Biomarkers in ACS and Heart Failure: Should Men and Women Be Interpreted Differently?

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BACKGROUND: Sex-based differences exist in the circulating concentrations of certain novel and established biomarkers in patients with acute coronary syndromes (ACS) and heart failure (HF). However, to date, few studies have compared the diagnostic and prognostic utility of these markers in men vs women.

CONTENT: This mini-review contains a discussion of the published reports of studies that have explored whether differences in biomarker concentrations exist between men and women with ACS or HF. It also examines those studies that have compared the utility of biomarkers for diagnosis or risk stratification in men vs men. Because biomarkers are often used to make therapeutic and triage decisions in patient care, the potential clinical implications for any observed differences in biomarker reference limits for men and women is discussed.

SUMMARY: Although the concentration distributions may differ between men and women for certain biomarkers in clinical use, the clinical implications of these observations remain unclear. Because elements of the pathophysiology of ACS and HF may differ between the sexes, further research is needed to better evaluate the diagnostic and prognostic utility of biomarkers in men vs women.

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Although age-adjusted mortality rates have progressively declined during the past few decades, cardiovascular (CV) disease remains the leading cause of death in both men and women in the Western world. As our armamentarium of therapeutic options in the management of acute coronary syndromes (ACS) and heart failure (HF) continues to grow, there is continued interest in developing strategies that may help to identify those individuals at increased risk of adverse outcomes and those who may derive greater net benefit from evidence-based therapies. To that end, several established and novel biomarkers have been shown to be useful for risk stratification in patients with ACS and HF. Further, a subset of these biomarkers may help to identify those patients who derive enhanced benefit from particular treatment strategies. Although emerging data suggest that the pathophysiology of ACS and HF may differ between the sexes, little is known regarding the relative diagnostic and prognostic utility of biomarkers in men vs women.

Cardiac Troponin T and I in ACS

Cardiac troponin T (cTnT) and cTnI remain the gold standard for the diagnosis of myocardial infarction (MI) (1). In particular, a rise and/or fall in cardiac troponin concentrations with at least 1 value above the 99th percentile is a key feature for the diagnosis of a new event under the Universal Definition (1). In addition to the diagnostic utility of cardiac troponin, several studies have demonstrated that higher concentrations of cardiac troponin are independently associated with an increased risk of CV events in patients with ACS (2). Moreover, cardiac troponin concentrations may be useful for helping to identify those patients who may derive enhanced benefit from particular therapies in ACS, including glycoprotein IIb/IIIa receptor blockers or routine cardiac catheterization (2). However, few studies have compared the relative diagnostic or prognostic utility of cardiac troponin in men vs women.

This question may be particularly relevant given that several studies have shown that cardiac troponin...
Concentrations are significantly higher in men than in women in healthy individuals (Fig. 1) (3) and in those patients with stable coronary artery disease (CAD) (4). To date, it remains incompletely understood why mean cardiac troponin concentrations are higher in men than in women because cardiac troponin is presumed to be restricted to the cardiomyocyte. In contrast, the mean values of circulating creatine kinase MB (CK-MB) concentrations are higher in men than women owing to the higher skeletal muscle mass in the average male study participant, because CK-MB comprises 1%–2% of the creatine kinase originating from skeletal muscle.

It is plausible that a higher mean cardiac mass may be responsible for the higher cardiac troponin concentrations in males because the weight-adjusted cardiac troponin content has been shown to be similar in male and female myocardial tissue (5). However, it remains unknown whether the release and clearance kinetics of cardiac troponin are similar in men and women in response to ischemic injury. Intriguing data from a small study demonstrated that the perioperative release of cardiac troponin was 3-fold greater in men than women in response to a similar ischemic insult during cardiac surgery, despite similar exposure to cardiopulmonary bypass and aortic cross-clamping times in age and risk-factor matched male and female study participants with a comparable body mass index (6). It is therefore plausible that sex-based differences may exist with respect to cardiac troponin release at the level of the cardiomyocyte; however, this requires further study. Another contributing factor to sex-based differences in cardiac troponin concentrations in stable or unstable CAD may be related to the fact that women are less likely than men to have obstructive macrovascular CAD at catheterization (7) and less extensive disease (8), and therefore differences in cardiac troponin release could reflect pathophysiological differences in CAD between the sexes, including a higher prevalence of microvascular disease, endothelial dysfunction, or more diffuse atherosclerosis in women. To that end, the higher prevalence of a patent epicardial vessel in women with ACS could potentially influence the washout kinetics of cardiac troponin from the interstitium and therefore blunt the peak concentration of cardiac troponin detected in the periphery (9).

Importantly, it remains unclear whether the observed differences in mean cardiac troponin concentrations in men vs women have clinical implications. Under the Universal Definition of MI, a rise and/or fall in cardiac troponin exceeding the 99th percentile in a normal reference population is required to be diagnos-
tic of a new event (1). Since the MI reference limit is often employed to make key therapeutic and triage decisions in patient care, it is of upmost importance to correctly identify this threshold for a given patient population. However, the established approach toward determining manufacturer assay reference limits is to assess the distribution of cardiac troponin concentrations in a healthy population with a roughly even split between men and women. Therefore, in many instances the stated manufacturer reference limit exists beyond the “true” 99th percentile in a healthy female population (10–12) and for some assays can be as much as 5 times higher in healthy men vs women (12).

More recently, the advent of high-sensitivity cardiac troponin assays has allowed for the ascertainment of very low concentrations of circulating cardiac troponin with greater precision at concentrations that were previously undetectable with earlier generations of the assay. High-sensitivity assays have revealed that low concentrations of cardiac troponin are present in a large proportion of healthy-appearing individuals (13, 14), including 98% of patients with stable CAD (4). Men have been shown to have more extensive CAD than women, and the extent of coronary atherosclerosis has been shown to be associated with increasing circulating concentrations of high-sensitivity cardiac troponin (8). Given their higher precision at the lower range, it is plausible that the high-sensitivity assays will only further unmask sex differences that may not have been as apparent with less sensitive generations of assays. Consistent with analytical validations conducted with earlier assays, independent evaluations of high-sensitivity cardiac troponin assays have demonstrated that the 99th percentile appears to be consistently higher in men than women for several of the high-sensitivity cardiac troponin assays (3, 12, 15). Research is ongoing to help determine whether serial changes in cardiac troponin concentrations may therefore be a more reliable indicator of myocardial injury in women, compared to an approach that requires a single diagnostic cutoffpoint. This type of strategy would place greater emphasis on the relative changes in cardiac troponin concentrations in the setting of ischemic symptoms, rather than relying on an absolute threshold.

Beyond the diagnostic utility of cardiac troponin, the relative prognostic value of cardiac troponin in men vs women has not been rigorously evaluated to date. In a small substudy of individuals with unstable coronary disease who underwent a symptom-limited stress test, the prognostic value of cTnT with an older generation assay was comparable in both men and women (16). Similarly, in TACTICS-TIMI (Treat Angina With Aggrastat and Determine the Cost of Therapy With an Invasive or Conservative Strategy–Thrombolysis in Myocardial Infarction) 18, the prognostic value of cardiac troponin after NSTE-ACS was broadly equivalent in men and women, although the marker appeared to be a stronger predictor of recurrent MI in women than men (17), a finding supported by a second independent analysis (18). In contrast, the prognostic value of periprocedural myonecrosis in men vs women has yielded conflicting results (19, 20).

At this time, it is unknown whether the absence of sex-specific decision limits for the diagnosis of MI contributes to the underdiagnosis and undertreatment of ACS in women compared to men. Several studies have demonstrated that women represent a high-risk group who receive fewer guideline-recommended therapies in the setting of ACS, including less frequent cardiac catheterization and use of secondary prevention therapies. After the 2000 European Society of Cardiology and American College of Cardiology guidelines placed greater emphasis on the use of cardiac troponin for the diagnosis of ACS, a study in Israel suggested that implementation of the revised recommendations was associated with a reduction in the sex-based mortality difference in patients with ACS despite the fact that an early invasive strategy and secondary prevention continued to be underutilized in female patients. In part this difference appeared to be explained by increased use of guideline-based therapies in both men and women (21).

More recently, the 2007 American College of Cardiology (ACC)/American Heart Association (AHA) guidelines for the management of unstable angina and non-ST-elevation MI (NSTE-MI) established sex-specific recommendations such that a conservative treatment strategy is recommended for low-risk women, including those women with negative cardiac biomarkers (2). These recommendations are supported by analyses from randomized trials that have demonstrated an apparent lack of benefit from a routine invasive strategy in women without increased cardiac biomarkers of myonecrosis (7, 17). Identification of an appropriate MI decision limit for cardiac troponin in women is therefore crucial because a decision limit that is too high could lead to misclassification of women as being lower risk and influence key management decisions.

Supporting this concern, an analysis from the CRUSADE (Can Rapid risk stratification of Unstable angina patients Suppress ADverse outcomes with Early implementation of the ACC/AHA Guidelines) database demonstrated that patients with low concentrations of cardiac troponin or gray-zone increases that fell below the diagnostic cutoffpoint for MI were significantly less likely to receive guideline recommended
acute and discharge therapies in the setting of ACS (22). This hypothesis is also supported in part by the fact that women are significantly less likely than men to have cardiac troponin concentrations that rise above the MI decision limit despite a presentation suggestive of ACS (7). In the TACTICS-TIMI 18 trial, women with NSTE-ACS were nearly half as likely as men to have increased cTnT [odds ratio (OR), 0.53; 95% CI, 0.43–0.68] or cTnI (OR, 0.58; 95% CI, 0.46–0.73) concentrations at study entry after taking into account baseline differences (Fig. 2) (17).

Based on the observed differences in cardiac troponin concentrations in men and women, some individuals have advocated for separate sex-specific 99th percentile reference limits for all cardiac troponin assays (12); however, to date such recommendations have not yet been adopted. In part, the reluctance to embrace sex-specific cutpoints stems from a lack of studies demonstrating that a small shift in a diagnostic or prognostic cutpoint will have clinical implications. Higher concentrations of high-sensitivity cardiac troponin were associated with future coronary events in both healthy men and women (23), as well as all-cause mortality in a study of healthy perimenopausal women (24). Whether the prognostic value of high-sensitivity cardiac troponin in healthy populations differs by sex, however, is unclear. Although the newest generation of cardiac troponin assays has allowed a lower diagnostic cutoff through implementation of more analytically sensitive and precise assays, the diagnostic specificity of the assay results for identifying individuals with myonecrosis due to coronary plaque rupture may be reduced. To that end, several studies have demonstrated that women are less likely than men to have evidence of obstructive macrovascular disease at the time of coronary angiography despite a presumed diagnosis of ACS (7). It has been hypothesized that this may be due to a higher prevalence of microvascular disease in women or disease states that mimic ACS, including myocarditis and HF. Therefore it will be important to determine whether low concentrations of cardiac troponin carry the same diagnostic and prognostic value in both sexes and whether these low concentrations should be used to guide clinical decision-making.

**Fig. 2.** In a large clinical trial population of patients with NSTE-ACS, men were significantly more likely than women to have a significant increase in CK-MB or cardiac troponin concentration at presentation. After taking into account baseline differences, women were 47% less likely than men to have an increased cTnT (OR, 0.53; 95% CI, 0.43–0.68) and were 42% less likely to have an increased cTnI (OR, 0.58; 95% CI, 0.46–0.73) concentration at study entry [Wiviott et al. (17)]. CABG, coronary artery bypass graft; PTCA, percutaneous transluminal coronary angioplasty.
Other Biomarkers for Risk Stratification in ACS

Although there are few studies that have rigorously compared the diagnostic and prognostic utility of cardiac troponin in men vs women, this is especially true of other novel and established biomarkers in the setting of ACS. In TACTICS-TIMI 18, women with NSTE-ACS were significantly more likely than men to have an increased concentration of high-sensitivity C-reactive protein (CRP) or B-type natriuretic peptide (BNP), even after controlling for baseline differences. Despite these differences in baseline concentrations, the prognostic value of these markers for predicting CV events appeared to be broadly equivalent in both sexes (17). In addition, a multimarker approach that included cTnI or T, high-sensitivity CRP, or BNP was useful for helping to identify more women at increased odds of death or MI than any marker on its own and also helped to identify those individuals with a greater benefit from an invasive strategy. The prognostic value of the multimarker score for predicting the odds of death or MI was comparable between sexes (17).

Biomarkers in HF

In addition to their utility for patients with ACS, biomarkers have emerged as useful and powerful adjuncts to clinical judgment and guideline-directed medical therapy in the risk stratification and management of patients with HF. The most extensively studied markers have now been incorporated into current clinical practice guidelines (25), while new biomarkers continue to be identified and investigated for their abilities to reflect underlying pathophysiologic processes involved in HF development and progression. If the clinical use of HF biomarkers eventually becomes more widespread, it will become increasingly important to understand analytical factors to correctly interpret and apply the results. Specifically, one aspect to consider is how sex-related differences in biomarker concentrations may affect their interpretation. For example, while the biological variability of the natriuretic peptides has been studied in detail in laboratory studies (26), much remains unknown about the interaction between such variation and clinical applications over extended periods of time; similar issues will emerge as other emerging biomarkers are being studied relative to their importance as serial monitoring tools (27). Additionally, not factored into this entire question is how sex affects biological variation. This is not an insignificant question, because several biomarkers most widely used for CV disease evaluation have sex-related differences in their reference intervals. These analytical issues reveal the complexity of sex-based application of biomarker testing.

Biomarkers in ACS and Heart Failure

Mini-Reviews

NATRIURETIC PEPTIDES

BNP and amino-terminal pro BNP (NT-proBNP) are both released primarily in response to myocyte stretch and provide important information related to HF diagnosis, prognosis, and response to HF therapy. Several population-based studies have demonstrated that, in health, sex is one of the strongest predictors of circulating NP concentrations. These studies have shown that healthy women have higher concentrations than healthy men and have suggested that sex-specific reference limits could be helpful in guiding clinical use of NPs for diagnostic purposes (28, 29). However, cutoffs based on these reference intervals have not been conclusively established and have not yet been validated for use in the clinical setting.

The mechanisms that explain these differences are not entirely clear, but experimental and human studies have suggested that sex hormones may play a role. One murine-based study demonstrated that treatment with estradiol and progesterone stimulated NP gene expression in females (30), while another study demonstrated that testosterone suppressed volume-stimulated NP release from isolated perfused rat atria (31). Clinically, a study of postmenopausal women demonstrated a significant increase in mean BNP concentrations after transdermal estradiol administration (32), while observational studies have identified inverse correlations between circulating androgens and NP concentrations (Fig. 3) (33, 34). These findings have led to speculation that the generally higher NP concentrations in healthy women may be explained by a combination of estrogen-mediated stimulation and androgen-mediated suppression. Understanding these mechanisms and establishing sex-based reference intervals may improve the usefulness of NP measurement for screening purposes in low-risk, asymptomatic patients.

On the other hand, in disease states of acute and chronic HF, sex differences in NP concentrations are less obvious and are rather influenced by the sex-related differences in the epidemiology of HF. Studies have reported no statistically significant differences between BNP or NT-proBNP concentrations in female vs male patients with acute or chronic HF (35, 36), with women demonstrating slightly lower values. This paradox may be explained by the observation that women are more likely than men to have HF with preserved ejection fraction (37), which is a distinct pathophysiologic state associated with lower NP concentrations (38). Therefore, in HF, the sex-based difference in NP concentrations may be attenuated such that women with HF are more likely to have higher NP concentrations at baseline and smaller increases in the setting of preserved ejection fraction than men with HF, who may have lower concentrations at baseline but larger...
increases in the setting of systolic dysfunction. This difference in the prevalence of HF with preserved ejection fraction may be the major reason why studies have failed to show significant differences between NP concentrations in men and women with HF.

Importantly, despite these modifying factors, NPs retain their usefulness for the evaluation of female patients with acute dyspnea. In 1 large study, ROC curve analyses of BNP for diagnosis of acute HF as a function of sex demonstrated only slightly weaker diagnostic utility for females compared with males (areas under the curve, 0.870 and 0.918, respectively) \((39\)\), whereas another study of NT-proBNP demonstrated essentially no difference based on sex \((35\)\). Based on these findings, there is no clear evidence that sex-based criteria should be required when using NPs for diagnosis of acute HF in patients with dyspnea in the emergency department.

CARDIAC TROPONINS

As previously noted, cardiac troponin is the gold standard for the diagnosis of acute MI, but cardiac troponin is also often detectable in patients with HF regardless of whether underlying CAD is present \((40\)\). Furthermore, increased cardiac troponin concentrations are associated with worse clinical outcomes and increased mortality in both acute and chronic HF \((40\)\). With the advent of high-sensitivity cardiac troponin assays, cardiac troponin is now detectable in the vast majority of individuals independent of any known underlying disease state \((13, 14\)\), in whom its presence is associated with traditional CV risk factors and structural changes, as well as male sex. As noted previously, in presumably healthy populations, high-sensitivity cardiac troponin measured by multiple different assays has been shown to have significantly higher 99th percentile values for males than for females, which has led to the consideration of sex-specific 99th percentile reference intervals.

In the context of chronic HF, high-sensitivity cardiac troponin is measurable in almost all patients, and 1 study of patients with systolic HF showed that women were more likely than men to have concentrations above the sex-specific 99th percentile for the assay \((41\)\). It remains unclear whether this has clinical meaning, and further studies are needed to better understand this observation.

**Novel and Emerging Biomarkers**

In recent years, the field of biomarkers in the setting of HF has continued to expand and some are beginning to enter the realm of clinical practice. One of these is

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**Fig. 3.** Association between circulating free testosterone and NP concentrations.

(A) Data points represent median (25th and 75th percentiles) for each quartile (Q) of cFT (calculated free testosterone) concentration [adapted with permission from Chang et al. \((33\)\)]. (B) Least squares means and SE of log NT-proBNP are shown for each sex-specific quartile (I to IV) of FT (free testosterone) in men and women [reproduced with permission from Lam et al. \((34\)\)].
Galectin-3, a marker that has been hypothesized to play a direct role in the progression of HF via mediation of myocardial fibrosis, although the data to date remain inconclusive. Higher concentrations of galectin-3 have been associated with increased risk for incident HF in the general population \(^{(42)}\), as well as with an increased risk of adverse outcomes in patients with HF \(^{(43)}\). In large population-based studies, concentrations of galectin-3 have been generally higher in women than in men, and have been associated with traditional CV risk factors, with stronger correlations observed in women \(^{(42)}\). To date, sex-related differences in galectin-3 concentrations have not been well-established in HF.

Another new biomarker is soluble ST2 (sST2), which is strongly associated with myocardial fibrosis and hypertrophy through its interaction with interleukin-33. Higher concentrations of sST2 have been associated with greater CV risk of incident HF in the general population \(^{(44)}\) and in patients with acute HF \(^{(45)}\). In a large population-based study, higher circulating sST2 concentrations were associated with traditional CV risk factors and male sex \(^{(46)}\). Concentrations were significantly lower in women than in age-matched men, and even lower in women taking estrogen replacement, suggesting that these differences may represent the effects of sex hormones (Fig. 4). In the context of acute or chronic HF, our experience has been that sex-related differences in sST2 concentrations are less apparent; much like the NPs, no adjustments in reference intervals appear to be necessary when using sST2 in symptomatic women with HF.

Another novel biomarker of interest is growth differentiation factor-15 (GDF-15), a stress-responsive cytokine that is a marker for inflammation and is synthesized by multiple organs including the placenta. Higher concentrations of GDF-15 have been associated with greater risk of incident HF in the general population \(^{(44)}\) and are also associated with disease severity and prognosis in chronic HF \(^{(47)}\). Concentrations may be lower in women with HF than in men \(^{(36)}\), although the clinical implications of these observations are unknown.

An intriguing new biomarker that has been linked to risk for cardiometabolic disease specifically in women is proneurotensin (PNT). PNT is a stable precursor to neurotensin, a hormone that has been linked to satiety and obesity, and a receptor for PNT is a major human CAD susceptibility gene variant. A large population-based study demonstrated that PNT concentrations were significantly higher in women and predicted the onset of diabetes mellitus, CV disease including coronary events and stroke, breast cancer, and death; however, this relationship was observed only in women \(^{(48)}\). Though the mechanisms for these relationships and their clinical implications are not entirely clear, these initial epidemiological results suggest that PNT may be useful for identifying women at high overall CV risk in the absence of traditional risk factors. The role of PNT to predict HF bears examination, and further studies are ongoing to evaluate this novel potential marker of women’s health.

**Summary**

Sex-based variations may exist in the circulating concentrations of some established and novel biomarkers for diagnosis and risk assessment in patients with ACS or HF. However, it remains incompletely understood whether these observed differences are explained by dissimilarities in the prevalence of underlying comorbid conditions or whether they reflect fundamental discrepancies in the underlying pathobiology of ACS and HF in men vs women. To date, few studies have rigorously compared the diagnostic or prognostic utility of candidate biomarkers in both sexes. As such, it remains incompletely understood whether sex-specific reference limits should be adopted for biomarkers that are used in routine clinical practice. Although the clinical consequences remain unknown, such decisions would be anticipated to have the most relevance for those biomarkers that are used to help guide clinical decision-making. Research in this area is therefore needed to continue to optimize care and improve patient outcomes in at-risk patient groups. In addition, a better understanding of these differences may provide a unique window into the underlying biology of the disease process in both men and women.
References

28. Strong Connections in the Cardiac Interventions Forum, 2013. Acknowledgments: Those authors who did not receive grants had no conflicts of interest. None declared.
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34. Expert Testimony: None declared.


