Curr Emerg Hosp Med Rep (2016) 4:99–106
DOI 10.1007/s40138-016-0104-3

Novel Cardiac Biomarkers for Emergency Department Evaluation of Acute Coronary Syndrome: The Recent Evidence on Non-troponin Biomarkers and Their Limitations

Taylor R. Spencer[1,2] · Mandeep S. Sidhu[3,4] · Jonathan Bisaillon[5] · C. Christopher King[1]

Published online: 4 July 2016

Abstract
Purpose of Review Evaluation of chest pain in the emergency department remains a common diagnostic challenge. Significant concerns persist regarding the potential for missed acute coronary syndrome and the limitations of troponin as a diagnostic biomarker. Therefore, recent research has explored and shed further light on a variety of novel biomarkers for use in the evaluation of chest pain and possible acute coronary syndrome.
Recent Findings This article reviews the most recent literature regarding four of the biomarkers that have generated the most interest: matrix metalloproteinases, copeptin, ischemia-modified albumin, and heart-type fatty acid binding protein. Pregnancy-associated plasma protein A is also considered. Research has studied these both independently, and in conjunction with troponin assays. This review additionally addresses the potential role of risk stratification in applying these biomarkers.
Summary There remain concerns about the limitations of a troponin-based diagnostic strategy and the potential for missed myocardial infarction. However, despite the promise of a variety of novel biomarkers, the limitations of these alternatives remain apparent.

Keywords Cardiac biomarkers · Chest pain evaluation · Acute coronary syndrome · Emergency department

Introduction

The initial evaluation of chest pain and possible acute coronary syndrome (ACS) in the emergency department (ED) includes laboratory testing to identify non-ST elevation myocardial infarction (NSTEMI). The specific serum biomarkers for ACS have evolved over time, and troponin is the primary biomarker currently used in most ED settings.

The troponin complex is made up of three subunits involved in myocardial sarcomeric contraction. Cardiac isoforms—cardiac troponin T (cTnT) and cardiac troponin I (cTnI)—are subunits exclusively expressed in myocardial tissue. Elevated serum levels are therefore markers of myocardial injury. But non-ACS elevations in troponin may result from a wide range of conditions, including sepsis, renal disease, and pulmonary embolism. Analytic issues can produce false-positive results in healthy subjects [1]. The conventional cTnT has been reported to be 72 % sensitive and 95 % specific. High-sensitivity troponin assays increase sensitivity to 94 % with a decrease in specificity to 73 % [2]. cTnI shows similar tradeoffs with conventional and high-sensitivity assays [3]. As a diagnostic test, troponin is effective and widely utilized, but many have explored its limitations [4–6]. These limitations

This article is part of the Topical collection on *Acute Coronary Syndrome*.

✉ Taylor R. Spencer
spencet@mail.amc.edu

[1] Department of Emergency Medicine, Albany Medical Center, Albany, NY, USA

[2] Department of Emergency Services, Ellis Hospital, Schenectady, NY, USA

[3] Division of Cardiology, Department of Medicine, Albany Medical Center, Albany, NY, USA

[4] Division of Cardiology, Department of Medicine, Stratton Veterans Affairs Medical Center, Albany, NY, USA

[5] Internal Medicine Residency, University of Massachusetts, Worchester, MA, USA

highlight the need for alternative biomarkers to improve the accuracy of ACS diagnosis.

While reviews of alternative diagnostic cardiac biomarkers have previously been published, we focus on the strongest evidence from the past three years. The search for a better biomarker has been challenging. To illustrate this, Lin's 2012 review of 58 studies that evaluated 37 novel biomarkers found clinical value for only five biomarkers: matrix metalloproteinase-9, copeptin, ischemia-modified albumin, heart-type fatty acid binding protein, and B-type natriuretic peptide [7•]. They represent discrete aspects in the pathogenesis of ACS, such as plaque instability to myocardial ischemia and necrosis. The latest evidence on each of these alternative biomarkers is explored here.

We have excluded from our review B-type Natriuretic Peptide (BNP) and its N-terminal fragment, N-terminal Pro-B-type Natriuretic Peptide (NT-pro-BNP), which are secreted in response to overloaded cardiac ventricles. Emerging evidence suggests that BNP or NT-pro-BNP is a potential prognostic tool rather than a diagnostic tool [8–14]. As a diagnostic tool, they only modestly predict severity of underlying coronary disease and ischemia [7•, 15–17]. There seems to be little new evidence for supporting BNP or NT-pro-BNP in ACS diagnosis.

Biomarkers

Matrix Metalloproteinases

Metalloproteinases (MMP) hydrolyze components of the extracellular matrix and possibly identify ACS and plaque instability. There are 23 recognized MMPs [18]. MMP-9 levels correlate with Framingham risk score, and elevations may identify risk for future myocardial infarction [19]. MMP-2 and -9 have been evaluated in the diagnosis of STEMI [20, 21], or ACS more generally [22]. It is theorized that "increased expression of MMP-2 and MMP-9 metalloproteinases and their tissue inhibitor (TIMP-2) is responsible for disturbed equilibrium of the metalloproteinase/tissue inhibitors system and as a consequence, for destabilization of atherosclerotic plaque and occurrence of the acute coronary syndrome" [22]. Small studies have found possible utility of MMP-9 in ACS diagnosis [23, 24]. However, countervailing evidence was provided by a study finding no elevation in blood mononuclear cells' expression of MMP-9 on admission in acute myocardial infarction (AMI) [25].

Pregnancy-associated plasma protein A (PAPP-A), another metalloproteinase, may have a diagnostic role [26], including potential utility in unstable angina not detected by troponin elevation [27]. In fact, PAPP-A has figured more prominently in recent literature than the matrix metalloproteinases. Yet PAPP-A is only 90 % sensitive [28], and a five-study meta-analysis encompassing 2050 patients found it to be inferior to troponin [29]. In the only study from that meta-analysis to explore supplementing troponin with PAPP-A, there were only marginal non-significant improvements in diagnostic accuracy [29, 30]. Currently, there is an inadequate support for any of the metalloproteinases in ACS diagnosis for routine clinical practice.

Copeptin

Copeptin, the C-terminus of the vasopressin prohormone, is a surrogate of vasopressin release and indicates neurohormonal stress activation and vasoactive response. In a recent small study, copeptin blood levels mirror cTnT in AMI [31]. But the area under the curve (AUC) in ROC analysis of copeptin in NSTEMI was only 0.71. A cutoff of 10.25 pmol/l showed a sensitivity of 88.8 % and specificity of 69.8 % [31]. Other investigators found equally poor diagnostic characteristics when copeptin was used alone [32, 33•, 34].

A recent meta-analysis of 9244 patients from 14 studies found that patients with AMI and an initially negative troponin had a higher copeptin level than those without AMI (22.8 vs. 8.3 pmol/l) [35••]. Rather than being used alone, copeptin may complement troponin in early chest pain, identifying ACS missed by troponin. Piyanuttapull states that "the additional use of copeptin to cTnT allows for a rapid triage of chest pain patients to an early diagnosis of non-ST elevation myocardial infarction" [31]. In one study of 1927 patients in which NSTEMI had a 6 % rule-in rate, elevated copeptin identified approximately half of NSTEMI patients with initially negative cTnI (10 of 19 patients) [36••]. Furthermore, in 12 studies on 6988 chest pain patients without STEMI, adding copeptin to troponin had a sensitivity of 95 % (compared to 87 % sensitivity for troponin alone for AMI). However, this was at the expense of considerable loss of specificity (57 % for troponin plus copeptin, compared to 84 % for troponin) [37]. Charpentier et al. added copeptin to troponin in 641 ED patients which increased the sensitivity from 55.3 to 90.4 % in their cohort and improved the negative predictive value (NPV) from 92.8 to 97.6 % [38]. The AUC of the combination of copeptin and cTnI was significantly higher than for cTnI alone on presentation (0.89 vs. 0.77) [38], and similar results have been noted in diabetic patients [34]. Others report a NPV of 98 % in patients with known coronary disease presenting within 6 h of symptom onset [39]. A large meta-analysis seems to reinforce these results [35••].

While it is better with copeptin, one must still ask if the combined NPV is high enough to be applied in our current

medico-legal environment. Charpentier et al. contend that "the sensitivity of this combination even using a conventional troponin assay remains insufficient to safely rule out NSTEMI at the time of presentation" [38]. A NPV around 98 % [38, 39] is certainly high but still not perfect. Moreover, the lost specificity and perhaps only modest increase in identified cases cannot be ignored. The large CHOPIN trial (copeptin helps in the early detection of patients with acute myocardial infarction) encompassed 16-sites and 1927 patients in EDs with less than six hours of chest discomfort onset and without electrocardiographic evidence of ST segment elevation. Combined normal initial cTnI and copeptin was seen in 58 % of patients, with a NPV of 99.2 %. But almost one-third (503/1646) of patients with normal troponin had an increased copeptin, and just ten of these patients ruled in as NSTEMI [36••]. A persistent copeptin elevation on serial testing may identify those with actual infarction [40]; but even with a negative copeptin and troponin, almost as many patients (9/1143) ruled in, so copeptin barely identified half of the unrecognized NSTEMI [36••].

In the COPED–MIRRO trial—a multicenter, prospective, observational, longitudinal cohort study of 1018 patients—copeptin plus troponin on arrival did not fully rule out AMI without necessitating additional evaluation [32]. In the Randomized Assessment of Treatment using Panel Assay of Cardiac Markers (RATPAC) trial, a randomized controlled trial of point-of-care cardiac markers in the ED that included 2243 subjects, copeptin did not add significantly to troponin in suspected-AMI [10]. Thus larger trials have cast doubt on the utility of copeptin in routine clinical practice.

High-sensitivity assays may provide better results. When high-sensitivity (hs) cTnT was used in combination with copeptin, the summary sensitivity and specificity were 98–50 % [37]. With multiple hs-cTnI assays in comparison, the sensitivity combined with copeptin may be slightly less–ranging from 89.4 to 97.8 %—with modest increases in specificity. The best reported AUC for hs-cTnI and copeptin was 0.94 [41]. In discussing their study of 1170 patients which showed a trend toward improved diagnostic accuracy using hs-cTnT combined with copeptin, Potoki et al. stated "copeptin provides independent prognostic information, largely by overcoming the challenging interpretation of mild increases in hs-cTnT" [42]. However, others have argued that copeptin does not enhance the diagnosis of NSTEMI over hs-cTnT alone [10, 34, 43, 44, 45•] and that the sensitivity of this combination is still not high enough to definitively exclude AMI [46].

The addition of ultrasensitive (us) copeptin has also been studied. The "ultrasensitive copeptin in addition to medium sensitivity cardiac troponin for the early diagnosis of non-ST-elevation Acute Coronary Syndromes" (COPACS) trial included 190 subjects enrolled within the first 6 h of onset of non-traumatic chest pain who did not have ST elevation on EKG. If validated, this might support an expedited cardiac rule out in the ED, and do so with a numerically higher sensitivity (100 vs 89.7 %, although not reaching statistical significance in this study). Unfortunately, the sensitivity increase is at the expense of specificity—only 74.2 % for the combination. The single-sample strategy of combined us-copeptin and cTnI is statistically non-inferior to serial cTnI for NSTEMI, although the AUC was actually numerically decreased from 0.939 to 0.871 [47]. Using high-sensitivity troponin assays instead of conventional cTnI, the AUC in one recent study improved to 0.93 for hs-cTnT plus us-copeptin, compared to 0.89 for hs-cTnT alone. In this case, sensitivity improved from 76 to 96 % and NPV from 95 to 98.9 % [48].

Regardless of which assays are used, a high NPV for a combination of troponin and copeptin would be advantageous for ruling out ACS. The NPV approaches 100 % in some studies [47, 49–51], but other investigators have found that even high-sensitivity assays for both troponin and copeptin have yielded NPVs similar to standard troponin and copeptin alone [36••, 37, 39]. To summarize, based on the literature that currently exists, there is inadequate evidence to support the routine use of copeptin—either alone or combined with troponin—as a diagnostic tool for the evaluation of AMI.

Ischemia-Modified Albumin

Ischemia changes the structure of serum albumin into ischemia-modified albumin (IMA). This modified protein is measured by its decreased binding of cations [52]. Since troponin will not rise in reversible myocardial ischemia without myocardial necrosis, IMA might be sensitive for parts of the ACS spectrum missed by troponin, notably angina.

A specific weakness of troponin is its poor sensitivity early in the course of symptom onset and clinical progression. Alternative biomarkers might rise earlier and address this weakness. Liebetrau et al. used a surgical treatment for hypertrophic cardiomyopathy as a model for AMI. IMA concentrations increased significantly 30 min after induction of this model of AMI versus baseline, with values of 26.0 U/ml (Interquartile range [IQR] 21.8–38.6 U/ml) versus 15.6 U/ml (IQR 10.1–24.7 U/ml), and then decreased after 75 min [53].

IMA was examined in a small cohort of ED patients in a third-world population in India presenting with suspected ACS within 6 h of onset of symptoms [54]. In the group determined to be non-ischemic, the mean IMA value was 56.38 ± 23.89 U/ml. In unstable angina, IMA levels were

89.00 ± 7.76 U/ml, and in AMI levels reached 87.50 ± 9.62 U/ml. Using a cutoff of ≤80 U/ml, IMA was normal in 21 of 24 patients without ischemia, and elevated in all but 2 of 25 patients with unstable angina and 6 of 40 patients with AMI. This yielded a sensitivity of 92 % and specificity of 87 %, with NPV of 94 % [54].

In another recent study where the sensitivity of IMA was only 88 %, this was improved to 96 % when used in combination with cTnI [55]. In 2014, Bhakthavatsala Reddy et al. found in a study of 89 patients that in 16 patients an early diagnosis could be made when compared with cTnT [54]. However, the results of other studies have been less favorable [26, 56–58]. Here again, conflicting findings make it difficult to recommend adoption of this marker for clinical practice.

Heart-Type Fatty Acid Binding Protein

Heart-type Fatty Acid Binding Protein (hFABP) is a small cytoplasmic protein released from cardiac myocytes following cardiac injury. As with IMA, levels of hFABP significantly increase 15 min after induction of a surgical model of "AMI" to reach a level of 9.0 ng/mL (IQR 7.0–15.4 ng/ml) compared to a baseline of 4.6 ng/mL (IQR 3.4–7.1 ng/ml). hFABP showed a continuous increase until the 8th hour with a decline afterward [53]. As previously noted in the discussion of IMA, the generalizability of this model to ED evaluation of undifferentiated chest pain is unclear. But with its promising biokinetics, hFABP may prove to have better sensitivity in the early presentation of chest pain [59].

Despite this promise, hFABP has not yet been shown to improve the diagnosis of an ED patient with chest pain. Freund and colleagues' recent data found that hFABP does not provide useful additional information to cTnI for ruling out AMI with a NPV of 96 % versus a NPV of 95 % for cTnI alone [60]. Others have also failed to find any improvements in sensitivity, specificity, PPV, and NPV for the diagnosis ACS by adding hFABP [61]. In contrast, in the previously described RATPAC study, the combination of hFABP and troponin actually increased diagnostic sensitivity [10]. Further, adding copeptin to this combination of cTnI and hFABP has been shown in one study to provide a net NPV of 95.8 % and AUC of 0.88 [62]. But it is noteworthy that the NPV is not significantly different from the NPV for cTnI alone by Freund and colleagues [60]. Jacobs et al. still argue that the combination of cTnI, hFABP, and copeptin actually was superior to cTnI alone. These authors point to the first three hours—when troponin's limitations are most apparent—and when the NPV of 92.9 % for the cTnI–hFABP–copeptin combination was clearly superior to the NPV of 84.6 % for cTnI alone [62].

But if most others find that hFABP does not add to the diagnostic sensitivity to standard troponin, it also generally fails to add to the high-sensitivity troponin based on emerging evidence. In 2013, Reiter and colleagues demonstrated this in a large 1247-patient multicenter study [45]. Smaller studies also revealed that the diagnostic characteristics did not significantly improve by addition of hFABP [63–67], but adding hFABP to hs-cTnT did increase the sensitivity from 83 to 96.8 % and the NPV 95.6–98.9 % for NSTEMI, enhancing the rule-out capability at the expense of significant loss of specificity [63]. Only the small study by Gami and colleagues found the combination could reach a NPV of 100 % while maintaining a specificity of 88.89 % [68]. Others have not reproduced such promising diagnostic characteristics. Thus, despite favorable kinetics, hFABP does not reliably live up to its promise of adding to the sensitivity of the troponins currently used—or at least not without compromising specificity.

The Patient Selection Challenge

The most recent research suggests that cardiac biomarkers—both troponin assays and novel biomarkers—do not in themselves provide clarity in ED evaluation of chest pain. The "holy grail" of perfect sensitivity without sacrificing specificity and ideal biokinetics has not yet been found.

The answer may not be a better assay alone but the selection of appropriate patients using risk stratification tools, algorithms, and risk score models to improve diagnostic accuracy. In cases of STEMI, MMP-9 level correlated with Global Registry of Acute Coronary Event (GRACE) risk score, although individually each demonstrated relatively poor diagnostic characteristics [69]. At a minimum, the correlation between the biomarker and the risk score suggests they may be measuring the same thing, although this relationship does not determine whether the use of GRACE risk scoring in conjunction with MMP-9 would add any diagnostic value, or whether this would extend to NSTEMI as well.

A 2014 study of 537 ED patients found that adding copeptin to cTnI improved the NPV for ruling out AMI. But it did so in low-to-intermediate risk patients specifically. When applied to patients with a GRACE risk score below 140 (11.3 % rate of AMI in this group), copeptin plus high-sensitivity troponin had an AUC of 0.925 and NPV of 98.6 %. At a lower cutoff below 108 (4.4 % AMI rate), the AUC was 0.935 and the NPV for AMI reached 100 % (95 % CI 97.5–100.0) [70]. Thus a single troponin and copeptin measurement might safely rule out AMI in a low-to-intermediate risk population [71, 72]. The Thrombolysis in Myocardial Infarction (TIMI) score has also been

proposed as a risk-stratifying option when using copeptin [46].

The Manchester Acute Coronary Syndromes (MACS) decision rule was formulated for suspected cardiac chest pain in the ED [73]. Among its eight variables were high-sensitivity cTnT and hFABP. These biomarkers were used in conjunction with a series of electrocardiographic and clinical findings, in effect incorporating a clinical assessment. The decision rule categorizes patients into four different risk assessment categories. The AUC for major cardiac events was 0.95 in the derivation study [73, 74]. With the validation, the MACS rule had a sensitivity of 100.0 % (95 % CI 95.4–100.0 %) and NPV 100.0 % (97.1–100.0 %) for AMI [74]. Perhaps there is some promise for hFABP in the context of the MACS, with its incorporation of clinical features. But Freund and colleagues looked at hFABP in a low-risk group, based on empirical clinician judgment. While the NPV was strong, this held true for troponin alone as well. Even in the low-risk group, there was no significant improvement using hFABP [60].

Risk stratification may theoretically enhance the ability to rule out ACS. It would seem that the most promising applications for the novel biomarkers might not be in simply replacing or complementing troponin. Rather, the novel biomarkers may be the most useful to enhance the diagnostic capabilities of risk stratification tools and troponin together. Nevertheless, more robust evidence is needed, including determining which biomarkers and which risk stratification tools are most effective.

Conclusion

The diagnosis of acute chest pain in the ED setting is a common clinical challenge requiring substantial health care resource utilization. To improve the effectiveness and efficiency of care, significant efforts have been made to identify novel biomarkers that will aid in the diagnosis of AMI.

This article reviews literature on non-troponin cardiac biomarkers from the past three years, specifically those that were previously identified as having early promise [7•]. However, despite an array of options being explored, no clear solution exists, and it is possible that ACS may be too complex to be reduced to a laboratory test alone. While hope still exists for identifying a breakthrough diagnostic biomarker strategy, definitive answers and consensus remain elusive. The novel biomarkers cannot yet be endorsed for standard use in routine clinical practice.

The contradictions and limitations in the recent literature reviewed here on potential replacements or supplements to the troponin assay suggest that the search for the "perfect" biomarker, or combination of biomarkers, may be futile. A few studies have incorporated clinical risk stratification with some promise, but this evidence is preliminary at best. Further research on existing biomarkers as well as the possible development of new biomarkers—will be needed before any significant advancements in the evaluation of patients presenting with symptoms of AMI in the ED can be made.

Acknowledgments The authors would like to thank Dr. William E. Boden.

Compliance with Ethical Guidelines

Conflict of Interest Drs Spencer, Sidhu, Bisaillon, and King declare no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain studies with human or animal subjects performed by the author.

References

Papers of particular interest, published recently, are highlighted as:
- • Of importance
- •• Of major importance

1. Korff S, Katus HA, Giannitsis E. Differential diagnosis of elevated troponins. Heart. 2006;92(7):987–93.
2. Al-Saleh A, Alazzoni A, Al Shalash S, Ye C, Mbuagbaw L, Thabane L, Jolly SS. Performance of the high-sensitivity troponin assay in diagnosing acute myocardial infarction: systematic review and meta-analysis. CMAJ Open. 2014;2(3):E199–207. doi:10.9778/cmajo.20130074.
3. Sabu T, Kavsak P, Devereaux PJ. Cardiac troponin: clinical considerations for high-sensitivity assays. Clin Lab News. 2013. https://www.aacc.org/publications/cln/articles/2013/april/troponin.aspx. Accessed 13 July 2015.
4. Mahajan VS, Jarolim P. How to interpret elevated cardiac troponin levels. Circulation. 2011;124(21):2350–4. doi:10.1161/CIRCULATIONAHA.111.023697.
5. Agzew Y. Elevated serum cardiac troponin in non-acute coronary syndrome. Clin Cardiol. 2009;32(1):15–20. doi:10.1002/clc.20445.
6. Jones ID, Slovis CM. Pitfalls in evaluating the low-risk chest pain patient. Emerg Med Clin N Am. 2010;28(1):183–201. doi:10.1016/j.emc.2009.10.002.
7. • Lin S, Yokoyama H, Rac VE, Brooks SC. Novel biomarkers in diagnosing cardiac ischemia in the emergency department: a systematic review. Resuscitation. 2012;83(6):684–91. doi:10.1016/j.resuscitation.2011.12.015. *This review article effectively summarizes the literature on novel biomarkers through 2010. Our current review expounds on this with more recent data.*
8. de Azevedo JC, Reis BC, Barreto NM, Junior F, Diogenes S, Prezotti LS, Procaci VR, Octaviano VW, Volschan A, Mesquita ET, Mesquita CT. BNP was associated with ischemic myocardial scintigraphy and death in patients at chest pain unit. Arq Bras Cardiol. 2015;104(1):16–23. doi:10.5935/abc.20140175.

9. Gravning J, Smedsrud MK, Omland T, Eek C, Skulstad H, Aaberge L, Bendz B, Kjekshus J, Mørkrid L, Edvardsen T. Sensitive troponin assays and N-terminal pro-B-type natriuretic peptide in acute coronary syndrome: prediction of significant coronary lesions and long-term prognosis. Am Heart J. 2013;165(5):716–24. doi:10.1016/j.ahj.2013.02.008.
10. Collinson PO, Gaze DC, Thokala P, Goodacre S. Randomised assessment of treatment using panel assay of cardiac markers–contemporary biomarker evaluation (RATPAC CBE). Health Technol Assess. 2013;17(15):1–122. doi:10.3310/hta17150.
11. Leistner DM, Klotsche J, Palm S, Pieper L, Stalla GK, Lehnert H, Silber S, März W, Wittchen HU, Zeiher AM. DETECT Study Group. Prognostic value of reported chest pain for cardiovascular risk stratification in primary care. Eur J Prev Cardiol. 2014;21(6):727–38. doi:10.1177/2047487312452503.
12. Lyngbæk S, Winkel P, Gøtze JP, Kastrup J, Gluud C, Kolmos HJ, Kjøller E, Jensen GB, Hansen JF, Hildebrandt P, Hilden J. CLARICOR Trial group. Risk stratification in stable coronary artery disease is possible at cardiac troponin levels below conventional detection and is improved by use of N-terminal pro-B-type natriuretic peptide. Eur J Prev Cardiol. 2014;21(10):1275–84. doi:10.1177/2047487313492099.
13. Guidez T, Maréchaux S, Pinçon C, Lamour H, Barrailler S, Decourcelle V, Braun S, Bouabdallaoui N, Bauchart JJ, Auffray JL, Hennache B, Juthier F, Vincentelli A, Asseman P, Van Belle E, Ennezat PV. Addition of B-type natriuretic peptide to the GRACE score to predict outcome in acute coronary syndrome: a retrospective (development) and prospective (validation) cohort-based study. Emerg Med J. 2012;29(4):274–9. doi:10.1136/emj.2010.104422.
14. Reesukumal K, Pratumvinit B. B-type natriuretic peptide not TIMI risk score predicts death after acute coronary syndrome. Clin Lab. 2012;58(9–10):1017–22.
15. Rajabiani A, Mohaghgheghi A, Kamal-Hedayat D, Sheikhbahaei S, Abdollahi A, Adl SR, Sateh H. Does serum n-terminal pro-brain natriuretic peptide level predict the severity of angiographic lesions in patients with acute coronary syndrome? J Tehran Heart Cent. 2013;8(3):152–7.
16. Conde D, Elissamburu P, Lalor N, Rodriguez L, Trivi M. B-type natriuretic peptide predicts complexity and severity of the coronary lesions in patients with acute coronary syndromes. Am J Emerg Med. 2013;31(8):1282. doi:10.1016/j.ajem.2013.05.004.
17. Qin YW, Teng X, He JQ, Du J, Tang CS, Qi YF. Increased plasma levels of intermedin and brain natriuretic peptide associated with severity of coronary stenosis in acute coronary syndrome. Peptides. 2013;42:84–8. doi:10.1016/j.peptides.2013.01.011.
18. Yamamoto K, Murphy G, Troeberg L. Extracellular regulation of metalloproteinases. Matrix Biol. 2015;44–46C:255–63. doi:10.1016/j.matbio.2015.02.007.
19. Hou ZH, Lu B, Gao Y, Cao HL, Yu FF, Jing N, Chen X, Cong XF, Roy SK, Budoff MJ. Matrix metalloproteinase-9 (MMP-9) and myeloperoxidase (MPO) levels in patients with nonobstructive coronary artery disease detected by coronary computed tomographic angiography. Acad Radiol. 2013;20(1):25–31. doi:10.1016/j.acra.2012.07.014.
20. Kuliczkowski W, Urbaniak J, Hallén J, Woźniak M, Poloński L, Mysiak A, Atar D, Zembala M, Serebruany V. Matrix metalloproteinases and the activity of their tissue inhibitors in patients with ST-elevation myocardial infarction treated with primary angioplasty. Kardiol Pol. 2013;71(5):453–63. doi:10.5603/KP.2013.0091.
21. Nilsson L, Hallén J, Atar D, Jonasson L, Swahn E. Early measurements of plasma matrix metalloproteinase-2 predict infarct size and ventricular dysfunction in ST-elevation myocardial infarction. Heart. 2012;98(1):31–6. doi:10.1136/heartjnl-2011-300079.
22. Dabek J, Glogowska-Ligus J, Szadorska B. Transcription activity of MMP-2 and MMP-9 metalloproteinase genes and their tissue inhibitor (TIMP-2) in acute coronary syndrome patients. J Postgrad Med. 2013;59(2):115–20. doi:10.4103/0022-3859.113836.
23. Kobayashi N, Hata N, Kume N, Yokoyama S, Shinada T, Tomita K, Kitamura M, Shirakabe A, Inami T, Yamamoto M, Seino Y, Mizuno K. Matrix metalloproteinase-9 for the earliest stage acute coronary syndrome. Circ J. 2011;75(12):2853–61.
24. Hamed GM, Fattah MF. Clinical relevance of matrix metalloproteinase 9 in patients with acute coronary syndrome. Clin Appl Thromb Hemost. 2015;21(8):705–11.
25. White DA, Fang L, Chan W, Morand EF, Kiriazis H, Duffy SJ, Taylor AJ, Dart AM, Du XJ, Gao XM. Pro-inflammatory action of MIF in acute myocardial infarction via activation of peripheral blood mononuclear cells. PLoS One. 2013;8(10):e76206. doi:10.1371/journal.pone.0076206. eCollection 2013. Erratum in: PLoS One. 2013;8(11). doi:10.1371/annotation/6e24b7bb-83c7-4887-962196b64acfb1c1
26. Bayir A, Kara H, Kiyici A, Özturk B, Sivrikaya A, Akyürek F. Pregnancy-associated plasma protein A and procalcitonin as markers of myocardial injury in patients with acute coronary syndrome. Turk J Med Sci. 2015;45(1):159–63.
27. Parveen N, Subhakumari KN, Krishnan S. Pregnancy Associated Plasma Protein-A (PAPP-A) Levels in Acute Coronary Syndrome: A Case Control Study in a Tertiary Care Centre. Indian J Clin Biochem. 2015;30(2):150–4. doi:10.1007/s12291-014-0421-9.
28. Gururajan P, Gurumurthy P, Nayar P, Rao GS, Babu RS, Sarasabharati A, Cherian KM. Pregnancy associated plasma protein-A (PAPP-A) as an early marker for the diagnosis of acute coronary syndrome. Indian Heart J. 2012;64(2):141–5. doi:10.1016/S0019-4832(12)60049-2.
29. Lippi G, Mattiuzzi C, Cervellin G. Pregnancy-associated plasma protein A (PAPP-A) for the early diagnosis of myocardial infarction: more doubts than certainties. Indian Heart J. 2012;64(6):625–6. doi:10.1016/j.ihj.2012.10.005.
30. Schaub N, Reichlin T, Meune C, Twerenbold R, Haaf P, Hochholzer W, Niederhauser N, Bosshard P, Stelzig C, Freese M, Reiter M, Gea J, Buser A, Mebazaa A, Osswald S, Mueller C. Markers of plaque instability in the early diagnosis and risk stratification of acute myocardial infarction. Clin Chem. 2012;58(1):246–56. doi:10.1373/clinchem.2011.172940.
31. Piyanuttapull S. Correlation of plasma copeptin levels and early diagnosis of acute myocardial infarction compared with troponin-T. J Med Assoc Thai. 2013;96(1):13–9.
32. Llorens P, Sánchez M, Herrero P, Martín-Sánchez FJ, Piñera P, Miró O. COPED study investigators. The utility of copeptin in the emergency department for non-ST-elevation myocardial infarction rapid rule out: COPED–MIRRO study. Eur J Emerg Med. 2014;21(3):220–9. doi:10.1097/MEJ.0b013e3283632f8b.
33. • Collinson P, Gaze D, Goodacre S. Comparison of contemporary troponin assays with the novel biomarkers, heart fatty acid binding protein and copeptin, for the early confirmation or exclusion of myocardial infarction in patients presenting to the emergency department with chest pain. Heart. 2014;100(2):140–5. doi:10.1136/heartjnl-2013-304716. *This moderate size study of an assay of novel biomarkers in low risk patients found that copeptin did not improve chest pain diagnosis over high sensitivity troponin alone, although addition of hFABP may still have a benefit when high-sensitivity troponin is used.*
34. Zellweger C, Wildi K, Twerenbold R, Reichlin T, Naduvilekoot A, Neuhaus JD, Balmelli C, Gabutti M, Al Afify A, Ballarino P, Jäger C, Druey S, Hillinger P, Haaf P, Vilaplana C, Darbouret B, Ebmeyer S, Rubini Gimenez M, Moehring B, Osswald S, Mueller C. Use of copeptin and high-sensitive cardiac troponin T for diagnosis and prognosis in patients with diabetes mellitus and

suspected acute myocardial infarction. Int J Cardiol. 2015;190:190–7. doi:10.1016/j.ijcard.2015.04.134.

35. •• Lipinski MJ, Escárcega RO, D'Ascenzo F, Magalhães MA, Baker NC, Torguson R, Chen F, Epstein SE, Miró O, Llorens P, Giannitsis E, Lotze U, Lefebvre S, Sebbane M, Cristol JP, Chenevier-Gobeaux C, Meune C, Eggers KM, Charpentier S, Twerenbold R, Mueller C, Biondi-Zoccai G, Waksman R. A systematic review and collaborative meta-analysis to determine the incremental value of copeptin for rapid rule-out of acute myocardial infarction. Am J Cardiol. 2014;113(9):1581–91. doi:10.1016/j.amjcard.2014.01.436. *This large scale meta-analysis including 14 studies provides perhaps the strongest evidence to support adding copeptin to troponin. This improved the rule-out capability in particular.*
36. •• Maisel A, Mueller C, Neath SX, Christenson RH, Morgenthaler NG, McCord J, Nowak RM, Vilke G, Daniels LB, Hollander JE, Apple FS, Cannon C, Nagurney JT, Schreiber D, deFilippi C, Hogan C, Diercks DB, Stein JC, Headden G, Limkakeng AT Jr, Anand I, Wu AH, Papassotiriou J, Hartmann O, Ebmeyer S, Clopton P, Jaffe AS, Peacock WF. Copeptin helps in the early detection of patients with acute myocardial infarction: primary results of the CHOPIN trial (copeptin Helps in the early detection of patients with acute myocardial infarction). J Am Coll Cardiol. 2013;62(2):150–60. doi:10.1016/j.jacc.2013.04.011. *This large prospective multicenter trial suggested a benefit of adding copeptin to troponin, especially for the early rule out of myocardial infarction in an emergency department population. The high negative predictive value found would suggest particular utility for early diagnosis and disposition.*
37. Raskovalova T, Twerenbold R, Collinson PO, Keller T, Bouvaist H, Folli C, Giavarina D, Lotze U, Eggers KM, Dupuy AM, Chenevier-Gobeaux C, Meune C, Maisel A, Mueller C, Labarère J. Diagnostic accuracy of combined cardiac troponin and copeptin assessment for early rule-out of myocardial infarction: a systematic review and meta-analysis. Eur Heart J Acute Cardiovasc Care. 2014;3(1):18–27. doi:10.1177/2048872613514015.
38. Charpentier S, Maupas-Schwalm F, Cournot M, Elbaz M, Botella JM, Lauque D. Combination of copeptin and troponin assays to rapidly rule out non-ST elevation myocardial infarction in the emergency department. Acad Emerg Med. 2012;19(5):517–24. doi:10.1111/j.1553-2712.2012.01350.x.
39. Ray P, Charpentier S, Chenevier-Gobeaux C, Reichlin T, Twerenbold R, Claessens YE, Jourdain P, Riou B, Mueller C. 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. 2012;30(3):440–8. doi:10.1016/j.ajem.2011.12.008.
40. Marston NA, Shah KS, Mueller C, Neath SX, Christenson RH, McCord J, Nowak RM, Daniels LB, Hollander JE, Apple F, Nagurney J, Schreiber D, deFilippi C, Diercks D, Limkakeng A, Anand IS, Wu AH, Jaffe AS, Peacock WF, Maisel AS. Serial sampling of copeptin levels improves diagnosis and risk stratification in patients presenting with chest pain: results from the CHOPIN trial. Emerg Med J. 2015;33(1):23–9. doi:10.1136/emermed-2015-204692.
41. Wildi K, Zellweger C, Twerenbold R, Jaeger C, Reichlin T, Haaf P, Faoro J, Giménez MR, Fischer A, Nelles B, Druey S, Krivoshei L, Hillinger P, Puelacher C, Herrmann T, Campodarve I, Rentsch K, Steuer S, Osswald S, Mueller C. Incremental value of copeptin to highly sensitive cardiac Troponin I for rapid rule-out of myocardial infarction. Int J Cardiol. 2015;1(190):170–6. doi:10.1016/j.ijcard.2015.04.133.
42. Potocki M, Reichlin T, Thalmann S, Zellweger C, Twerenbold R, Reiter M, Steuer S, Bassetti S, Drexler B, Stelzig C, Freese M, Winkler K, Haaf P, Balmelli C, Hochholzer W, Osswald S, Mueller C. Diagnostic and prognostic impact of copeptin and high-sensitivity cardiac troponin T in patients with pre-existing coronary artery disease and suspected acute myocardial infarction. Heart. 2012;98(7):558–65. doi:10.1136/heartjnl-2011-301269.
43. Duchenne J, Mestres S, Dublanchet N, Combaret N, Marceau G, Caumon L, Dutoit L, Ughetto S, Motreff P, Sapin V, Schmidt J. Diagnostic accuracy of copeptin sensitivity and specificity in patients with suspected non-ST-elevation myocardial infarction with troponin I below the 99th centile at presentation. BMJ Open. 2014;4(3):e004449. doi:10.1136/bmjopen-2013-004449.
44. Sukul D, Bonaca MP, Ruff CT, Kosowsky J, Conrad M, Murphy SA, Sabatine MS, Jarolim P, Morrow DA. Diagnostic performance of copeptin in patients with acute nontraumatic chest pain: BWH-TIMI ED chest pain study. Clin Cardiol. 2014;37(4):227–32. doi:10.1002/clc.22244.
45. • Reiter M, Twerenbold R, Reichlin T, Mueller M, Hoeller R, Moehring B, Haaf P, Wildi K, Merk S, Bernhard D, Mueller CZ, Freese M, Freidank H, Campodarve Botet I, Mueller C. Heart-type fatty acid-binding protein in the early diagnosis of acute myocardial infarction. Heart. 2013;99(10):708–14. doi:10.1136/heartjnl-2012-303325. *This large prospective observation study suggest high-sensitivity troponin may be more useful than alternative novel biomarkers in chest pain diagnosis, and questions any added utility of novel biomarkers such as hFABP and copeptin.*
46. Charpentier S, Lepage B, Maupas-Schwalm F, Cinq-Frais C, Bichard-Bréaud M, Botella JM, Elbaz M, Lauque D. Copeptin improves the diagnostic performance of sensitive troponin I-Ultra but cannot rapidly rule out non-ST-elevation myocardial infarction at presentation to an emergency department. Ann Emerg Med. 2013;61(5):549 e1–558 e1. doi:10.1016/j.annemergmed.2012.12.018.
47. Ricci F, Di Scala R, Massacesi C, Di Nicola M, Cremonese G, De Pace D, Rossi S, Griffo I, Cataldo I, Martinotti S, Rotondo D, Jaffe AS, Zimarino M, De Caterina R. Ultra-sensitive Copeptin and cardiac troponin in diagnosing non-st-elevation acute coronary syndromes-the COPACS study. Am J Med. 2015;129(1):105–14. doi:10.1016/j.amjmed.2015.06.033.
48. Sebbane M, Lefebvre S, Kuster N, Jreige R, Jacques E, Badiou S, Dumont R, Cristol JP, Dupuy AM. Early rule out of acute myocardial infarction in ED patients: value of combined high-sensitivity cardiac troponin T and ultrasensitive copeptin assays at admission. Am J Emerg Med. 2013;31(9):1302–8. doi:10.1016/j.ajem.2013.04.033.
49. Bahrmann P, Bahrmann A, Breithardt OA, Daniel WG, Christ M, Sieber CC, Bertsch T. Additional diagnostic and prognostic value of copeptin ultra-sensitive for diagnosis of non-ST-elevation myocardial infarction in older patients presenting to the emergency department. Clin Chem Lab Med. 2013;51(6):1307–19. doi:10.1515/cclm-2012-0401.
50. Lotze U, Lemm H, Heyer A, Müller K. Combined determination of highly sensitive troponin T and copeptin for early exclusion of acute myocardial infarction: first experience in an emergency department of a general hospital. Vasc Health Risk Manag. 2011;7:509–15. doi:10.2147/VHRM.S21753.
51. Giannitsis E, Kehayova T, Vafaie M, Katus HA. Combined testing of high-sensitivity troponin T and copeptin on presentation at prespecified cutoffs improves rapid rule-out of non-ST-segment elevation myocardial infarction. Clin Chem. 2011;57(10):1452–5. doi:10.1373/clinchem.2010.161265.
52. Bar-Or D, Curtis G, Rao N, Bampos N, Lau E. Characterization of the Co(2+) and Ni(2+) binding amino-acid residues of the N-terminus of human albumin. An insight into the mechanism of a new assay for myocardial ischemia. Eur J Biochem. 2001;268(1):42–7.
53. Liebetrau C, Nef HM, Dörr O, Gaede L, Hoffmann J, Hahnel A, Rolf A, Troidl C, Lackner KJ, Keller T, Hamm CW, Möllmann

H. Release kinetics of early ischaemic biomarkers in a clinical model of acute myocardial infarction. Heart. 2014;100(8):652–7. doi:10.1136/heartjnl-2013-305253.
54. Bhakthavatsala Reddy C, Cyriac C, Desle HB. Role of "Ischemia Modified Albumin" (IMA) in acute coronary syndromes. Indian Heart J. 2014;66(6):656–62. doi:10.1016/j.ihj.2014.12.005.
55. Patil SM, Banker MP, Padalkar RK, Pathak AP, Bhagat SS, Ghone RA, Phatake AS. The clinical assessment of ischaemia modified albumin and troponin I in the early diagnosis of the acute coronary syndrome. J Clin Diagn Res. 2013;7(5):804–8. doi:10.7860/JCDR/2013/5288.2944.
56. Goodacre S, Thokala P, Carroll C, Stevens JW, Leaviss J, Al Khalaf M, Collinson P, Morris F, Evans P, Wang J. Systematic review, meta-analysis and economic modelling of diagnostic strategies for suspected acute coronary syndrome. Health Technol Assess. 2013;17(1 (v–vi)):1–188. doi:10.3310/hta17010.
57. Sokhanvar S, Mellati AO, Mousavinasab SN, Taran L, Vahdani B, Golmmohamadi Z. Ischemia-modified albumin (IMA) in differential diagnosis of transient myocardial ischemia from non ischemic chest pain. Bratisl Lek Listy. 2012;113(10):612–5.
58. Lippi G, Cervellin G, Plebani M. Ischemia-modified albumin in the era of high-sensitivity troponin immunoassays: useful or hype? Scand J Clin Lab Invest. 2013;73(7):598–9. doi:10.3109/00365513.2013.807357.
59. Gerede DM, Güleç S, Kiliçkap M, Kaya CT, Vurgun VK, Özcan ÖU, Göksülük H, Erol Ç. Comparison of a qualitative measurement of heart-type fatty acid-binding protein with other cardiac markers as an early diagnostic marker in the diagnosis of non-ST-segment elevation myocardial infarction. Cardiovasc J Afr. 2015;26:1–6. doi:10.5830/CVJA-2015-028.
60. Freund Y, Chenevier-Gobeaux C, Leumani F, Claessens YE, Allo JC, Doumenc B, Cosson C, Bonnet P, Riou B, Ray P. Heart-type fatty acid binding protein and the diagnosis of acute coronary syndrome in the ED. Am J Emerg Med. 2012;30(8):1378–84. doi:10.1016/j.ajem.2011.10.001.
61. Banu S, Tanveer S, Manjunath CN. Comparative study of high sensitivity troponin T and heart-type fatty acid-binding protein in STEMI patients. Saudi J Biol Sci. 2015;22(1):56–61. doi:10.1016/j.sjbs.2014.05.011.
62. Jacobs LH, van Borren M, Gemen E, van Eck M, van Son B, Glatz JF, Daniels M, Kusters R. Rapidly rule out acute myocardial infarction by combining copeptin and heart-type fatty acid-binding protein with cardiac troponin. Ann Clin Biochem. 2015;52(Pt 5):550–61.
63. Dupuy AM, Cristol JP, Kuster N, Reynier R, Lefebvre S, Badiou S, Jreige R, Sebbane M. Performances of the heart fatty acid protein assay for the rapid diagnosis of acute myocardial infarction in ED patients. Am J Emerg Med. 2015;33(3):326–30. doi:10.1016/j.ajem.2014.11.012.
64. Banu S, Tanveer S, Manjunath CN. Comparative study of high sensitivity troponin T and heart-type fatty acid-binding protein in STEMI patients. Saudi J Biol Sci. 2015;22(1):56–61. doi:10.1016/j.sjbs.2014.05.011.
65. Kellens S, Verbrugge FH, Vanmechelen M, Grieten L, Van Lierde J, Dens J, Vrolix M, Vandervoort P. Point-of-care heart-type fatty acid binding protein versus high-sensitivity troponin T testing in emergency patients at high risk for acute coronary syndrome. Eur Heart J Acute Cardiovasc Care. 2015;5(2):177–84.
66. Schoenenberger AW, Stallone F, Walz B, Bergner M, Twerenbold R, Reichlin T, Zogg B, Jaeger C, Erne P, Mueller C. Incremental value of heart-type fatty acid-binding protein in suspected acute myocardial infarction early after symptom onset. Eur Heart J Acute Cardiovasc Care. 2015;5(2):185–92.
67. Bank IE, Dekker MS, Hoes AW, Zuithoff NP, Verheggen PW, de Vrey EA, Wildbergh TX, Timmers L, de Kleijn DP, Glatz JF, Mosterd A. Suspected acute coronary syndrome in the emergency room: Limited added value of heart type fatty acid binding protein point of care or ELISA tests: the FAME-ER (Fatty Acid binding protein in Myocardial infarction Evaluation in the Emergency Room) study. Eur Heart J Acute Cardiovasc Care. 2015.
68. Gami BN, Patel DS, Haridas N, Chauhan KP, Shah H, Trivedi A. Utility of heart-type fatty acid binding protein as a new biochemical marker for the early diagnosis of acute coronary syndrome. J Clin Diagn Res. 2015;9(1):BC22–4.
69. Guo JL, Yang YN, Ma YT, Li XM, Sun HP, Xie X, Liu F. Values of matrix metalloproteinase-9 in early diagnosis and short-term prognosis of ST-segment elevation myocardial infarction. Zhonghua Yi Xue Za Zhi. 2012;92(38):2681–4.
70. Searle J, Slagman A, Stockburger M, Vollert JO, Müller C, Muller R, Möckel M. Use of copeptin in emergency patients with cardiac chief complaints. Eur Heart J Acute Cardiovasc Care. 2014;4(5):393–402.
71. 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 JO, Giannitsis E. 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. 2015;36(6):369–76. doi:10.1093/eurheartj/ehu178.
72. Bohyn E, Dubie E, Lebrun C, Jund J, Beaune G, Lesage P, Belle L, Savary D. Expeditious exclusion of acute coronary syndrome diagnosis by combined measurements of copeptin, high-sensitivity troponin, and GRACE score. Am J Emerg Med. 2014;32(4):293–6. doi:10.1016/j.ajem.2013.11.043.
73. Body R, Carley S, McDowell G, Pemberton P, Burrows G, Cook G, Lewis PS, Smith A, Mackway-Jones K. The Manchester Acute Coronary Syndromes (MACS) decision rule for suspected cardiac chest pain: derivation and external validation. Heart. 2014;100(18):1462–8. doi:10.1136/heartjnl-2014-305564.
74. Body R, Burrows G, Carley S, Lewis PS. The Manchester Acute Coronary Syndromes (MACS) decision rule: validation with a new automated assay for heart-type fatty acid binding protein. Emerg Med J. 2014;. doi:10.1136/emermed-2014-204235.