., McClure, L.A., Howard, V.J., Jenny, N.S., Lakoski, S.G., Howard, G., 2009. Implications of increased C-reactive protein for cardiovascular risk stratification in black and white men and women in the US. Clin. Chem. https://doi.org/10.1373/clinchem.2008.122093

Destgeer, G., Ouyang, M., Wu, C.-Y., Di Carlo, D., 2020. Formation of uniform reaction volumes using concentric amphiphilic microparticles. bioRxiv Preprint, DOI: 10.1101/2020.03.15.992321. https://doi.org/10.1101/2020.03.15.992321

Dong, R., Zhang, M., Hu, Q., Zheng, S., Soh, A., Zheng, Y., Yuan, H., 2018. Galectin-3 as a novel biomarker for disease diagnosis and a target for therapy (Review). Int. J. Mol. Med. 41, 599–614. https://doi.org/10.3892/ijmm.2017.3311

Ezekowitz, J.A., O'Meara, E., McDonald, M.A., Abrams, H., Chan, M., Ducharme, A., Giannetti, N., Grzeslo, A., Hamilton, P.G., Heckman, G.A., Howlett, J.G., Koshman, S.L., Lepage, S., McKelvie, R.S., Moe, G.W., Rajda, M., Swiggum, E., Virani, S.A., Zieroth, S., Al-Hesayen, A., Cohen-Solal, A., D'Astous, M., De, S., Estrella-Holder, E., Fremes, S., Green, L., Haddad, H., Harkness, K., Hernandez, A.F., Kouz, S., LeBlanc, M.-H., Masoudi, F.A., Ross, H.J., Roussin, A., Sussex, B., 2017. 2017 Comprehensive Update of the Canadian Cardiovascular Society Guidelines for the Management of Heart Failure. Can. J. Cardiol. 33, 1342–1433. https://doi.org/10.1016/j.cjca.2017.08.022

Fellahi, J.L., Hanouz, J.L., Le Manach, Y., Gué, X., Monier, E., Guillou, L., Riou, B., 2009. Simultaneous measurement of cardiac troponin I, B-type natriuretic peptide, and C-reactive protein for the prediction of long-term cardiac outcome after cardiac surgery. Anesthesiology. https://doi.org/10.1097/ALN.0b013e3181a1f720

Fong, S.W., Few, L.L., See Too, W.C., Khoo, B.Y., Nik Ibrahim, N.N.I., Yahaya, S.A., Yusof, Z., Mohd Ali, R., Abdul Rahman, A.R., Yvonne-Tee, G.B., 2015. Systemic and coronary levels of CRP, MPO, sCD40L and PlGF in patients with coronary artery disease. BMC Res. Notes 8, 679. https://doi.org/10.1186/s13104-015-1677-8

Gaggin, H.K., Motiwala, S., Bhardwaj, A., Parks, K.A., Januzzi, J.L., 2013. Soluble Concentrations of the Interleukin Receptor Family Member ST2 and β-Blocker Therapy in Chronic Heart Failure. Circ. Hear. Fail. 6, 1206–1213. https://doi.org/10.1161/CIRCHEARTFAILURE.113.000457

Gaze, D.C., 2016. Rapid Cardiovascular Diagnostics, in: Proof and Concepts in Rapid Diagnostic Tests and Technologies. InTech. https://doi.org/10.5772/63910

Genest, J., McPherson, R., Frohlich, J., Anderson, T., Campbell, N., Carpentier, A., Couture, P., Dufour, R., Fodor, G., Francis, G.A., Grover, S., Gupta, M., Hegele, R.A., Lau, D.C., Leiter, L., Lewis, G.F., Lonn, E., Mancini, G.B.J., Ng, D., Pearson, G.J., Sniderman, A., Stone, J.A., Ur, E., 2009. 2009 Canadian Cardiovascular Society/Canadian guidelines for the diagnosis and treatment of dyslipidemia and prevention of cardiovascular disease in the adult - 2009 recommendations. Can. J. Cardiol. 25, 567–79.

Goff, D.C., Lloyd-Jones, D.M., Bennett, G., Coady, S., D'Agostino, R.B., Gibbons, R., Greenland, P., Lackland, D.T., Levy, D., O'Donnell, C.J., Robinson, J.G., Schwartz, J.S., Shero, S.T., Smith, S.C., Sorlie, P., Stone, N.J., Wilson, P.W.F., 2014. 2013 ACC/AHA Guideline on the Assessment of Cardiovascular Risk. Circulation 129, S49–S73. https://doi.org/10.1161/01.cir.0000437741.48606.98

Goodacre, S.W., Bradburn, M., Cross, E., Collinson, P., Gray, A., Hall, A.S., 2011. The Randomised Assessment of Treatment using Panel Assay of Cardiac Markers (RATPAC) trial: a randomised controlled trial of point-of-care cardiac markers in the emergency department. Heart 97, 190–196. https://doi.org/10.1136/hrt.2010.203166

Greenland, P., Alpert, J.S., Beller, G.A., Benjamin, E.J., Budoff, M.J., Fayad, Z.A., Foster, E., Hlatky, M.A., Hodgson, J.M., Kushner, F.G., Lauer, M.S., Shaw, L.J., Smith, S.C., Taylor, A.J., Weintraub, W.S., Wenger, N.K., 2010. 2010 ACCF/AHA Guideline for Assessment of Cardiovascular Risk in Asymptomatic Adults. J. Am. Coll. Cardiol. 56, e50–e103. https://doi.org/10.1016/j.jacc.2010.09.001

Griffiths, J., Humphrey, J., Pearson, S.-A., Pallot, D., 2015. USING POINT-OF-CARE CARDIAC MARKERS IN LOW RISK CHEST PAIN IN THE EMERGENCY DEPARTMENT. Emerg. Med. J. 32, e6.1-e6. https://doi.org/10.1136/emermed-2015-204880.15

Inoue, K., Suwa, S., Ohta, H., Itoh, S., Maruyama, S., Masuda, N., Sugita, M., Daida, H., 2011. Heart Fatty Acid-Binding Protein Offers Similar Diagnostic Performance to High-Sensitivity Troponin T in Emergency Room Patients Presenting With Chest Pain. Circ. J. https://doi.org/10.1253/circj.CJ-11-0598

Jacobs, A.K., Kushner, F.G., Ettinger, S.M., Guyton, R.A., Anderson, J.L., Ohman, E.M., Albert, N.M., Antman, E.M., Arnett, D.K., Bertolet, M., Bhatt, D.L., Brindis, R.G., Creager, M.A., DeMets, D.L., Dickersin, K., Fonarow, G.C., Gibbons, R.J., Halperin, J.L., Hochman, J.S., Koster, M.A., Normand, S.-L.T., Ortiz, E., Peterson, E.D., Roach, W.H., Sacco, R.L., Smith, S.C., Stevenson, W.G., Tomaselli, G.F., Yancy, C.W., Zoghbi, W.A., Harold, J.G., He, Y., Mangu, P.B., Qaseem, A., Sayre, M.R., Somerfield, M.R., Jacobs, A.K., Anderson, J.L., Halperin, J.L., Albert, N.M., Creager, M.A., DeMets, D.L., Ettinger, S.M., Guyton, R.A., Hochman, J.S., Kushner, F.G., Ohman, E.M., Stevenson, W.G., Yancy, C.W., 2013. ACCF/AHA Clinical Practice Guideline Methodology Summit Report. Circulation 127, 268–310. https://doi.org/10.1161/CIR.0b013e31827e8e5f

Jiang, N., Ahmed, R., Damayantharan, M., Ünal, B., Butt, H., Yetisen, A.K., 2019. Lateral and Vertical Flow Assays for Point-of-Care Diagnostics. Adv. Healthc. Mater. 8, 1900244. https://doi.org/10.1002/adhm.201900244

Jneid, H., Addison, D., Bhatt, D.L., Fonarow, G.C., Gokak, S., Grady, K.L., Green, L.A., Heidenreich, P.A., Ho, P.M., Jurgens, C.Y., King, M.L., Kumbhani, D.J., Pancholy, S., 2017. 2017 AHA/ACC Clinical Performance and Quality Measures for Adults With ST-Elevation and Non–ST-Elevation Myocardial Infarction. J. Am. Coll. Cardiol. 70, 2048–2090. https://doi.org/10.1016/j.jacc.2017.06.032

Joung, H.-A., Ballard, Z.S., Ma, A., Tseng, D.K., Teshome, H., Burakowski, S., Garner, O.B., Di Carlo, D., Ozcan, A., 2019. Paper-based multiplexed vertical flow assay for point-of-care testing. Lab Chip 19, 1027–1034. https://doi.org/10.1039/C9LC00011A

Karmali, K.N., Brown, T., Sanchez, T., Long, T., Persell, S.D., 2017. Point-of-care testing to promote cardiovascular disease risk assessment: A proof of concept study. Prev. Med. Reports 7, 136–139. https://doi.org/10.1016/J.PMEDR.2017.05.016

Keller, T., Tzikas, S., Zeller, T., Czyz, E., Lillpopp, L., Ojeda, F.M., Roth, A., Bickel, C., Baldus, S., Sinning, C.R., Wild, P.S., Lubos, E., Peetz, D., Kunde, J., Hartmann, O., Bergmann, A., Post, F., Lackner, K.J., Genth-Zotz, S., Nicaud, V., Tiret, L., Münzel, T.F., Blankenberg, S., 2010. Copeptin Improves Early Diagnosis of Acute Myocardial Infarction. J. Am. Coll. Cardiol. https://doi.org/10.1016/j.jacc.2010.01.029

Kemp, M., Donovan, J., Higham, H., Hooper, J., 2004. Biochemical markers of myocardial injury. BJA Br. J. Anaesth. 93, 63–73. https://doi.org/10.1093/bja/aeh148

Khan, S.Q., O'Brien, R.J., Struck, J., Quinn, P., Morgenthaler, N., Squire, I., Davies, J., Bergmann, A., Ng, L.L., 2007. Prognostic Value of Midregional Pro-Adrenomedullin in Patients With Acute Myocardial Infarction. The LAMP (Leicester Acute Myocardial Infarction Peptide) Study. J. Am. Coll. Cardiol. https://doi.org/10.1016/j.jacc.2006.12.038

King, K.R., Grazette, L.P., Paltoo, D.N., McDevitt, J.T., Sia, S.K., Barrett, P.M., Apple, F.S., Gurbel, P.A., Weissleder, R., Leeds, H., Iturriaga, E.J., Rao, A.K., Adhikari, B., Desvigne-Nickens, P., Galis, Z.S., Libby, P., 2016. Point-of-Care Technologies for Precision Cardiovascular Care and Clinical Research. JACC Basic to Transl. Sci. 1, 73–86. https://doi.org/10.1016/j.jacbts.2016.01.008

Kragelund, C., Grønning, B., Køber, L., Hildebrandt, P., Steffensen, R., 2005. N-Terminal Pro–B-Type Natriuretic Peptide and Long-Term Mortality in Stable Coronary Heart Disease. N. Engl. J. Med. 352, 666–675. https://doi.org/10.1056/NEJMoa042330

Lambert, L., Brown, K., Segal, E., Brophy, J., Rodes-Cabau, J., Bogaty, P., 2010. Association between timeliness of reperfusion therapy and clinical outcomes in ST-elevation myocardial infarction. JAMA - J. Am. Med. Assoc. https://doi.org/10.1001/jama.2010.712

Land, K.J., Boeras, D.I., Chen, X.S., Ramsay, A.R., Peeling, R.W., 2019. REASSURED diagnostics to inform disease control strategies, strengthen health systems and improve patient outcomes. Nat. Microbiol. https://doi.org/10.1038/s41564-018-0295-3

Lassus, J., Gayat, E., Mueller, C., Peacock, W.F., Spinar, J., Harjola, V.P., Van Kimmenade, R., Pathak, A., Mueller, T., Disomma, S., Metra, M., Pascual-Figal, D., Laribi, S., Logeart, D., Nouira, S., Sato, N., Potocki, M., Parenica, J., Collet, C., Cohen-Solal, A., Januzzi, J.L., Mebazaa, A., 2013. Incremental value of biomarkers to clinical variables for mortality prediction in acutely decompensated heart failure: The Multinational Observational Cohort on Acute Heart Failure (MOCA) study. Int. J. Cardiol. 168, 2186–2194. https://doi.org/10.1016/j.ijcard.2013.01.228

Leigh, J.A., Alvarez, M., Rodriguez, C.J., 2016. Ethnic Minorities and Coronary Heart Disease: an Update and Future Directions. Curr. Atheroscler. Rep. 18, 9. https://doi.org/10.1007/s11883-016-0559-4

Levine, G.N., O'Gara, P.T., Beckman, J.A., Al-Khatib, S.M., Birtcher, K.K., Cigarroa, J.E., de las Fuentes, L., Deswal, A., Fleisher, L.A., Gentile, F., Goldberger, Z.D., Hlatky, M.A., Joglar, J.A., Piano, M.R., Wijeysundera, D.N., 2019. Recent Innovations, Modifications, and Evolution of ACC/AHA Clinical Practice Guidelines: An Update for Our Constituencies: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. Circulation 139. https://doi.org/10.1161/CIR.0000000000000651

Li, F., Guo, L., Hu, Y., Li, Z., Liu, J., He, J., Cui, H., 2020. Multiplexed chemiluminescence determination of three acute myocardial infarction biomarkers based on microfluidic paper-based immunodevice dual amplified by multifunctionalized gold nanoparticles. Talanta 207, 120346. https://doi.org/10.1016/j.talanta.2019.120346

Li, Z., Ausri, I.R., Zilberman, Y., Tang, X.S., 2019. Towards label-free, wash-free and quantitative B-type natriuretic peptide detection for heart failure diagnosis. Nanoscale 11, 18347–18357. https://doi.org/10.1039/c9nr05386g

Lindahl, B., Venge, P., Wallentin, L., 1995. Early diagnosis and exclusion of acute myocardial infarction using biochemical monitoring. The BIOMACS Study Group. Biochemicals Markers of Acute Coronary Syndromes. Coron Artery Dis.

Maisel, A., Mueller, C., Nowak, R., Peacock, W.F., Landsberg, J.W., Ponikowski, P., Mockel, M., Hogan, C., Wu, A.H.B., Richards, M., Clopton, P., Filippatos, G.S., Di Somma, S., Anand, I., Ng, L., Daniels, L.B., Neath, S.-X., Christenson, R., Potocki, M., McCord, J., Terracciano, G., Kremastinos, D., Hartmann, O., von Haehling, S., Bergmann, A., Morgenthaler, N.G., Anker, S.D., 2010. Mid-Region Pro-Hormone Markers for Diagnosis and Prognosis in Acute Dyspnea: Results From the BACH (Biomarkers in Acute Heart Failure) Trial. J. Am. Coll. Cardiol. 55, 2062–2076. https://doi.org/10.1016/J.JACC.2010.02.025

McCann, C.J., Glover, B.M., Menown, I.B.A., Moore, M.J., McEneny, J., Owens, C.G., Smith, B., Sharpe, P.C., Young, I.S., Adgey, J.A., 2008. Novel biomarkers in early diagnosis of acute myocardial infarction compared with cardiac troponin T. Eur. Heart J. 29, 2843–2850. https://doi.org/10.1093/eurheartj/ehn363

Melanson, S.F., Lewandrowski, E.L., Januzzi, J.L., Lewandrowski, K.B., 2004. Reevaluation of myoglobin for acute chest pain evaluation: Would false-positive results on "first-draw" specimens lead to increased hospital admissions? Am. J. Clin. Pathol. https://doi.org/10.1309/80MCYMBL4DLNJDC3

Mendis, S., Puska, P., Norrving, B., 2011. Global Atlas on cardiovascular disease prevention and control Joint Publication of the World Health Organization the World Heart 1–166. https://doi.org/10.1016/S0022-3476(51)80275-0

Möckel, M., Searle, J., Hamm, C., Slagman, A., Blankenberg, S., Huber, K., Katus, H., Liebetrau, C., Müller, C., Muller, R., Peitsmeyer, P., von Recum, J., Tajsic, M., Vollert, J.O., Giannitsis, E., 2015. Early discharge using single cardiac troponin and copeptin testing in patients with suspected acute coronary syndrome (ACS): a randomized, controlled clinical process study. Eur. Heart J. 36, 369–376. https://doi.org/10.1093/eurheartj/ehu178

Moe, G.W., Ezekowitz, J.A., O'Meara, E., Lepage, S., Howlett, J.G., Fremes, S., Al-Hesayen, A., Heckman, G.A., Abrams, H., Ducharme, A., Estrella-Holder, E., Grzeslo, A., Harkness, K., Koshman, S.L., McDonald, M., McKelvie, R., Rajda, M., Rao, V., Swiggum, E., Virani, S., Zieroth, S., Arnold, J.M.O., Ashton, T., D'Astous, M., Chan, M., De, S., Dorian, P., Giannetti, N., Haddad, H., Isaac, D.L., Kouz, S., Leblanc, M.-H., Liu, P., Ross, H.J., Sussex, B., White, M., Canadian Cardiovascular Society, 2015. The 2014 Canadian Cardiovascular Society Heart Failure Management Guidelines Focus Update: anemia, biomarkers, and recent therapeutic trial implications. Can. J. Cardiol. 31, 3–16. https://doi.org/10.1016/j.cjca.2014.10.022

Morgenthaler, N.G., Struck, J., Alonso, C., Bergmann, A., 2005. Measurement of midregional proadrenomedullin in plasma with an immunoluminometric assay. Clin. Chem. https://doi.org/10.1373/clinchem.2005.051110

Morgenthaler, N.G., Struck, J., Jochberger, S., Dünser, M.W., 2008. Copeptin: clinical use of a new biomarker. Trends Endocrinol. Metab. 19, 43–49. https://doi.org/10.1016/j.tem.2007.11.001

Morrow, D.A., Cannon, C.P., Jesse, R.L., Newby, L.K., Ravkilde, J., Storrow, A.B., Wu, A.H.B., Christenson, R.H., 2007. National Academy of Clinical Biochemistry Laboratory Medicine Practice Guidelines: Clinical Characteristics and Utilization of Biochemical Markers in Acute Coronary Syndromes. Circulation 115. https://doi.org/10.1161/CIRCULATIONAHA.107.182882

Morrow, D.A., Sabatine, M.S., Brennan, M.-L., de Lemos, J.A., Murphy, S.A., Ruff, C.T., Rifai, N., Cannon, C.P., Hazen, S.L., 2008. Concurrent evaluation of novel cardiac biomarkers in acute coronary syndrome: myeloperoxidase and soluble CD40 ligand and the risk of recurrent ischaemic events in TACTICS-TIMI 18. Eur. Heart J. 29, 1096–1102. https://doi.org/10.1093/eurheartj/ehn071

MPO-Practitioner-One-Pager-CHL-D006b [WWW Document], n.d. URL www.clevelandheartlab.com (accessed 4.5.19).

Mueller, T., Gegenhuber, A., Leitner, I., Poelz, W., Haltmayer, M., Dieplinger, B., 2016. Diagnostic and prognostic accuracy of galectin-3 and soluble ST2 for acute heart failure. Clin. Chim. Acta 463, 158–164. https://doi.org/10.1016/J.CCA.2016.10.034

MYTHILI, S., MALATHI, N., 2015. Diagnostic markers of acute myocardial infarction. Biomed. Reports. https://doi.org/10.3892/br.2015.500

Neumann, J.T., Sörensen, N.A., Schwemer, T., Ojeda, F., Bourry, R., Sciacca, V., Schaefer, S., Waldeyer, C., Sinning, C., Renné, T., Than, M., Parsonage, W., Wildi, K., Makarova, N., Schnabel, R.B., Landmesser, U., Mueller, C., Cullen, L., Greenslade, J., Zeller, T., Blankenberg, S., Karakas, M., Westermann, D., 2016. Diagnosis of myocardial infarction using a high-sensitivity Troponin i 1-hour algorithm. JAMA Cardiol. https://doi.org/10.1001/jamacardio.2016.0695

O'Brien, P., Reagan, W., York, M., Jacobsen, M., 2011. Review of Qualification Data for Cardiac Troponins. Cent. Drug Eval. Res. U.S. Food Drug Adm. https://doi.org/10.1016/j.yrtph.2013.07.006

O'Donoghue, M.L., Morrow, D.A., Cannon, C.P., Jarolim, P., Desai, N.R., Sherwood, M.W., Murphy, S.A., Gerszten, R.E., Sabatine, M.S., 2016. Multimarker Risk Stratification in Patients With Acute Myocardial Infarction. J. Am. Heart Assoc. https://doi.org/10.1161/JAHA.115.002586

O'Gara, P.T., Kushner, F.G., Ascheim, D.D., Casey, D.E., Chung, M.K., de Lemos, J.A., Ettinger, S.M., Fang, J.C., Fesmire, F.M., Franklin, B.A., Granger, C.B., Krumholz, H.M., Linderbaum, J.A., Morrow, D.A., Newby, L.K., Ornato, J.P., Ou, N., Radford, M.J., Tamis-Holland, J.E., Tommaso, C.L., Tracy, C.M., Woo, Y.J., Zhao, D.X., 2013. 2013 ACCF/AHA Guideline for the Management of ST-Elevation Myocardial Infarction. J. Am. Coll. Cardiol. 61, e78–e140. https://doi.org/10.1016/j.jacc.2012.11.019

Osman, J., Tan, S.C., Lee, P.Y., Low, T.Y., Jamal, R., 2019. Sudden Cardiac Death (SCD) – risk stratification and prediction with molecular biomarkers. J. Biomed. Sci. 26, 39. https://doi.org/10.1186/s12929-019-0535-8

Ouyang, M., Di Carlo, D., 2019. Nanoplasmonic swarm biosensing using single nanoparticle colorimetry. Biosens. Bioelectron. 132, 162–170. https://doi.org/10.1016/J.BIOS.2019.02.056

Parikh, R., Mathai, A., Parikh, S., Sekhar, G.C., Thomas, R., 2008. Understanding and using sensitivity, specificity and predictive values. Indian J. Ophthalmol. 56, 45–50. https://doi.org/10.4103/0301-4738.37595

Pearson, T.A., Mensah, G.A., Alexander, R.W., Anderson, J.L., Cannon, R.O., Criqui, M., Fadl, Y.Y., Fortmann, S.P., Hong, Y., Myers, G.L., Rifai, N., Smith, S.C., Taubert, K., Tracy, R.P., Vinicor, F., 2003. Markers of Inflammation and Cardiovascular Disease. Circulation 107, 499–511. https://doi.org/10.1161/01.CIR.0000052939.59093.45

Peeling, R.W., Holmes, K.K., Mabey, D., Ronald, A., 2006. Rapid tests for sexually transmitted infections (STIs): The way forward. Sex. Transm. Infect. https://doi.org/10.1136/sti.2006.024265

Pelsers, M.M.A.L., Hermens, W.T., Glatz, J.F.C., 2005. Fatty acid-binding proteins as plasma markers of tissue injury. Clin. Chim. Acta 352, 15–35. https://doi.org/10.1016/j.cccn.2004.09.001

Perk, J., De Backer, G., Gohlke, H., Graham, I., Reiner, Z., Verschuren, M., Albus, C., Benlian, P., Boysen, G., Cifkova, R., Deaton, C., Ebrahim, S., Fisher, M., Germano, G., Hobbs, R., Hoes, A., Karadeniz, S., Mezzani, A., Prescott, E., Ryden, L., Scherer, M., Syvanne, M., Scholte Op Reimer, W.J.M., Vrints, C., Wood, D., Zamorano, J.L., Zannad, F., Cooney, M.T., Bax, J., Baumgartner, H., Ceconi, C., Dean, V., Deaton, C., Fagard, R., Funck-Brentano, C., Hasdai, D., Hoes, A., Kirchhof, P., Knuuti, J., Kolh, P., McDonagh, T., Moulin, C., Popescu, B.A., Reiner, Z., Sechtem, U., Sirnes, P.A., Tendera, M., Torbicki, A., Vahanian, A., Windecker, S., Funck-Brentano, C., Sirnes, P.A., Aboyans, V., Ezquerra, E.A., Baigent, C., Brotons, C., Burell, G., Ceriello, A., De Sutter, J., Deckers, J., Del Prato, S., Diener, H.-C., Fitzsimons, D., Fras, Z., Hambrecht, R., Jankowski, P., Keil, U., Kirby, M., Larsen, M.L., Mancia, G., Manolis, A.J., McMurray, J., Pajak, A., Parkhomenko, A., Rallidis, L., Rigo, F., Rocha, E., Ruilope, L.M., van der Velde, E., Vanuzzo, D., Viigimaa, M., Volpe, M., Wiklund, O., Wolpert, C., 2012. European Guidelines on cardiovascular disease prevention in clinical practice (version 2012): The Fifth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by re. Eur. Heart J. 33, 1635–1701. https://doi.org/10.1093/eurheartj/ehs092

Plüddemann, A., Thompson, M., Price, C.P., Wolstenholme, J., Heneghan, C., 2012. Point-of-care testing for the analysis of lipid panels: primary care diagnostic technology update. Br. J. Gen. Pract. 62, e224-6. https://doi.org/10.3399/bjgp12X630241

Ponikowski, P., Voors, A.A., Anker, S.D., Bueno, H., Cleland, J.G.F., Coats, A.J.S., Falk, V., González-Juanatey, J.R., Harjola, V.-P., Jankowska, E.A., Jessup, M., Linde, C., Nihoyannopoulos, P., Parissis, J.T., Pieske, B., Riley, J.P., Rosano, G.M.C., Ruilope, L.M., Ruschitzka, F., Rutten, F.H., van der Meer, P., Filippatos, G., McMurray, J.J. V, Aboyans, V., Achenbach, S., Agewall, S., Al-Attar, N., Atherton, J.J., Bauersachs, J., John Camm, A., Carerj, S., Ceconi, C., Coca, A., Elliott, P., Erol, Ç., Ezekowitz, J., Fernández-Golfín, C., Fitzsimons, D., Guazzi, M., Guenoun, M., Hasenfuss, G., Hindricks, G., Hoes, A.W., Iung, B., Jaarsma, T., Kirchhof, P., Knuuti, J., Kolh, P., Konstantinides, S., Lainscak, M., Lancellotti, P., Lip, G.Y.H., Maisano, F., Mueller, C., Petrie, M.C., Piepoli, M.F., Priori, S.G., Torbicki, A., Tsutsui, H., van Veldhuisen, D.J., Windecker, S., Yancy, C., Zamorano, J.L., Zamorano, J.L., Aboyans, V., Achenbach, S., Agewall, S., Badimon, L., Barón-Esquivias, G., Baumgartner, H., Bax, J.J., Bueno, H., Carerj, S., Dean, V., Erol, Ç., Fitzsimons, D., Gaemperli, O., Kirchhof, P., Kolh, P., Lancellotti, P., Lip, G.Y.H., Nihoyannopoulos, P., Piepoli, M.F., Ponikowski, P., Roffi, M., Torbicki, A., Vaz Carneiro, A., Windecker, S., Sisakian, H.S., Isayev, E., Kurlianskaya, A., Mullens, W., Tokmakova, M., Agathangelou, P.,

Melenovsky, V., Wiggers, H., Hassanein, M., Uuetoa, T., Lommi, J., Kostovska, E.S., Juillière, Y., Aladashvili, A., Luchner, A., Chrysohoou, C., Nyolczas, N., Thorgeirsson, G., Marc Weinstein, J., Di Lenarda, A., Aidargaliyeva, N., Bajraktari, G., Beishenkulov, M., Kamzola, G., Abdel-Massih, T., Celutkiene, J., Noppe, S., Cassar, A., Vataman, E., Abir-Khalil, S., van Pol, P., Mo, R., Straburzynska-Migaj, E., Fonseca, C., Chioncel, O., Shlyakhto, E., Otasevic, P., Goncalvesová, E., Lainscak, M., Díaz Molina, B., Schaufelberger, M., Suter, T., Yilmaz, M.B., Voronkov, L., Davies, C., 2016. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure. Eur. Heart J. 37, 2129–2200. https://doi.org/10.1093/eurheartj/ehw128

Primo, E.N., Kogan, M.J., Verdejo, H.E., Bollo, S., Rubianes, M.D., Rivas, G.A., 2018. Label-Free Graphene Oxide-Based Surface Plasmon Resonance Immunosensor for the Quantification of Galectin-3, a Novel Cardiac Biomarker. ACS Appl. Mater. Interfaces 10, 23501–23508. https://doi.org/10.1021/acsami.8b03039

Ray, P., Charpentier, S., Chenevier-Gobeaux, C., Reichlin, T., Twerenbold, R., Claessens, Y.E., Jourdain, P., Riou, B., Mueller, C., 2012. Combined copeptin and troponin to rule out myocardial infarction in patients with chest pain and a history of coronary artery disease. Am. J. Emerg. Med. https://doi.org/10.1016/j.ajem.2011.12.008

Rehman, S.U., Mueller, T., Januzzi, J.L., 2008. Characteristics of the Novel Interleukin Family Biomarker ST2 in Patients With Acute Heart Failure. J. Am. Coll. Cardiol. 52, 1458–1465. https://doi.org/10.1016/j.jacc.2008.07.042

Reiter, M., Twerenbold, R., Reichlin, T., Mueller, M., Hoeller, R., Moehring, B., Haaf, P., Wildi, K., Merk, S., Bernhard, D., Mueller, C.Z., Freese, M., Freidank, H., Campodarve Botet, I., Mueller, C., 2013. Heart-type fatty acid-binding protein in the early diagnosis of acute myocardial infarction. Heart. https://doi.org/10.1136/heartjnl-2012-303325

Richards, A.M., Di Somma, S., Mueller, T., 2015. ST2 in stable and unstable ischemic heart diseases. Am. J. Cardiol. https://doi.org/10.1016/j.amjcard.2015.01.041

Ridker, P.M., 2014. Inflammation, C-Reactive Protein, and Cardiovascular Disease: Moving Past the Marker Versus Mediator Debate. Circ. Res. 114, 594–595. https://doi.org/10.1161/CIRCRESAHA.114.303215

Robinson, J.G., Ray, K., 2016. Moving Toward the Next Paradigm for Cardiovascular Prevention. Circulation 133, 1533–6. https://doi.org/10.1161/CIRCULATIONAHA.116.022134

Roffi, M., Patrono, C., Collet, J.-P., Mueller, C., Valgimigli, M., Andreotti, F., Bax, J.J., Borger, M.A., Brotons, C., Chew, D.P., Gencer, B., Hasenfuss, G., Kjeldsen, K., Lancellotti, P., Landmesser, U., Mehilli, J., Mukherjee, D., Storey, R.F., Windecker, S., Baumgartner, H., Gaemperli, O., Achenbach, S., Agewall, S., Badimon, L., Baigent, C., Bueno, H., Bugiardini, R., Carerj, S., Casselman, F., Cuisset, T., Erol, Ç., Fitzsimons, D., Halle, M., Hamm, C., Hildick-Smith, D., Huber, K., Iliodromitis, E., James, S., Lewis, B.S., Lip, G.Y.H., Piepoli, M.F., Richter, D., Rosemann, T., Sechtem, U., Steg, P.G., Vrints, C., Luis Zamorano, J., Zamorano, J.L., Aboyans, V., Achenbach, S., Agewall, S., Badimon, L., Barón-Esquivias, G., Baumgartner, H., Bax, J.J., Bueno, H., Carerj, S., Dean, V., Erol, Ç., Fitzsimons, D., Gaemperli, O., Kirchhof, P., Kolh, P., Lancellotti, P., Lip, G.Y., Nihoyannopoulos, P., Piepoli, M.F., Ponikowski, P., Roffi, M., Torbicki, A., Carneiro, A.V., Windecker, S., Chilingaryan, A., Weidinger, F., Najafov, R., Sinnaeve, P.R., Terzic, I., Postadzhiyan, A., Milicic, D., Eftychiou, C., Widimsky, P., Bang, L., El Etriby, A., Marandi, T., Pietilä, M., Kedev, S., Koning, R., Aladashvili, A., Neumann, F.-J., Tsioufis, K., Becker, D., Guðnason, T., Matetzky, S., Bolognese, L., Mussagaliyeva, A., Beishenkulov, M., Latkovskis, G., Serpytis, P., Pereira, B., Magri, C.J., Grosu, A., Abir-Khalil, S., Larsen, A.I., Budaj, A., Mimoso, J.M.V., Ginghina, C., Averkov, O., Nedeljkovic, M.A., Studencan, M., Barrabés, J.A., Held, C., Rickli, H., Peters, R.J., Mourali, M.S., Atalar, E., Swanson, N., Parkhomenko, A., 2016. 2015 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation. Eur. Heart J. 37, 267–315. https://doi.org/10.1093/eurheartj/ehv320

Roh, Y.H., Lee, H.J., Kim, J.Y., Kim, H.U., Kim, S.M., Bong, K.W., 2020. Precipitation-based Colorimetric Multiplex Immunoassay in Hydrogel Particles. Lab Chip. https://doi.org/10.1039/d0lc00325e

RTI International, 2016. Projections of Cardiovascular Disease Prevalence and Costs: 2015–2035 Technical Report.

Rubini Giménez, M., Twerenbold, R., Boeddinghaus, J., Nestelberger, T., Puelacher, C., Hillinger, P., Wildi, K., Jaeger, C., Grimm, K., Heitzelmann, K.F., Sabti, Z., Badertscher, P., Cupa, J., Honegger, U., Schaerli, N., Kozhuharov, N., du Fay de Lavallaz, J., Lopez, B., Salgado, E., Miró, Ò., Martín-Sánchez, F.J., Adrada, E.R., Morawiec, B., Parenica, J., Ganovska, E., Neugebauer, C., Rentsch, K., Lohrmann, J., Osswald, S., Reichlin, T., Mueller, C., 2016. Clinical Effect of Sex-Specific Cutoff Values of High-Sensitivity Cardiac Troponin T in Suspected Myocardial Infarction. JAMA Cardiol. https://doi.org/10.1001/jamacardio.2016.2882

Savic-Radojevic, A., Pljesa-Ercegovac, M., Matic, M., Simic, D., Radovanovic, S., Simic, T., 2017. Novel Biomarkers of Heart Failure, in: Advances in Clinical Chemistry. https://doi.org/10.1016/bs.acc.2016.09.002

Sawicki, M., Sypniewska, G., Kozinski, M., Gruszka, M., Krintus, M., Obonska, K., Pilaczynska-Cemel, M., Kubica, J., 2011. Diagnostic efficacy of myeloperoxidase for the detection of acute coronary syndromes. Eur. J. Clin. Invest. https://doi.org/10.1111/j.1365-2362.2010.02457.x

Searle, J., Shih, J., Muller, R., Vollert, J.O., Müller, C., Danne, O., Datwyler, S., Möckel, M., 2013. The role of myeloperoxidase (MPO) for prognostic evaluation in sensitive cardiac troponin I negative chest pain patients in the emergency department. Eur. Hear. J. Acute Cardiovasc. Care 2, 203–210. https://doi.org/10.1177/2048872613484688

Sinha, A., Tai, T.Y., Li, K.H., Gopinathan, P., Chung, Y. Da, Sarangadharan, I., Ma, H.P., Huang, P.C., Shiesh, S.C., Wang, Y.L., Lee, G. Bin, 2019. An integrated microfluidic system with field-effect-transistor sensor arrays for detecting multiple cardiovascular biomarkers from clinical samples. Biosens. Bioelectron. 129, 155–163. https://doi.org/10.1016/j.bios.2019.01.001

Spaeth, B.A., Shephard, M.D., Omond, R., 2017. Clinical Application of Point-of-Care Testing in the Remote Primary Health Care Setting. Qual. Prim. Care 25, 164–175.

Spinarova, L.L., Parenica, J., Spinar, J., Spinarova, M., Labr, K., Malek, F., Ostadal, P., Ludka, O., Goldbergova-Pavkova, M., Tomandl, J., 2018. Prognostic impact of copeptin and mid-regional pro-adrenomedullin in chronic heart failure with regard to comorbidities, in: EUROPEAN JOURNAL OF HEART FAILURE. WILEY 111 RIVER ST, HOBOKEN 07030-5774, NJ USA, p. 432.

Su, Y., Xu, S., Zhang, J., Chen, X., Jiang, L.P., Zheng, T., Zhu, J.J., 2019. Plasmon Near-Field Coupling of Bimetallic Nanostars and a Hierarchical Bimetallic SERS "hot Field": Toward Ultrasensitive Simultaneous Detection of Multiple Cardiorenal Syndrome Biomarkers. Anal. Chem. 91, 864–872. https://doi.org/10.1021/acs.analchem.8b03573

Tajsic, M., Járai, R., Koch, J., Stangl, K., Wojta, J., Dreger, H., Huber, K., 2018. Midregional pro-A-type natriuretic peptide as part of a dual biomarker strategy for the early rule out of non-ST segment elevation acute coronary syndrome – The WilCop study. Int. J. Cardiol. https://doi.org/10.1016/j.ijcard.2018.09.084

Tang, W.H.W., Tong, W., Troughton, R.W., Martin, M.G., Shrestha, K., Borowski, A., Jasper, S., Hazen, S.L., Klein, A.L., 2007. Prognostic Value and Echocardiographic Determinants of Plasma Myeloperoxidase Levels in Chronic Heart Failure. J. Am. Coll. Cardiol. https://doi.org/10.1016/j.jacc.2007.02.053

Thygesen, K., Alpert, J.S., Jaffe, A.S., Chaitman, B.R., Bax, J.J., Morrow, D.A., White, H.D., Infarction, E.G. on behalf of the J.E.S. of C. (ESC)/American C. of C. (ACC)/American H.A. (AHA)/World H.F. (WHF) T.F. for the U.D. of M., 2018. Fourth Universal Definition of Myocardial Infarction (2018). J. Am. Coll. Cardiol. 72, 2231–2264. https://doi.org/10.1016/j.jacc.2018.08.1038

Thygesen, K., Mair, J., Mueller, C., Huber, K., Weber, M., Plebani, M., Hasin, Y., Biasucci, L.M., Giannitsis, E., Lindahl, B., Koenig, W., Tubaro, M., Collinson, P., Katus, H., Galvani, M., Venge, P., Alpert, J.S., Hamm, C., Jaffe, A.S., 2012. Recommendations for the use of natriuretic peptides in acute cardiac care: A position statement from the Study Group on Biomarkers in Cardiology of the ESC Working Group on Acute Cardiac Care. Eur. Heart J. 33, 2001–2006. https://doi.org/10.1093/eurheartj/ehq509

Twerenbold, R., Boeddinghaus, J., Nestelberger, T., Wildi, K., Rubini Gimenez, M., Badertscher, P., Mueller, C., 2017. Clinical Use of High-Sensitivity Cardiac Troponin in Patients With Suspected Myocardial Infarction. J. Am. Coll. Cardiol. 70, 996–1012. https://doi.org/10.1016/J.JACC.2017.07.718

Tzikas, S., Keller, T., Ojeda, F.M., Zeller, T., Wild, P.S., Lubos, E., Kunde, J., Baldus, S., Bickel, C., Lackner, K.J., Münzel, T.F., Blankenberg, S., 2013. MR-proANP and MR-proADM for risk stratification of patients with acute chest pain. Heart. https://doi.org/10.1136/heartjnl-2012-302956

van Kimmenade, R.R., Januzzi, J.L., Ellinor, P.T., Sharma, U.C., Bakker, J.A., Low, A.F., Martinez, A., Crijns, H.J., MacRae, C.A., Menheere, P.P., Pinto, Y.M., 2006. Utility of Amino-Terminal Pro-Brain Natriuretic Peptide, Galectin-3, and Apelin for the Evaluation of Patients With Acute Heart Failure. J. Am. Coll. Cardiol. https://doi.org/10.1016/j.jacc.2006.03.061

Vegter, E.L., Van Der Meer, P., De Windt, L.J., Pinto, Y.M., Voors, A.A., 2016. MicroRNAs in heart failure: From biomarker to target for therapy. Eur. J. Heart Fail. 18, 457–468. https://doi.org/10.1002/ejhf.495

Vila, G., Riedl, M., Maier, C., Struck, J., Morgenthaler, N.G., Handisurya, A., Prager, G., Ludvik, B., Clodi, M., Luger, A., 2009. Plasma MR-proADM correlates to BMI and decreases in relation to leptin after gastric bypass surgery. Obesity. https://doi.org/10.1038/oby.2009.22

Viswanathan, K., Kilcullen, N., Morrell, C., Thistlethwaite, S.J., Sivananthan, M.U., Hassan, T.B., Barth, J.H., Hall, A.S., 2010. Heart-Type Fatty Acid-Binding Protein Predicts Long-Term Mortality and Re-Infarction in Consecutive Patients With Suspected Acute Coronary Syndrome Who Are Troponin-Negative. J. Am. Coll. Cardiol. https://doi.org/10.1016/j.jacc.2009.12.062

Weber, M., Hamm, C., 2006. Role of B-type natriuretic peptide (BNP) and NT-PROBNP in clinical routine. Heart. https://doi.org/10.1136/hrt.2005.071233

Wen, Y., Yuan, J., Chen, J., Zhao, Y., Niu, Y., Yu, C., 2018. Amperometric myeloperoxidase immunoassay based on the use of CuPdPt nanowire networks. Microchim. Acta 185, 1–8. https://doi.org/10.1007/s00604-017-2563-y

Wu, R., Gao, W., Yao, K., Ge, J., 2019. Roles of Exosomes Derived From Immune Cells in Cardiovascular Diseases. Front. Immunol. 10, 648. https://doi.org/10.3389/fimmu.2019.00648

Xu, W., Wang, L., Zhang, R., Sun, X., Huang, L., Su, H., Wei, X., Chen, C.-C., Lou, J., Dai, H., Qian, K., 2020. Diagnosis and prognosis of myocardial infarction on a plasmonic chip. Nat. Commun. 11, 1654. https://doi.org/10.1038/s41467-020-15487-3

Yancy, C.W., Jessup, M., Bozkurt, B., Butler, J., Casey, D.E., Colvin, M.M., Drazner, M.H., Filippatos, G.S., Fonarow, G.C., Givertz, M.M., Hollenberg, S.M., Lindenfeld, J., Masoudi, F.A., McBride, P.E., Peterson, P.N., Stevenson, L.W., Westlake, C., 2017. 2017 ACC/AHA/HFSA Focused Update of the 2013 ACCF/AHA Guideline for the Management of Heart Failure. J. Am. Coll. Cardiol. https://doi.org/10.1016/j.jacc.2017.04.025

Yancy, C.W., Jessup, M., Bozkurt, B., Butler, J., Casey, D.E., Drazner, M.H., Fonarow, G.C., Geraci, S.A., Horwich, T., Januzzi, J.L., Johnson, M.R., Kasper, E.K., Levy, W.C., Masoudi, F.A., McBride, P.E., McMurray, J.J.V., Mitchell, J.E., Peterson, P.N., Riegel, B., Sam, F., Stevenson, L.W., Tang, W.H.W., Tsai, E.J., Wilkoff, B.L., 2013. 2013 ACCF/AHA Guideline for the Management of Heart Failure: A Report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. J. Am. Coll. Cardiol. 62, e147–e239. https://doi.org/10.1016/J.JACC.2013.05.019

Ye, X.D., He, Y., Wang, S., Wong, G.T., Irwin, M.G., Xia, Z., 2018. Heart-type fatty acid binding protein (H-FABP) as a biomarker for acute myocardial injury and long-term post-ischemic prognosis review-article. Acta Pharmacol. Sin. https://doi.org/10.1038/aps.2018.37

You, M., Lin, M., Gong, Y., Wang, S., Li, A., Ji, L., Zhao, H., Ling, K., Wen, T., Huang, Y., Gao, D., Ma, Q., Wang, T., Ma, A., Li, X., Xu, F., 2017. Household Fluorescent Lateral Flow Strip Platform for Sensitive and Quantitative Prognosis of Heart Failure Using Dual-Color Upconversion Nanoparticles. ACS Nano 11, 6261–6270. https://doi.org/10.1021/acsnano.7b02466

Zhang, D., Huang, L., Liu, B., Su, E., Chen, H.Y., Gu, Z., Zhao, X., 2018. Quantitative detection of multiplex cardiac biomarkers with encoded SERS nanotags on a single T line in lateral flow assay. Sensors Actuators, B Chem. 277, 502–509. https://doi.org/10.1016/j.snb.2018.09.044

Zong, C., Zhang, D., Yang, H., Wang, S., Chu, M., Li, P., 2017. Chemiluminescence immunoassay for cardiac troponin T by using silver nanoparticles functionalized with hemin/G-quadruplex DNAzyme on a glass chip array. Microchim. Acta 184, 3197–3204. https://doi.org/10.1007/s00604-017-2331-z

A Review of Biosensor Technologies for Blood Biomarkers Toward Monitoring Cardiovascular Diseases at the Point of Care

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Highlights

- Comprehensive insights including clinical aspects and commercial devices related to facilitating multi-marker panel selection for the development of biosensors targeting cardiac biomarkers.
- Extensive review on both gold-standard and emerging cardiac biomarkers used in ACS, MI, and HF based on authoritative guidelines for clinical practice and recent clinical studies.
- Detailed discussion on multi-marker panels and how the combination of markers may lead to improved diagnosis, prognosis, and risk stratification of ACS/MI or HF.
- Focused survey on commercial *in vitro* diagnostic devices for the detection of both single cardiac biomarkers and multi-marker panels within various settings including centralized labs and at the point-of-care.
- Identification of recent biosensor technologies, their sensing modalities, and the strengths and weaknesses of these approaches in addressing gaps in the commercial approaches.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: