Sex-Related Aspects of Biomarkers in Cardiac Disease

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Biomarkers in Cardiac Disease
Art work by Piet Michiels, Leuven, Belgium

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Abstract

Biomarkers play an important role in the clinical management of cardiac care. In particular, cardiac troponins (cTn) and natriuretic peptides

P. L. M. Kerkhof, V. M. Miller (eds.), *Sex-Specific Analysis of Cardiovascular Function*,
Advances in Experimental Medicine and Biology 1065, https://doi.org/10.1007/978-3-319-77932-4_33

are the cornerstones for the diagnosis of acute myocardial infarction (AMI) and for the diagnosis of heart failure (HF), respectively. Current guidelines do not make a distinction between women and men. However, the commonly used "one size fits all" algorithms are topic of debate to improve assessment of prognosis, particularly in women. Due to the high-sensitivity assays (hs-cTn), lower cTn levels (and 99th percentile upper reference limits) were observed in women as compared with men. Sex-specific diagnostic thresholds may improve the diagnosis of AMI in women, though clinical relevance remains controversial and more trials are needed. Also other diagnostic aspects are under investigation, like combined biomarkers approach and rapid measurement strategies. For the natriuretic peptides, previous studies observed higher concentrations in women than in men, especially in premenopausal women who might benefit from the cardioprotective actions. Contrary to hs-cTn, natriuretic peptides are particularly incorporated in the ruling-out algorithms for the diagnosis of HF and not ruling-in. Clinical relevance of sex differences here seems marginal, as clinical research has shown that negative predictive values for ruling-out HF were hardly effected when applying a universal diagnostic threshold that is independent from sex or other risk factors. Apart from the diagnostic issues of AMI in women, we believe that in the future most sex-specific benefits of cardiac biomarkers can be obtained in patient follow-up (guiding therapy) and prognostic applications, fitting modern ideas on preventive and personalized medicine.

Keywords

Acute myocardial infarction · Age dependence · Biomarkers · Cardiac troponin I · Cardiac troponin T · Estrogen · Heart failure · Natriuretic peptides · BNP · NT-proBNP · Pregnancy · Review

Introduction

Biomarkers play an important role in the clinical management of cardiac care and risk stratification for cardiovascular diseases (CVD) [72, 84] and heart failure [68, 94]. Although over the last decades the number of deaths attributable to CVD has declined globally [59], the reduction on CVD-related deaths is less pronounced for women than men [65]. With respect to sex-specific analysis, the common cardiac care guidelines do not make a distinction between women and men on the use of cardiac biomarkers [68, 72, 84, 94]. However, the awareness has been raised that sex inequalities, and hence, the use of the "one size fits all" algorithms that comprises cardiac biomarkers, may hamper the diagnosis and treatment in women [22, 72].

A number of biomarkers such as lactate dehydrogenase, creatine kinase (CK), or creatine kinase muscle-brain (CKMB) type were used in the past for the diagnosis of acute myocardial infarction (AMI) (Fig. 33.1). Though most of these biomarkers are run in every clinical laboratory today, also for other purposes, their main disadvantage is the lack in cardiac specificity and so none of them are preferred anymore in the diagnosis of cardiac diseases [2, 72, 84].

At this point, cardiac troponins (cTn) are, due to their outperformance on cardiac specificity, incorporated in common guidelines and clinical practice for diagnosis, risk stratification, and prognosis of acute coronary syndrome (ACS) (Fig. 33.1) [72, 84].

That no sex-based differences are made in cTn algorithms can be explained from a historical point of view, since the "older" assays were not able to detect any sex differences. Due to the analytical improvement of these laboratory assays over the last years, nowadays, even basal levels of cTn in healthy individuals can be measured [5]. This improvement resulted in the observation

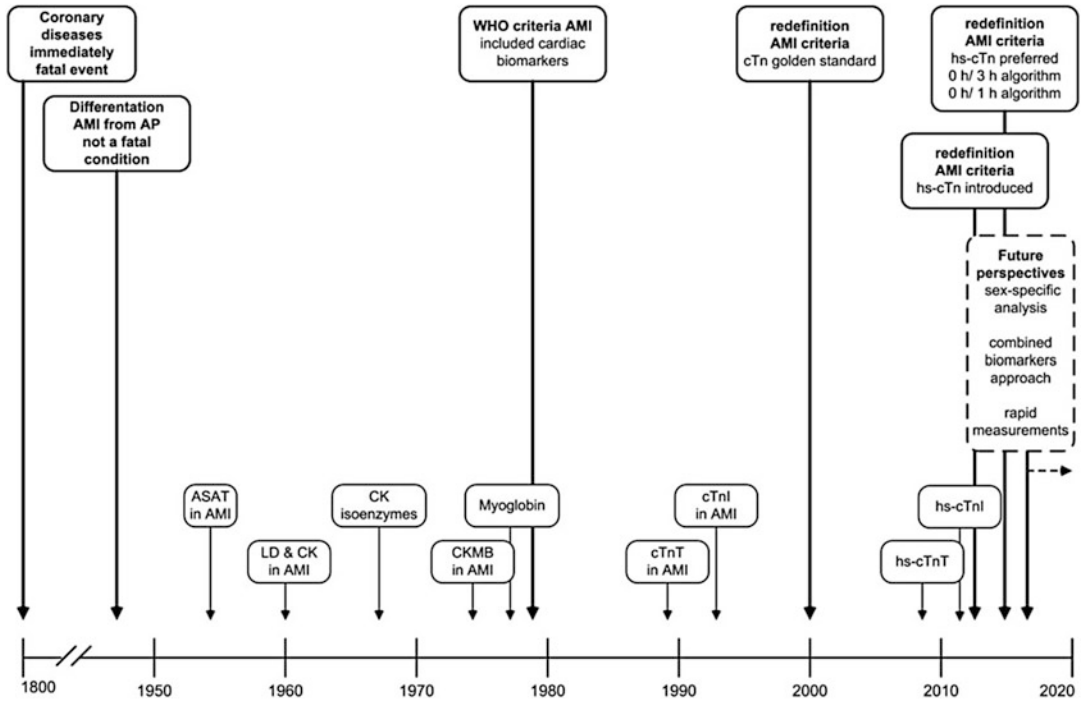

Fig. 33.1 History of cardiac biomarkers for the diagnosis of acute myocardial infarction (AMI). Abbreviations: *AMI* acute myocardial infarction, *AP* angina pectoris, *ASAT* aspartate aminotransferase, *CK* creatine kinase, *CKMB* creatine kinase muscle-brain type, *cTnI* cardiac troponin I, *cTnT* cardiac troponin T, *hs-cTnI* high-sensitivity cardiac troponin I, *hs-cTnT* high-sensitivity cardiac troponin T, *LD* lactate dehydrogenase, *WHO* World Health Organization

that disparities between women and men on cTn levels became apparent [58]. Hence, the technical progress has led to the ongoing debate whether the "one size fits all" strategy is sufficiently sensitive for women.

Also regarding the use of natriuretic peptides, no sex-based differences are incorporated in the guidelines and clinical practice [68, 94], despite the fact that it has clearly been shown that natriuretic peptide concentrations differ between men and women [70, 71].

In this chapter we will provide an overview of the current knowledge and existing gaps on sex differences of cardiac biomarkers. We focus on cTn as the gold standard for the diagnosis of AMI, and we focus on natriuretic peptides (B-type natriuretic peptic, BNP and N-terminal proBNP, NT-proBNP) in the area of diagnosis and prognosis of heart failure (HF).

Cardiac Troponins

The Basics of Cardiac Troponins

The contractile apparatus of cardiac and skeletal muscle cells consists of thick and thin filaments that slide along each other upon muscle contraction and relaxation. Thick filaments are built up from myosin; thin filaments are built up from actin with tropomyosin wrapped around and at regular sites a troponin complex of troponin T (TnT), troponin I (TnI), and troponin C (TnC). TnT, TnI, and TnC are tropomyosin binding, inhibition, and calcium binding components, respectively [4]. Upon stimulation of the myocytes, calcium ions are released and bound to TnC, and subsequently there is a conformational change of the troponin complex, resulting

in accessible binding sites between actin and myosin and leading to contraction of the muscles. TnT and TnI isoforms are characterized by a cardiac-specific N-terminal extension, while TnC in cardiac and slow skeletal muscle is similar (Swiss-Prot, P19429, P45379, and P63316, respectively), which makes them highly valuable for detection of cardiac injury and thus for the management of cardiac diseases. It has been estimated that around 5–10% of the total cTnT and cTnI content is present free and unbound in the cytosol, but this statement has been questioned by others. TnC, on the other hand, is not a cardiac-specific biomarker and will be therefore left out of consideration in this chapter.

After cardiac injury, e.g., in acute myocardial infarction (AMI), cardiac troponin T (cTnT) and cardiac troponin I (cTnI) are released in the bloodstream and detectable for diagnostic and prognostic purposes [72, 84]. In AMI patients, a main peak is typically shown within 1 day after onset of symptoms of acute chest pain and remains elevated for approximately 1 week, as depicted in Fig. 33.2. It was hypothesized that the first peak reflects the relatively fast release of cytosolic cTn, while the second broader peak represents the slower dissociation of cTn from the sarcomeres. If this is true, it is quite remarkable that cTnT and cTnI show a slight difference in their return to baseline. We believe this is due to differences between cTnT and cTnI clearance, as there are differences observed between cTnT and cTnI in patients with reduced kidney function [26], but also both proteins differ in their biochemical composition and their molecular weights (cTnT and cTnI being 37 kDa and 26 kDa, respectively), plus they are both susceptible to degradation [16, 47].

Cardiac Troponins in Healthy Women and Men

In the past, due to the restriction of conventional cTn assays, clinicians and scientists could easily discriminate "patients with AMI" from "patients without AMI" by the ability to detect positive cTn levels in case of clinical cardiac injury. However, a higher prevalence of cTn levels, particularly cTnT, was also seen in patients with kidney diseases [1, 13, 35]. Furthermore, also in particular subsets of individuals with several cardiac phenotypes like left ventricular hypertrophy (LVH) and congestive heart failure (CHF), elevated troponins were present [87]. The prominent factor that shed new light on the thought that cTn are not only the result of clinical cardiac injury was the technical improvement of the cTn assays. At this point, using the so-called "high-sensitivity" assays, we now know that cTn levels are also present in apparently healthy individuals [5]. In particular subgroups, and now

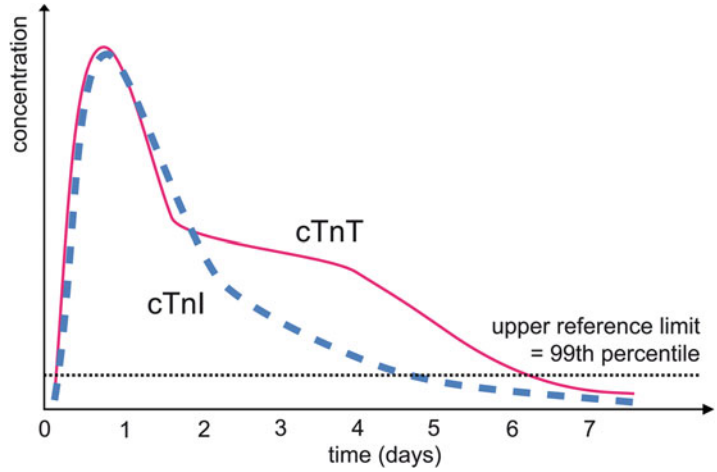

Fig. 33.2 Release patterns of cardiac troponin after AMI. Reprinted with permission from InTechOpen [39]. Abbreviations: *AMI* acute myocardial infarction, *cTnI* cardiac troponin I, *cTnT* cardiac troponin T

to an even larger and more obvious extent, a higher prevalence of cTn elevations is observed, mainly in individuals who are at risk for cardiovascular events, like in individuals with diabetes mellitus, hypertension, and reduced kidney function [35, 45, 54, 79, 87]. The current guidelines listed the conditions whereby elevated levels of cTn are observed other than AMI to consider for further diagnostic workup (Table 33.1) [72].

With the shift to more sensitive cTn assays, also sex inequalities of cTn levels in apparently healthy women and men became evident [58]. Although it is nowadays well established that basal levels of cTn are significantly lower in women than men, the underlying mechanisms of these divergence are still not completely unraveled and understood, but these are most likely multifactorial [30].

The pathogenesis of coronary artery disease (CAD) between women and men seems to differ. Women with CAD are more often presented with endothelial dysfunction than men, while men are more often present with more localized, i.e., less diffuse, coronary disease [40, 88]. The sex hormone estrogen seems to play a protective role in the pathogenesis of several cardiac pathologies [30]. It turned out that estrogen attenuates the processes of atherosclerosis, LVH, and cardiomyocyte apoptosis, possibly resulting into lower cTn levels in women than men [28, 67, 88].

Another important contributor to sex-based differences of cTn levels seems to be the left ventricular (LV) anatomy. The LV mass differs between women and men, whereby men have a higher LV mass as compared with women [24]. Previous studies showed a strong relationship between LV mass and circulating cTn levels [23, 61], and the higher cardiac mass of men probably leads to more release of cTn, which results into a higher basal circulating cTn levels in men than women.

Cardiac Troponins for the Diagnosis of Acute Myocardial Infarction

The cornerstones in the diagnosis of AMI are the clinical presentation, electrocardiography

Table 33.1 Conditions or procedures whereby elevated levels of cTn are observed other than AMI

Most frequent conditions
Tachyarrhythmias
Heart failure
Critical illness (e.g., shock/sepsis/burns)
Myocarditis (incl. myocardial extension of endocarditis or pericarditis)
Takotsubo cardiomyopathy
Structural heart disease (e.g., aortic stenosis)
Aortic dissection
Pulmonary embolism, pulmonary hypertension
Renal dysfunction and associated cardiac disease
Other conditions or procedures
Coronary spasm
Acute neurological event (e.g., stroke or subarachnoid hemorrhage)
Cardiac contusion or cardiac procedures (CABG, PCI, ablation, pacing, cardioversion, or endomyocardial biopsy)
Hypo- and hyperthyroidism
Infiltrative diseases (e.g., amyloidosis, hemochromatosis, sarcoidosis, scleroderma)
Myocardial drug toxicity or poisoning (e.g., doxorubicin, 5-fluorouracil, Herceptin, snake venoms)
Extreme endurance efforts
Rhabdomyolysis

Adapted from ESC guidelines 2016 [72]
Abbreviations: *AMI* acute myocardial infarction, *CABG* coronary artery bypass graft, *cTn* cardiac troponin, *PCI* percutaneous coronary intervention

(ECG) findings, and cardiac troponin measurements (Fig. 33.3). For patients with ST-elevation myocardial infarction (STEMI), the ECG findings are the gold standard to establish AMI diagnosis. However, patients with non-ST-elevation myocardial infarction (NSTEMI) show no diagnostic or inconclusive ECG elevations, whereby the clinical presentation of the patient, in conjunction with cTn measurements, establishes the diagnosis of AMI.

The current guidelines require a significant "rise and/or fall" of high-sensitivity cardiac troponin T (hs-cTnT) or I (hs-cTnI) between serial measurements with at least one value above the 99th percentile upper reference limit of hs-cTn from a healthy reference population [72, 84]. Although the ESC guidelines recommend this 0 h/3 h algorithm, they incorporated also an 0 h/1 h algorithm as alternative approach [72]. The 0 h/1 h algorithm handles other diagnostic thresholds for hs-cTnT and hs-cTnI, dependent of which hs-cTn assay is used [72].

The diagnosis of AMI in women is particularly challenging, as women with suspected ACS are more likely to present with an atypical clinical presentation and/or inconclusive ECG, which may hamper the diagnosis of AMI [15, 32, 43]. Besides, women have a higher prevalence of unrecognized (silent) AMIs as compared with men (women 54% vs men 33%) [25]. All in all this may lead to worse prognosis after AMI in women, and therefore an optimized diagnostic cTn algorithm is of great clinical importance.

Over the last years, we may already have booked some progress on closing the diagnostic gap between women and men. As stated previously, the common guidelines prefer hs-cTn assays above the conventional cTn assays, which have been introduced in Europe around 2012 [84]. Hs-cTn assays should meet two analytical criteria: (1) the 99th percentile upper reference limit of hs-cTn should not be lower than the 10% coefficient of variation (CV) cutoff, and (2) the proportion of individuals with measurable

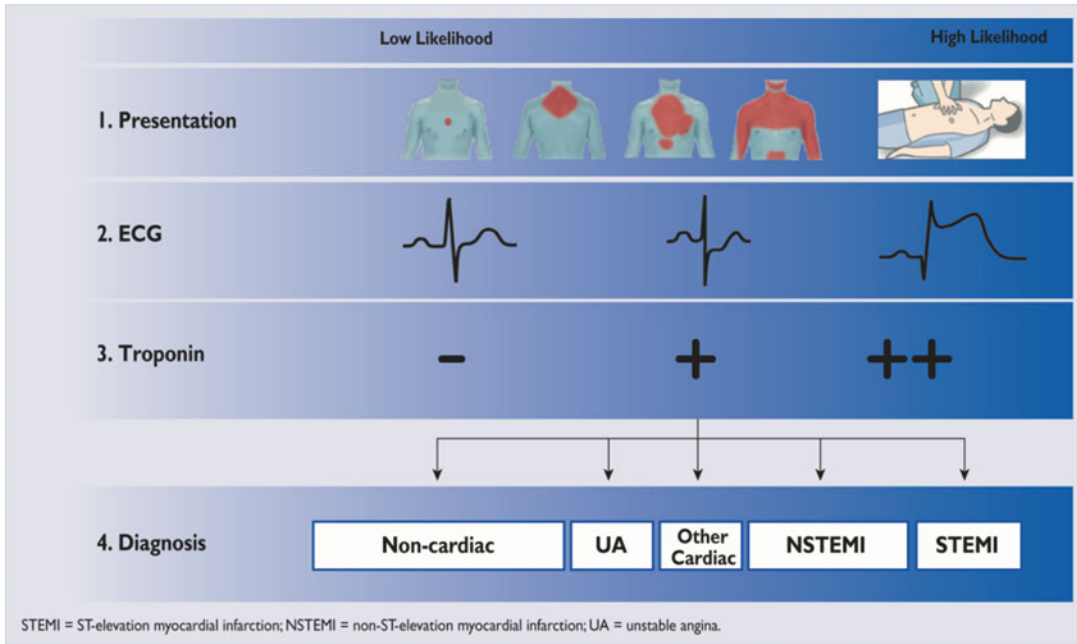

Fig. 33.3 Diagnostic assessment tools for AMI. Reprinted with permissions from Oxford University Press [72]. Abbreviations: *ECG* electrocardiography, *NSTEMI* non-ST-elevation myocardial infarction, *STEMI* ST-elevation myocardial infarction, *UA* unstable angina

cTn concentrations (above the assay's limit of detection) should be ≥50% [7]. Troponin assays that do not meet these criteria are defined as conventional cTn assays. Today there are two assays available which meet the high-sensitivity criteria: the hs-cTnT assay from Roche and Architect hs-cTnI assay from Abbott [7]. Whereas clinical cutoff values of conventional cTn assays were limited to 10% CV, the technical improvement of hs-cTn assays has led that the clinical cutoff values of hs-cTn assays are lowered corresponding to the 99th percentile upper reference limits from a reference population (hs-cTnT, 14 ng/L; hs-cTnI, 26 ng/L, package insert). This enabled that cTn concentrations could now be detected also in other high-risk CVD patients, as described above (Table 33.1). Somewhat misleading is that the higher prevalence of elevated cTn levels found by hs-cTn assays resulted into more "false-positive" results. Most of these unexpected hs-cTn elevations are "true positive" for (subclinical) myocardial injury (rather than AMI) and thus still require diagnostic workup [86]. Thus, the change from conventional cTn assays to hs-cTn assays, and thereby the lowering of the AMI cutoff values, has led to a higher proportion of individuals suitable for beneficial therapies [31]. Thus far, no studies investigated the clinical impact of change over from conventional to hs-cTn assays for diagnosis of AMI in women and men separately. However, hypothetically, the change over from cTn to hs-cTn assays may have led that unrecognized AMIs in women who were missed in the past could now be detected with hs-cTn assays. At this moment the available evidence supporting this hypothesis is scarce and this topic is currently under investigation.

Another important aspect is that women have lower troponin levels as compared with men [58]. A number of studies determined sex-specific 99th percentile upper reference limits of hs-cTnT and hs-cTnI and showed that women-specific thresholds are remarkably lower as compared with men-specific thresholds [5, 37, 44, 58]. As currently a universal diagnostic threshold of hs-cTn is recommended for diagnosing AMI, which is higher than women-specific thresholds, this may contribute to underdiagnosis of AMI in women. In addition, the recommended universal hs-cTnT and hs-cTnI thresholds (14 ng/L vs 26 ng/L, respectively) are not biologically equivalent [89]. A direct comparison of (sex-specific) 99th percentile upper reference limits of hs-cTnT and hs-cTnI from a single reference population revealed numerically similar 99th percentile upper reference limits (hs-cTnT, 15 ng/L; hs-cTnI, 13 ng/L) and enhanced further investigation into sex-specific analysis and downward adjustment of hs-cTnI threshold (Fig. 33.4) [44].

Whether these sex inequalities are of such clinical relevance that the use of universal hs-cTn thresholds hampers the diagnosis of AMI in women, and sex-specific thresholds should be incorporated in diagnostic cTn algorithms, remains controversial. The consideration of sex-specific analysis should be carefully weighted, as this implementation also will result into more complexity of acute cardiac care management. Thus far, the limited number of studies who investigated these issue showed contradictory findings [9, 73, 76, 80, 81, 85]. Two studies recommended to remain the universal thresholds, as they did not observe differences in sex-specific diagnostic and prognostic performance of hs-cTnT on clinical outcome [9], even when sex-specific thresholds were applied [73]. Contrary to these findings, Schofer et al. found sex disparities in the diagnostic performance of AMI using hs-cTnI, particularly into the rule-in performance [80]. The hypothesis that sex-specific thresholds reduce the underdiagnosis of AMI in women is reinforced by the findings of Shah et al., showing that the use of sex-specific hs-cTnI thresholds resulted into a doubling of AMI diagnosis in women but also that the prevalence of AMI diagnosis became similar for women and men [81]. They concluded that sex-specific clinical decision limits of hs-cTnI should be considered for further investigation to close the diagnostic gap between women and men. Two studies showed that applying sex-specific hs-cTnI thresholds did not lead to excessively false-positive AMI diagnosis [76, 85]. Altogether, this finding suggests that sex inequalities may be more relevant for the hs-cTnI assay than for the

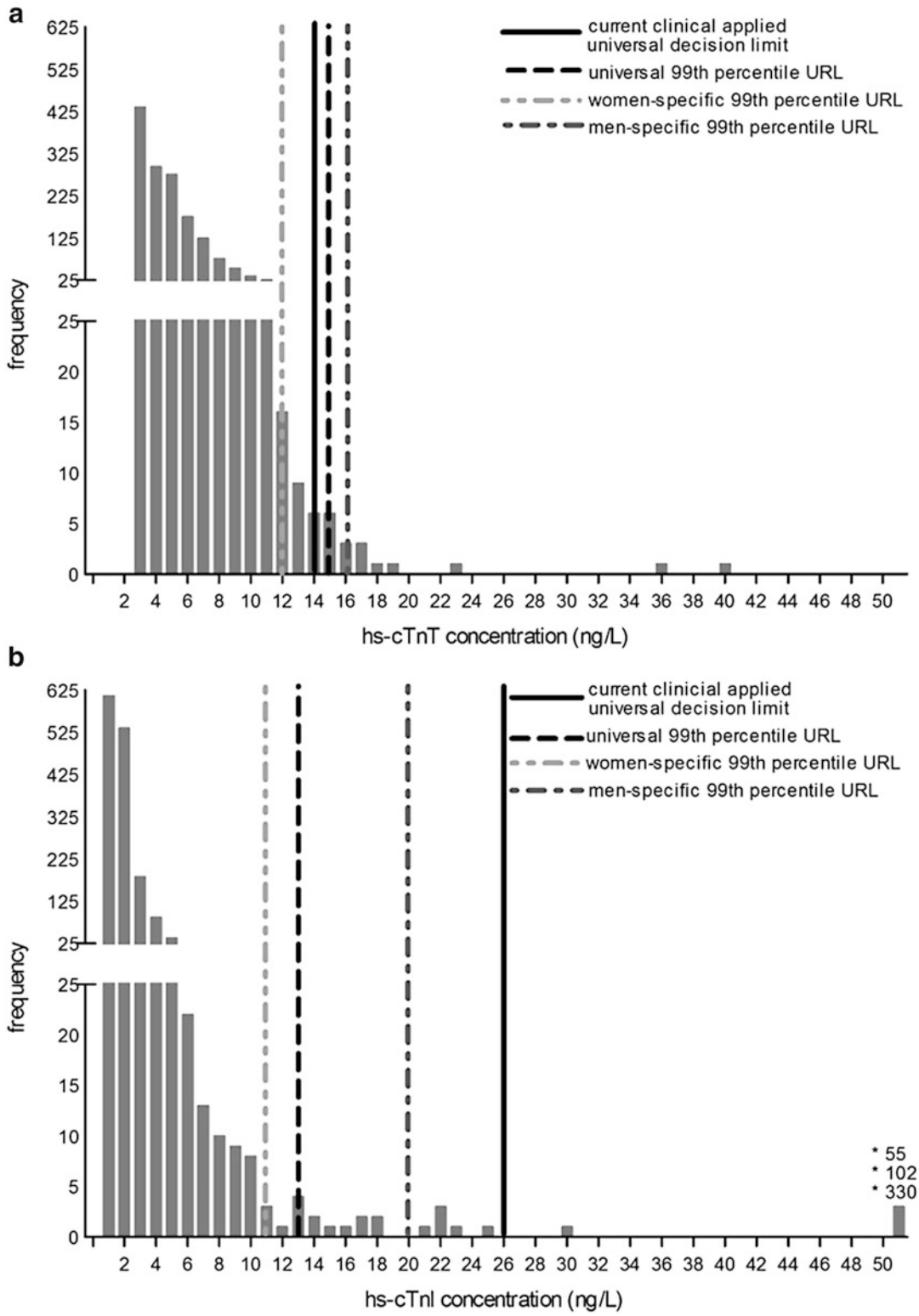

Fig. 33.4 Universal and sex-specific 99th percentile upper reference limits of hs-cTnT (**a**) and hs-cTnI (**b**). Adapted from Kimenai et al. with permission from BMJ Publishing Group Ltd. [44]. Abbreviations: *hs-cTnI* high-sensitivity cardiac troponin I, *hs-cTnT* high-sensitivity cardiac troponin T, *URL* upper reference limit

hs-cTnT assay. Large multicenter randomized controlled trials (e.g., clinical trials.gov NCT01852123) are currently ongoing that should clarify the clinical impact of incorporation of sex-specific cardiac troponin thresholds in the diagnostic algorithm of AMI.

Cardiac Troponins As Prognostic Biomarker

Despite conventional cTn assays were able to detect troponin levels in the clinical range seen in AMI patients, they were not suitable for further risk stratification or prognosis in lower-risk populations. With the shift to hs-cTn assays, cTn could also be detected in the general population, which makes cTn a new promising biomarker for risk stratification and prognosis of CVD morbidity and mortality in the general population. Thus far, a number of epidemiological studies have shown that basal hs-cTn levels are associated with cardiac morbidity and mortality in the general population [11, 23, 82, 90].

Due to fact that there are pathophysiological differences between sexes resulting into different cTn levels in women and men (Sect. "Cardiac troponins in healthy women and men"), the clinical impact of sex on the associations between hs-cTn and CVD mortality is a topic of debate [21, 29, 63, 77]. While some studies showed no interaction of sex on the relationships with troponin and CVD mortality [29, 77], other studies did [21, 63] (Table 33.2). The inconsistency between these studies is probably a result of the divergence of age. Besides the sex effect, also age modifies the association between hs-cTn and mortality. In individuals $\geq$70 years, the impact of sex seems not of clinical importance, as nonsignificant interaction terms of sex were found (all-cause mortality, $P_{\text{interaction}} = 0.74$; incident CVD, $P_{\text{interaction}} = 0.71$) [29]. However, in the lower age range, both for hs-cTnT and hs-cTnI, higher prognostic values on CVD mortality and all-cause mortality were observed in women than in men, whereby the risk on mortality of both sexes increased in older individuals $\geq$65 years [21, 51, 63, 96].

The prognostic impact of sex-specific hs-cTn thresholds in the subgroup of patients with suspected ACS on CVD mortality is also not clarified yet [20, 60, 73, 81]. Shah et al. observed that both women and men susceptive of AMI that were reclassified as having AMI after using sex-specific thresholds had the highest risk on death and recurrent AMI after 1 year as compared with subjects without having AMI [81]. In line with these results, Cullen et al. showed that particularly for women, sex-specific hs-cTnI thresholds improved the identification of women for CVD events [20, 81]. However, thus far, these observations are not confirmed for sex-specific hs-cTnT thresholds [60, 73]. Mueller-Hennessen stated that age might be the crucial factor that should be taken into account, instead of sex-specific analysis [60]. Further study is needed to draw conclusions about this statement, and whether or not sex-specific thresholds of hs-cTnT and/or hs-cTnI improve the long-term prognosis in individuals with suspected ACS.

Natriuretic Peptides

The Basics of Natriuretic Peptides

The heart muscle has an endocrine phenotype as it produces the cardiac hormones atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP). ANP (28 amino acids (aa), Swiss-Prot P01160) is primarily derived from cardiomyocytes from the atria, and BNP (32 aa, Swiss-Prot P16860) is derived from cardiomyocytes from the ventricles, although it was first identified in porcine brain (brain-type). ANP and BNP are quite similar peptides that are released upon stimulation by stretching of the myocyte, as often seen with an overfilled heart, thereby causing excretion of sodium and water. Besides promoting natriuresis and diuresis functionalities, their counterregulatory function also comprises vasodilation and inhibition of the renin-angiotensin-aldosterone and sympathetic nervous system. The release of ANP is regulated by its release from storage granules into the

Table 33.2 Characteristics and main findings of studies investigating clinical impact of sex on the relationship with hs-cTn and CVD morbidity and mortality in the general population

Publication	Population	Median follow-up	Women/men	Outcome (HR, 95% CI)
Zeller 2014	Scottish Heart Health Extended Cohort (30–74 years)	20 years	7742/7598	**CVD events per cubic root hs-cTnI increase**
				Women: 1.35 (1.18–1.53)
				Men: 1.26 (1.15–1.38)
				Coronary mortality per cubic root hs-cTnI increase
				Women: 1.53 (1.23–1.92)
				Men: 1.35 (1.21–1.50)
Dallmeier 2015	The ActiFE study (≥65 years)	4.0 years	618/804	**All-cause mortality per ln hs-cTnT increase**
				Women: 3.67 (2.31–5.81)
				Men: 2.15 (1.61–2.87)
				All-cause mortality per ln hs-cTnI increase
				Women: 3.33 (2.15–5.18)
				Men: 1.92 (1.55–2.38)
Eggers 2015	PIVUS study (70 years)	10 years	502/502	**All-cause mortality per ln hs-cTnI increase**
				Women: 1.59 (1.11–2.28)
				Men: 1.38 (1.12–1.70)
				Cardiac morbidity: incident CVD per ln hs-cTnI increase
				Women: 1.23 (0.82–1.86)
				Men: 1.14 (0.88–1.47)
Omland 2015	HUNT study (≥20 years)	13.9 years	5281/4431	**CVD mortality per 1-SD in ln hs-cTnI increase**
				Women: 1.44 (1.31–1.58)
				Men: 1.10 (1.00–1.20)
				All-cause mortality per 1-SD in ln hs-cTnI increase
				Women: 1.33 (1.24–1.42)
				Men: 1.08 (1.01–1.15)

Abbreviations: *AMI* acute myocardial infarction, *CVD* cardiovascular disease, *HF* heart failure, *hs-cTnI* high-sensitivity cardiac troponin I, *hs-cTnT* high-sensitivity cardiac troponin T, *ln* natural log-transformed

circulation, while on the other hand the release of BNP is regulated on the level of its gene expression. C-type natriuretic peptide (CNP) (Swiss-Prot P23582) and D-type natriuretic peptide (DNP) are also part of the natriuretic peptide family but should not be considered as cardiac peptides in humans.

The bioactive hormonal end products ANP and BNP are derived from their precursors, first in the form of preprohormones and next as prohormones. Human proBNP (108 aa) is cleaved at position 73–76, leading to the inactive side-product N-terminal fragment proBNP 1–76 (NT-proBNP) and the active C-terminal fragment proBNP 77–108 being known as BNP or BNP-32. Intact proBNP has an expected mass of 11 kDa, but in the mid-region (proBNP 36–71), glycosylation occurs to a fully or partially extent leading to unclarified masses that are estimated to be around 25 kDa. For the bioactivity of BNP, the ring structure is essential, formed by a disulfide bond, as is synthesized in the endoplasmic

reticulum and as is necessary for receptor binding and biological activity.

Natriuretic peptide concentrations in the blood circulation are fully integrated into clinical practice as important diagnostic and prognostic biomarkers. A number of different commercial immunoassays are available, especially for BNP and NT-proBNP; for NT-proBNP there is just one source of antibodies and calibrators (Roche Diagnostics). In 2005 the IFCC initiated recommendations on analytical and pre-analytical quality specifications and to improve assay harmonization [6, 8, 75]. BNP is especially susceptible to degradation that may affect antibody affinity. Only EDTA-anticoagulated plastic tubes are acceptable and samples should be immediately put on ice and measured as soon as possible, since BNP is not stable at room temperature and at 2–8 °C it is stable for just a few hours [95]. For NT-proBNP the main issue is the potential of cross-reactivity with split products of NT-proBNP and proBNP [50]. The extent of glycosylation does not play a role since antibodies are selected outside the region with glycosylation sites. NT-proBNP is measurable in serum or plasma (10% lower in EDTA plasma) and is stable for at least 48 h at room temperature [95].

Upon stretching of cardiomyocytes, proBNP is split into equimolar amounts of BNP and NT-proBNP, and indeed, they are used for similar clinical purposes as is pointed out further in this chapter [68, 94]. However, their concentrations in the blood circulation are absolutely different and values are not interchangeable, also for other reasons than the previously discussed pre-analytics and stability. Namely, BNP and NT-proBNP are both cleared by the kidneys, but BNP is besides that also cleared by natriuretic peptide receptor type C (NPR-C) and neural endopeptidases. This results in a half-life for BNP of approximately 25 min, and for NT-proBNP, this is estimated to be twice as long with approximately 120 min. Finally, both peptides have different molecular weights, with the conversion factor for BNP from pmol/L to pg/mL being 3.460 and for NT-proBNP 8.457.

Natriuretic Peptides in Healthy Women and Men

Early studies already illustrated significantly higher natriuretic peptide concentrations in women than in men [19, 70, 71] suggesting a close relation between the cardiac endocrine function and the sex steroid hormones. Figure 33.5 illustrates a typical NT-proBNP distribution as found in our reference population [58]. The sex effect remained true even after correction for differences in body composition and LV mass [49].

Interestingly, sex-based differences in natriuretic peptide concentrations seem to be more pronounced for premenopausal women than for postmenopausal women. It was observed that BNP and NT-proBNP concentrations were lower in postmenopausal women as compared to premenopausal women [48, 49] though this difference could not be confirmed by others [17]. Also, BNP and NT-proBNP concentrations in postmenopausal women seem to become closer to the concentrations as found in men, as was especially true for older adults of 70–75 years and older [21, 56], while others reported higher NT-proBNP concentrations for women independent of their hormonal and menopausal status [19]. Table 33.3 illustrates that we also found higher NT-proBNP concentrations for women when compared to men, irrespective of their age, with the lowest NT-proBNP concentrations for women who almost reached or just reached their menopause (40–50 years). The latter was found by some [19] but not by all [21, 56] as discussed in more detail in Sect "Natriuretic peptides and age".

It has been hypothesized that the sex effect on natriuretic peptides is caused by a stimulatory effect of estrogens and an inhibitory effect of androgens, as schematically illustrated in Fig. 33.6. NT-proBNP levels indeed were inversely associated with androgen concentrations (total and free testosterone) [17, 48], though the association with estradiol was found to be weak or not significant at all [17, 74]. The latter is not completely surprising because of the following issues. The biological variation in

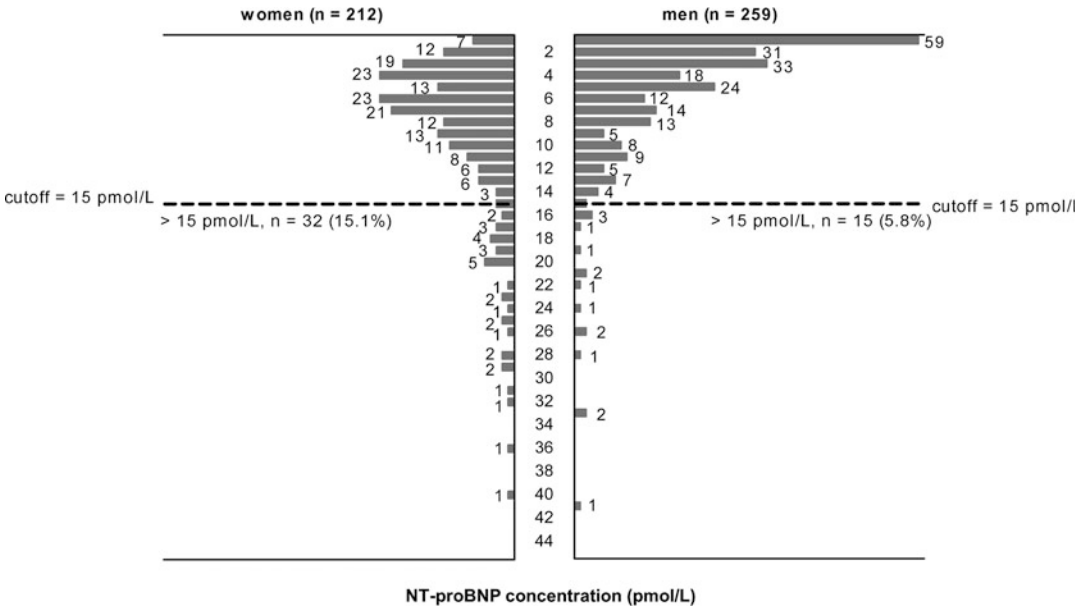

Fig. 33.5 Distribution of NT-proBNP concentrations (pmol/L) in healthy women (left, $n = 212$, mean age 50 ± 11 years) and men (right, $n = 259$, mean age 53 ± 10 years) [58]. Indicated are the number of individuals with elevated concentrations above the universal diagnostic threshold (125 pg/mL/15 pmol/L) used to rule out chronic HF

Table 33.3 NT-proBNP (pmol/L) concentrations in a healthy population [58], stratified by sex and age

Age	*n*	Total	Women	Men
NT-proBNP (pmol/L), median [IQR]				
<30 years	17	3.7 [2.0–6.3]	5.6 [3.1–6.6]	1.6 [0.9–3.5]
30–39 years	49	5.2 [2.4–9.7]	7.6 [3.2–10.7]	3.9 [1.4–6.7]
40–49 years	144	4.0 [1.9–6.5]	4.8 [3.7–8.0]	2.7 [1.1–5.1]
50–59 years	177	6.2 [2.8–9.5]	7.2 [5.0–12.1]	3.4 [1.7–8.5]
≥60 years	84	9.6 [4.9–13.5]	11.7 [6.6–17.3]	7.6 [4.2–12.4]
NT-proBNP ≥ 15 pmol/L, *n* (%)				
<30 years	17	1 (6)	1 (9)	0 (0)
30–39 years	49	5 (10)	4 (14)	1 (5)
40–49 years	144	5 (3)	4 (6)	1 (1)
50–59 years	177	22 (12)	15 (19)	7 (7)
≥60 years	84	17 (20)	10 (33)	7 (13)

NT-proBNP (pmol/L) concentrations are expressed as median (IQR) and as n (%) (>125 pg/mL or 15 pmol/L, universal diagnostic threshold to rule out chronic HF)
Abbreviation: *HF* heart failure

estradiol concentrations is great, up to fivefold during a menstrual cycle. Strikingly, NT-proBNP concentrations were lower in the midcycle phase than in the follicular or luteal phase and thus completely opposite to the fluctuations of estradiol [48]. Moreover, estradiol ranges in cycling women are ≥2 times greater than in men, while testosterone ranges in women are hardly to mention in comparison to the concentrations found in men (total testosterone, factor 10; free testosterone, factor 30). There might also be an alternative explanation via the indirect role of sex hormone-

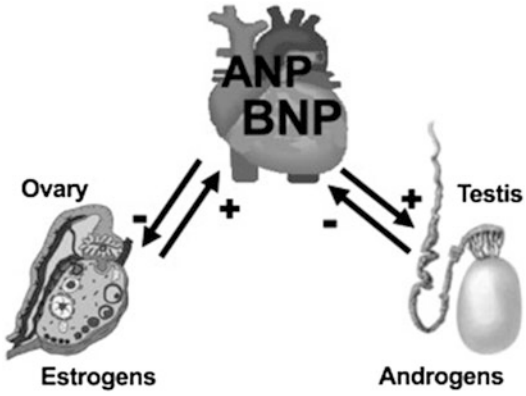

Fig. 33.6 Schematic representation of the relationship between cardiac endocrine and gonadal functions in women and men, showing a stimulatory effect of estrogens and an inhibitory effect of androgens. Reprinted with permission from Elsevier [18]. Abbreviations: *ANP* atrial natriuretic peptide, *BNP* brain-type natriuretic peptide, *SHBG* sex hormone-binding globulin

binding globulin (SHBG) which synthesis is stimulated by estrogens and inhibited by androgens [18] and SHBG was indeed positively associated with BNP and NT-proBNP concentrations [17]. Anyway, the explanation for this sex hormone phenomenon is not understood yet. The current hypothesis is that fertile women benefit from the cardioprotective actions of natriuretic peptides, including vasodilation and diuresis/natriuresis, as is also noticed by the lower risk for cardiovascular events for women during their cycling [59].

Clinical studies demonstrate that also interventions that affect sex hormones in women and men fit with previous observations. For instance, BNP and NT-proBNP concentrations were higher in premenopausal women who receive estrogens for contraception [48] or postmenopausal women who receive estrogens for hormone replacement therapy [52], as compared to premenopausal women. Moreover, androgen receptor blockage and, to a lesser extent, androgen suppression in men with prostate cancer also result in increased NT-proBNP concentrations [27].

Natriuretic Peptides During Pregnancy

Of special clinical interest is the relation of natriuretic peptides with pregnancy. Normal pregnancy goes along with median concentrations about twice that of nonpregnant controls, rising early in pregnancy and remaining high throughout gestation until ≈72 h after delivery [14]. Complications including acute HF may be triggered by eclampsia or preeclampsia and in such situations NT-proBNP might possibly help in the diagnosis [83] though this is not (yet) widely established in routine clinical care [38]. It has been proven though that in preeclampsia NT-proBNP may reflect ventricular stress and subclinical cardiac dysfunction worsening if fetal growth restriction is present [36].

Natriuretic Peptides and Age

Also the influence of age on baseline natriuretic peptide concentrations is very strong [8] and might even more be important in clinical patients than in healthy individuals [19]. Table 33.3 shows that we indeed found substantially higher NT-proBNP concentrations for older adults. Moreover, as mentioned before, NT-proBNP concentrations in our population were higher for women than for men and this was true for all age categories ($P < 0.05$), similar to what was found by some [19], while others found equal NT-proBNP concentrations for postmenopausal women and men [21, 56]. When focusing on the age effect, it is immediately clear that many of the older healthy individuals exceed the diagnostic cutoff that is used to rule out (chronic) HF. This is especially the case for adults with an age of 70–75 years and older, as also illustrated by Fig. 33.7. It is therefore crucial to remind that the current cutoffs are designed for ruling out HF but that other diagnostic tools are necessary for further diagnostic workup in the ruling in [68, 94].

Other clinical factors that influence natriuretic peptide concentrations are body mass index or

obesity, ethnicity, and non-HF pathologies [68, 91, 94] and are not described here in great detail. Explicit attention should be given to renal impairment which substantially increases NT-proBNP concentrations and to a lesser extent also BNP concentrations [3, 55].

Natriuretic Peptides for the Diagnosis of Heart Failure

The definition of HF is mainly based on the measurement of the left ventricular ejection fraction (LVEF) and includes a wide range of patients, from those with normal to reduced LVEF. Table 33.4 shows that natriuretic peptides are also part of the criteria for HF, and with the recent ESC and ACC/AHA guideline updates [68, 95] their role became even more important for diagnostic purposes, establishing disease severity, and prognosis.

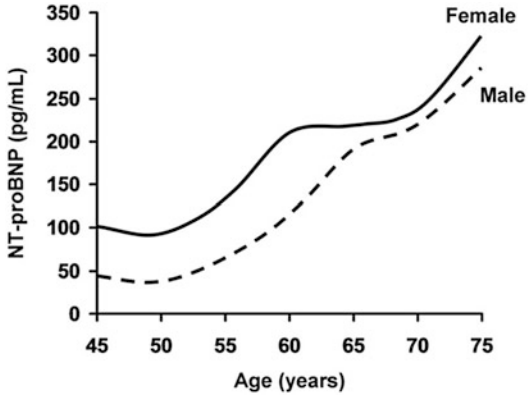

Fig. 33.7 Age- and sex-specific 80th percentile upper reference limits for NT-proBNP concentrations in healthy individuals and stage A/B HF subjects. The universal diagnostic threshold to rule out chronic HF is set at 125 pg/mL or 15 pmol/L. Reprinted with permission from Elsevier [56]

In the diagnostic workup, two algorithms are prescribed as summarized in the following part [68]. They use either BNP or NT-proBNP, though absolute values and diagnostic thresholds are not interchangeable [94], and to a lesser extent also mid-regional proANP (MR-proANP). There are also several analytical and clinical aspects like sex and age that should be considered when using natriuretic peptides for clinical use [8].

- In the non-acute setting [68], the diagnosis of HF is assessed by clinical history, physical examination, and ECG. If all elements are normal, HF is highly unlikely and other diagnoses need to be considered. If at least one element is abnormal, natriuretic peptides should be measured, if available, to identify those who need echocardiography. The upper limit of normal in the non-acute setting for BNP is 35 pg/mL (10 pmol/L), and for NT-proBNP, it is 125 pg/mL (15 pmol/L).
- In the acute setting [68], the diagnostic workup is somewhat different. Upon presentation with acute dyspnea and the suspicion of acute HF, several diagnostic tests are recommended to differentiate cardiac causes of acute dyspnea from noncardiac causes. Natriuretic peptides should be assessed in all patients, and here higher thresholds should be used, namely, for BNP 100 pg/mL (35 pmol/L) and for

Table 33.4 Definition of heart failure (HF)

Criteria	HFrEF	HFmrEF	HFpEF
1	Symptoms ± signs	Symptoms ± signs	Symptoms ± signs
2	LVEF <40%	LVEF 40–49%	LVEF ≥50%
3		1. Elevated levels of natriuretic peptides[a]	1. Elevated levels of natriuretic peptides[a]
		2. At least one additional criterion[b]	2. At least one additional criterion[b]

Adapted from ESC guidelines 2016; see also for further details [68]
Abbreviations: *HFrEF* heart failure with reduced ejection fraction, *HFmrEF* heart failure with midrange ejection fraction, *HFpEF* heart failure with preserved ejection fraction, *LVEF* left ventricular ejection fraction
[a]BNP >35 pg/mL (10 pmol/L); NT-proBNP >125 pg/mL (15 pmol/L)
[b]Relevant structural disease or diastolic dysfunction

NT-proBNP 300 pg/mL (35 pmol/L) and/or MR-proANP 120 pmol/L. Other diagnostic tests that are recommended include ECG; X-thorax; other laboratory assessments like cTn, urea, creatinine, electrolytes (sodium, potassium), glucose, complete blood count, liver function tests, and thyroid-stimulating hormone (TSH); and finally, echocardiography.

The use of natriuretic peptides is especially recommended for ruling out HF in patients who present with dyspnea, since their negative predictive values are very high (0.94–1.00), both in the non-acute and the acute settings [41, 46, 68]. This remains true even when using a universal diagnostic threshold that are regardless of age [41] or sex [46] or any of the other risk factors. It was first thought to use age-dependent cutoffs (US Food and Drug Administration, FDA-cleared cutoffs), 125 pg/mL (15 pmol/L) for patients younger than 75 years and 450 pg/mL (50 pmol/L) for patients of 75 years and older, and/or renal-dependent cutoffs for eGFR <60 ml/min/1.72m^2 [3, 55]. However, clinical research has inevitably shown that negative predictive values were hardly affected when applying a single and relatively low cutoff that is independent from risk factors and thus easier to use in daily practice and still safe for all types of patients.

In contrast, natriuretic peptides are less appropriate for establishing the diagnosis of HF with positive predictive values in the non-acute setting of only 0.44–0.57 and in the acute setting of 0.66–0.67 [68]. This could be mainly explained by the numerous cardiovascular and non-cardiovascular causes of elevated natriuretic peptides, as summarized in Table 33.5 [68, 91, 94] with atrial fibrillation, age, and renal failure being important, if not the most important factors [68].

The severity of CHF is significantly associated with natriuretic peptide concentrations as, for instance, determined by the New York Heart Association classification [41, 53]. Concentrations are lower for HF patients with preserved ejection fraction (HFpEF) than for patients with reduced ejection fraction (HFrEF) [12], but diagnostic values apply similarly to both groups [68].

Table 33.5 Causes of elevated natriuretic peptide concentrations

Cardiac
HF, including RV syndromes
Acute coronary syndromes
Heart muscle disease, including LVH
Valvular heart disease
Pericardial disease
Atrial fibrillation
Myocarditis
Cardiac surgery
Cardioversion
Toxic-metabolic myocardial insults, including cancer chemotherapy
Noncardiac
Advancing age
Anemia
Renal failure
Pulmonary: obstructive sleep apnea, severe pneumonia
Pulmonary hypertension
Critical illness
Bacterial sepsis
Severe burns

Adapted from ACC guidelines 2017 [94]
Abbreviations: *HF* heart failure, *LVH* left ventricular hypertrophy, and *RV* right ventricular

Natriuretic Peptides for the Management of Heart Failure

Over the last 15 years, it has been hypothesized that natriuretic peptides might be helpful as objective measures in the management of HF patients. Most of the prospective clinical trials investigated whether it is useful to titrate the pharmacologic therapy to a fixed target concentration. Results of single studies have been quite conflicting, unfortunately, whereas meta-analyses overall show promising effects. The most recent and largest meta-analysis by Savarese et al. included 2686 patients from 12 studies [78]. They reported benefits on both hospitalization and mortality of the HF patients. Significant results were obtained specifically for NT-proBNP-guided studies, in contrast to the BNP-guided studies. The final evidence was expected from the GUIDE-IT (Guiding Evidence Based Therapy Using Biomarker Intensified Treatment in Heart Failure) study by investigating high-risk patients with relatively advanced HFrEF [33], but NT-proBNP-guided therapy turned out not to be more effective in hospitalization or mortality than usual care strategy nor were doses of medical therapy different between the groups; this study was therefore stopped for futility when 894 of the 1100 patients were enrolled [34].

Sex-based differences for natriuretic peptide-guided therapy have not been reported, though this is different for the influence of age. The benefits for NT-proBNP guidance appear at least to be true for individuals of 75 years or younger [10, 12, 66] and for patients with HFrEF but not for HFpEF [12]. It is conceivable that the more frequent presence of comorbidities in elderly may prevent or even promote potentially harmful up-titration of drugs.

Unfortunately, the concept of serial sampling of natriuretic peptides is quite limited because of a great biological variation which is of course crucial for successful HF management. Changes in concentration of more than 50 to 100% are necessary to be sure that the changes observed are "real" (exceeding reference change values) and are indeed due to therapy interventions [92, 93]. It was recently validated and confirmed that the biological variation is independent from the clinical condition of the patient, thus being equal in healthy individuals and chronic HF patients [57].

Natriuretic Peptides for Prevention, Prognosis, and Risk Stratification

Numerous prognostic markers of death and hospitalization have been identified in HF patients. Extensive review and meta-analysis illustrate that especially age and to a lesser extent also sex are strong predictors for cardiac death and hospitalization in patients with HF, if not the most important ones [64, 69]. In the top ten of the most prominent predictors is also space for a couple of biomarkers that are related to renal function (sodium, creatinine, urea), oxygen supply (hemoglobin), and cardiac function (NT-proBNP) [64].

The use of natriuretic peptides as prognostic predictors remains however challenging for daily practice [68]. Nevertheless, the recent American guideline included new level 1 recommendations on the use of natriuretic peptides as powerful predictors with a specific role for (1) screening in patients at risk for developing HF (presence of hypertension, diabetes mellitus, or vascular disease), (2) baseline levels on admission to the hospital in case of acutely decompensated HF, (3) a predischarge level during hospitalization, and (4) in case of chronic HF to consider also biomarkers of myocardial injury (cTn) or fibrosis [94]. It seems almost logical that sex and probably also age are very important predictors that should always be considered when using these applications, but future evidence has to proof this first.

An interesting sex aspect is the benefit that fertile women gather from their higher baseline natriuretic peptide concentrations, as previously discussed in Sect. "Natriuretic peptides in healthy women and men" [59]. The survival advantage of women though was canceled out in case of congenital heart disease what might be related to the higher prevalence of severe pulmonary hypertension [62]. Also, despite that, marked sex differences were found in acute HF patients with

preserved versus reduced ejection fractions with opposite associations of anemia and LVEF requiring further attention [42]. Even in community-dwelling older adults, sex-based differences remain an ongoing research topic where associations of NT-proBNP with all-cause mortality were substantially stronger among women [21].

Conclusions and Future Perspectives

Taken together, the cardiac biomarkers cTn, NT-proBNP, and BNP are the cornerstones in the diagnosis and clinical management of AMI and HF, respectively. Current cardiac care guidelines do not make a distinction between women and men and make use of "one size fits all" algorithms.

The debate on sex-specific analysis mainly concerns the underdiagnosis of AMI in women. Due to the improvement of the cTn assays, a new era arose where cTn levels became measurable in apparently healthy individuals, whereby lower cTn levels (and 99th percentile upper reference limits) were observed in women as compared with men. The evidence for sex-specific diagnostic thresholds remains though controversial, and ongoing and future trials will clarify whether or not sex-specific analysis should be incorporated in the diagnostic algorithm of AMI.

Also other aspects of cTn algorithms are under investigation that may improve the diagnosis of AMI. For instance, combined biomarker approaches are investigated, as promising new biomarkers such as copeptin, heart fatty acid-binding protein, ST2, and growth differentiation factor-15 may have additive diagnostic value over hs-cTn. Up till now, however, the results are quite controversial, probably due to the already high diagnostic performance of hs-cTn. Furthermore, the hs-cTn assays allow further investigation to rapid measurement strategies, which could lead to faster diagnosis of AMI and, eventually, resulting into better prognosis after AMI.

For the natriuretic peptides, previous studies observed higher natriuretic peptide concentrations in women, especially in premenopausal women, than in men. Natriuretic peptides are particularly incorporated in the ruling-out algorithms for the diagnosis of HF and, in contrast, to hs-cTn, where clinical research has shown that negative predictive values for ruling out HF were hardly affected when applying a universal diagnostic threshold of natriuretic peptides that is independent from sex or other risk factors.

Finally, we believe that in the future most sex-specific benefits of cardiac biomarkers can be obtained in the field of patient follow-up and in the field of cardiac risk prevention and risk stratification. Both are unfinished areas and fit the modern ideas of preventive and personalized medicine: from guiding therapy and prognostic applications for patients who are already diagnosed to patients at risk and in the future possibly to even healthy individuals.

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