Review

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Cardiovascular risk evaluation in pregnancy: focus on cardiac specific biomarkers

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Abstract: Despite the evidence demonstrating the clinical utility of cardiac specific biomarkers in improving cardiovascular risk evaluation in several clinical conditions, even the most recent reviews and guidelines fail to consider their measurement in order to enhance the accuracy of the evaluation of cardiovascular risk in pregnant women. The aim of this review article was to examine whether the assay of cardiac specific biomarkers can enhance cardiovascular risk evaluation in pregnant women, first by reviewing the relationships between the physiological state of pregnancy and cardiac specific biomarkers. The clinical relevance of brain natriuretic peptide (BNP)/NT-proBNP and high-sensitivity cardiac troponin I/high-sensitivity cardiac troponin T (hs-cTnI/hs-cTnT) assay in improving cardiovascular risk evaluation is examined based on the results of clinical studies on subjects with normal and those with complicated pregnancy. Finally, the analytical approaches and clinical objectives related to cardio specific biomarkers are advocated in order to allow an early and more accurate evaluation of cardiovascular risk in pregnant women.

Keywords: pregnancy; cardiovascular risk; cardiac troponins; natriuretic peptides; myocardial injury; biological variation

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Introduction

Cardiovascular diseases are the leading cause of maternal mortality and fetal morbidity in European and North American countries [1–4]. Furthermore, a meta-analysis published in 2019 reported that women with a history of complications during pregnancy are at higher risk of cardiovascular diseases, in some cases, years after pregnancy [5]. In particular, hypertensive disorders, gestational diabetes, and preterm birth, which occur in 1–20 % of pregnancies, are the most frequent cardiovascular complications of pregnancy [6–8]. Preeclampsia, the most frequent condition, reportedly occurs in 4–5 % of cases [7]. Hypertension in pregnant women is of particular concern, given that it carries a higher risk of maternal and fetal morbidity and mortality, as well as a higher lifetime risk of cardiovascular disease in women with hypertension [6–8].

The 2023 Consensus Bundle on Cardiac Conditions in Obstetric Care published in US reports that the majority of women who died of cardiovascular complications during pregnancy or postpartum were not diagnosed with cardiac disease before death [3]. However, the majority of pregnancy-related deaths reportedly occur in non-Hispanic Black people [3]. This suggests that excessive mortality may reflect some US healthcare deficiencies in the identification of pre-pregnancy cardiovascular complications, especially in women from the social strata with the lowest income [3]. In view of the body of experimental and clinical evidence now available, as well recent guidelines, a more accurate evaluation of cardiovascular risk should be made in all women in early pregnancy [1–11].

Over the last 20 years, findings in an increasing number of experimental studies confirm that the measurement of cardiac natriuretic hormones (NPs) and cardiac troponin I (cTnI) and T (cTnT) can improve upon the accuracy of cardiovascular risk assessment in apparently healthy individuals, and in patients with cardiovascular diseases [12–14]. NPs are secreted and released specifically by atrial (especially ANP) and ventricular (especially BNP) cardiomyocytes in response to stressful stimuli that activate

the neuro-endocrine and immunological systems [12, 15–18]. Moreover, NPs (in particular, BNP and NT-proBNP) are considered the first-choice biomarkers in all national and international guidelines for early diagnosis, therapy monitoring and risk prediction in patients with heart failure [19–21].

In healthy adults, the measurement of cardiac troponins with high sensitivity methods (hs-cTnI and hs-cTnT) provides an estimate of the physiological or pathological turnover of myocardial cells (cardiomyocyte renewal) [22–24]. In 2018, the Fourth Universal Definition of Myocardial Infarction introduced the concept of myocardial injury, defined as a dynamic elevation of cardiac troponins with at least one value above the 99th percentile of biomarker values in a reference population (99th percentile upper reference limit – URL) [25]. Moreover, an early and more accurate assessment of cardiovascular risk in apparently healthy women and men in the general population is feasible using the measurement of cardiac specific biomarkers, especially cardiac troponin I and T, measured with high-sensitivity assay methods (hs-cTnI and hs-cTnT) [26–34]. In particular, these studies have demonstrated that apparently healthy men and women with circulating hs-cTnI and hs-cTnT levels in the upper tertile are at a high risk of cardiac mortality and Major Adverse Cardiovascular Events (MACE) [26–35]. A possible limitation of these studies is that the mean age of enrolled populations was >44 years.

Despite the evidence supporting the clinical utility of cardiac specific biomarkers assay in improving cardiovascular risk evaluation in healthy subjects and patients with several clinical conditions, the majority of reviews and guidelines fail to consider the measurement of cardiac specific biomarkers in improving on the evaluation of cardiovascular risk in pregnant women with preeclampsia, gestational hypertension, and cardiovascular diseases [7–11]. However, the most recent consensus document by Hameed et al. [3] reports that pregnant or post-partum women presenting with cardiac symptoms, such as shortness of breath, palpitations, or chest pain, should be evaluated for peripartum cardiomyopathy and significant cardiac conditions using an electrocardiogram, B-type natriuretic peptide test, or echocardiogram, or a combination of these, along with a cardiology consultation. Moreover, the 2018 ESC Guidelines for the management of cardiovascular diseases during pregnancy recommend the use of ECG and troponin level measurement in pregnant women with chest pain [35].

The aim of this review article was to discuss whether the assay of cardiac specific biomarkers can improve upon the evaluation of cardiovascular risk in pregnant women. The relationships between the physiological state of pregnancy and cardiac specific biomarkers are reviewed, and the clinical relevance of brain natriuretic peptide/N-terminal pro-brain natriuretic peptide (BNP/NT-proBNP) and hs-cTnI/hs-cTnT assay in enhancing cardiovascular risk evaluation is discussed on the basis of results of clinical studies on normal and complicated pregnancies. Finally, the analytical approaches and clinical objectives related to cardiac specific biomarkers, are advocated in order to enable an early, more accurate evaluation of cardiovascular risk in pregnant women.

Relationships between pregnancy and cardiac specific biomarkers

Natriuretic peptides

In healthy females and males, circulating levels of NPs and cardiac troponins show an opposite age trend, as shown in Figure 1 [12, 14–19, 36–41]. The age trend of circulating levels of NT-proBNP in these subjects (age 15–85 years) is reported in Figure 1A. NT-proBNP concentrations in healthy women are significantly higher (about two-fold) than in men of the same age, whereas the biomarker values increase in both categories after 55 years of age [12, 17–29, 36].

The great heterogeneity in results of studies measuring BNP and NT-proBNP in women with normal and those with complicated pregnancies is due to: a) the different numbers of healthy pregnant or non-pregnant women (the study control group); b) the presence of co-morbidity (especially cardiovascular diseases) before, or complications during pregnancy; c) different numbers and time intervals for sampling during pregnancy and peri- and post-partum; d) different methods used for ANP, BNP and NT-proBNP assaying [36, 42–54]. These variations preclude reliable comparison between the results found in different studies [42, 44, 52–54].

Several studies have studied possible variations in NPs levels during pregnancy and peri-partum [42, 44, 52–54]. In 2009, Franz et al. [42] reported that NT-proBNP levels are increased in healthy pregnancies (94 normotensive, healthy pregnant women, mean ± SD age: 32 ± 6 years) compared to non-pregnant controls (521 healthy women;

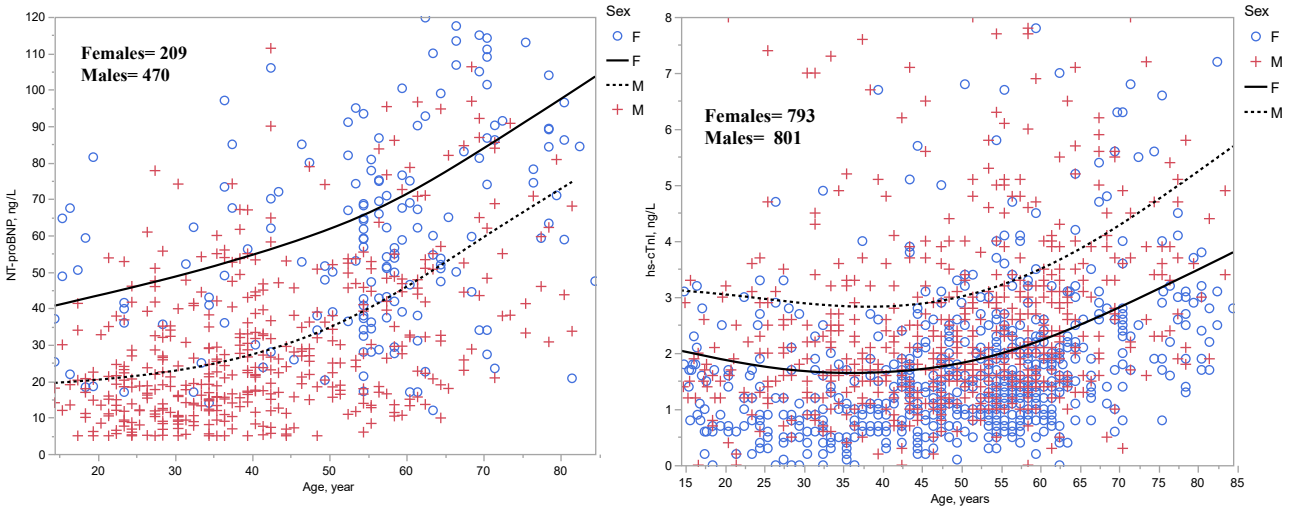

Figure 1: Age trends of circulating levels of NT-proBNP and hs-cTnI in healthy subjects (age 15–55 years). (A) Age trend (15–55 years) of NT-proBNP (Elecsys method, Roche Diagnostics) in healthy females (F) and males (M) enrolled in a reference population, as previously described [38–40]. (B) Age trend (15–55 years) of hs-cTnI (Architect method, Abbott Diagnostics) in healthy females (F) and males (M) enrolled in an Italian reference population, as previously described [39, 40]. The age trends were calculated by a spline function using the JMP 17.2.1 statistical program (SAS Institute Inc.). The 95 % confidence limits of spline functions are reported in the figures (shaded area).

mean ± SD age: 32 ± 7 years) at the first trimester sample. In 2017, Burlingame et al. [52] confirmed that levels of NPs (i.e., BNP and NT-proBNP) significantly increased in the first 48 h after delivery in 95 healthy pregnant women (mean age 31.2 years, SD 5.8 years). In 2018, Umazume et al. [44] measured BNP and NT-proBNP peptide (one blood sample per trimester) in 51 pregnant women (aged 34 ± 5 years) and in the post-partum period (one blood sample taken in the first postpartum week, and another one about 1 month after delivery) [44]. In particular, the levels of NPs, higher in the first trimester, tended to progressively decrease in the second and third trimesters, and markedly increased during the first postpartum days, with a return to normal values after the first week [44]. In 2020, Furenäs et al. [48] enrolled a total of 199 pregnant healthy women (age 18–44 years). Blood samples were obtained on four occasions: at 10–12 and also 20–25 gestational weeks, after delivery and 6 months post-partum; the results [48] confirmed that NT-proBNP levels change significantly during pregnancy and post-partum, the highest levels occurring during the first trimester. In 2021, Dockree et al. [49] aimed to define the reference interval for BNP and NT-pro BNP in each trimester of uncomplicated pregnancy. Findings made in a total of 260 healthy pregnant women (mean age 30.1 years, SD 5.0 years) enrolled confirmed that NT-proBNP levels change significantly throughout normal pregnancy, values being higher (median 68.8 ng/L) during the first, and lower during the third (median 40.6 ng/L) trimester [49]. The Authors found that the trend in BNP levels was similar to that of NT-proBNP, but the wide variation in values precluded any demonstration of significant variations throughout the course of normal pregnancy (median first trimester 16.5 ng/L, median third trimester 12.2 ng/L) [49]. They therefore suggested two cut-off values for NT-proBNP (first trimester<200 ng/L; third trimester<150 ng/L), but only one for BNP (<50 ng/L) [49]. In 2023, Minhas et al. [54] confirmed that 564 women (aged 20–40 years) without a history of cardiovascular diseases, enrolled from the general US population (n=2,134) during the first trimester of pregnancy, had significantly higher NT-proBNP than those in the third trimester and to age–matched non-pregnant women. In view of the results from these studies [42, 44, 47, 52–55], the dynamic nature of biomarkers should be taken into consideration for the clinical interpretation of circulating levels of BNP and NT-proBNP during normal pregnancy.

From an analytical perspective, BNP values measured in pregnant women should be considered method-dependent, even if no studies have specifically evaluated the systematic difference between BNP methods in large populations of pregnant women [49, 55, 56]. Moreover, no data are available on significant differences in circulating levels of BNP and NT-proBNP in pregnant women of different ethnicities.

In healthy subjects, the release of ANP by atrial cardiomyocytes is stimulated by mechanical stretch due to an increase in venous return via mechano-sensor receptors

[15–18]. However, it has been suggested that several biological factors and biomarkers stimulate or inhibit the production/secretion of NPs *in vivo* or in cardiomyocytes culture (Table 1) [12, 15–18, 57–62]. Regarding gender differences, according to some studies, estrogen administration can increase the production of cNPs in post-menopausal women [58, 59], while a recent study reported that NT-proBNP levels were higher in transgender women (assuming estrogens) than transgender men (assuming androgens) [63]. Accordingly, the physiological changes in BNP and NT-proBNP levels, usually observed throughout pregnancy and post-partum, are probably due to marked changes in cardiovascular and renal function, as well as increased plasma volume, partially related to an activation of the renin-angiotensin-aldosterone system, and also an increase in estradiol levels of about 100-fold in the third trimester compared to pre-pregnancy levels [42, 44, 49, 59–62]. In particular, cardiac output increases by 20 % in the first 8 weeks, and up to 40 % during the course of pregnancy, mainly due to peripheral vasodilation, while the blood pressure remains constant, even if peripheral resistance tends to decrease after the first trimester in women with a normal pregnancy [44, 49, 60, 61]. Childbirth further activates the neurohormonal system, resulting in a marked increase in natriuretic peptides and cardiac output [44, 49, 61, 62].

In their study on non-healthy pregnant women, Denoble et al. [50] reported that obese pregnant women (n=28) have lower NT-proBNP values than non-obese women (n=17) and are at a higher risk of developing hypertensive disorders.

Table 1: Biological factors and biomarkers suggested to affect the production/secretion of cardiac natriuretic hormones *in vivo* or in cell culture of cardiomyocytes (ref. [12, 15–18, 57–63]).

Stimulating factors	Inhibiting factors
Mechanical strain of myocardial tissue	Androgens
Hypoxia	Nitric oxide
Angiotensin II	Insulin
Endothelin-1	
Adrenergic agents (both α and β)	
Arginine-vasopressin	
Pro-inflammatory cytokines (such as IL-1, IL-6, TNF-α)	
Basic fibroblastic growth factor (bFGF)	
Prostaglandins (such as PGF2α and PGD2)	
Lypolysaccaride	
Chromogranin B	
Thyroid hormones	
Corticosteroids	
Estrogens	

IL, interleukin; TNF, tumor necrosis factor; bFGF, basic fibroblastic growth factor (bFGF); PG, prostaglandin.

Two meta-analyses, published in 2021 and 2022, respectively, report that persistently elevated levels of BNP or NT-proBNP during the second half of pregnancy may indicate the presence of complications (e.g. heart failure and pre-eclampsia) and are associated with dystocia and neonatal suffering [64, 65]. However, the few studies reporting these findings are heterogeneous in terms of the number of women enrolled, number and time of sampling during pregnancy, and analytical performances of BNP and NT-proBNP assay methods [64, 65].

More recently, Hauspurg et al. [66] investigated the hypothesis whether higher concentrations of NT-proBNP in early pregnancy would be associated with hypertensive disorders of pregnancy and hypertension 2–7 years post-partum. The study series consisted of 4,103 women (mean (SD) age, 27.0 (5.6) years) [66] with the following (clinical) characteristics: 1) a viable singleton pregnancy; 2) no previous pregnancy of more than 20 weeks' gestation; 3) between 6 and 14 weeks of gestation at enrollment (first study examination/visit/). Nine hundred and nine (22.2 %) had an Adverse Pregnancy Outcome (APO), and 817 (19.9 %) had hypertension at the follow-up visit. Women who experienced an APO were more likely to self-report as non-Hispanic Black people, and to have higher early-pregnancy BMI values than women without an APO [66]. Additionally, women with an APO, who had higher blood pressure in early pregnancy, were more likely to develop gestational diabetes, had earlier gestational ages at delivery, and lower infant birth weights. Pregnant women with the higher stages of hypertension had the lowest NT-proBNP concentrations in the first trimester, whereas women without hypertension at follow-up had higher concentrations of the biomarker in the first trimester of pregnancy. Higher NT-proBNP levels during the first month of pregnancy were also associated with a lower risk of hypertensive disorders (adjusted odds ratio: 0.81; 95 % CI: 0.73–0.91), this risk remaining constant after adjustment for age, self-reported race and ethnicity, early-pregnancy body mass index, smoking, and aspirin use [66]. Moreover, higher NT-proBNP concentrations in early pregnancy were also associated with a lower risk of incident hypertension two to 7 years after delivery (adjusted odds ratio: 0.84; 95 % CI: 0.77–0.93), an association that persisted after controlling for confounding factors, including hypertensive disorders of pregnancy. The authors suggest that normal early-pregnancy cardiovascular physiology, assessed on the basis of NT-proBNP concentration, may provide biologic insights into both pregnancy outcome and cardiovascular disease risk [66].

It is important to compare the results previously reported in the two meta-analyses [64, 65] with the more recent data reported by Hauspurg et al. [66]. Overall, these

data [64–66] indicate that the trend of NPs (BNP and NT-proBNP) concentrations expected in healthy pregnant women without APO is characterized by increased concentrations in the first trimester of pregnancy compared to pre-pregnancy levels, followed by a subsequent decline during the third trimester and, finally, a sharp increase in biomarker levels in the peri-partum period, lasting a few days after delivery. These changes in biomarkers levels should be considered as a physiological adaptation of the NP system to cardiovascular stress during pregnancy and partum [44, 48, 52, 53, 60–62, 66].

Hauspurg et al. [66] suggest that lower concentrations of NT-proBNP during the first trimester of pregnancy may reflect impaired adaptation to pregnancy or impaired pre-pregnancy cardiovascular function, resulting in increased vascular stiffness and a less robust volume expansion, which might be closely related to the risk of hypertensive disorders during pregnancy, and post-partum hypertension. This hypothesis is also in agreement with findings that lower concentrations of NPs are associated with higher BMI and waist circumference values, probably related to racial or ethnic differences (e.g., non-Hispanic Black people) [66, 67].

Cardiac troponins

Only during the last 10 years has a new generation of high-sensitivity immunoassay methods (hs-cTnI and hs-cTnT) been set up in order to accurately measure circulating levels of biomarkers in the majority of adult women (>18 years) [24, 38–40, 68, 69]. Overall, healthy women have significantly lower (about half) cTnI and cTnT levels than healthy men of the same age during the fertility age (about 18–45 years) (Figure 1B) [12, 24, 38–40, 68, 69]. The few studies using hs-cTnI or hs-cTnT methods to evaluate the physiological changes of cardiac troponin levels during normal or complicated pregnancy [44, 48, 70–76] have demonstrated no significant difference between hs-cTnI and hs-cTnT levels in pregnant, and those in non-pregnant, healthy women [48, 70–76].

In their systematic review of 2021, Dockree et al. [70] reported that in only 10 studies (9 for cTnI and 1 for cTnT) were circulating troponin levels measured in women with normal or complicated pregnancy, but in only two were hs-cTnI methods used [71, 75]. The results of this study suggest that the levels of cTnI and cTnT in healthy pregnant women are not significantly different from those in healthy non-pregnant women [70]. Moreover, the Authors observe that TnI is elevated in women with pre-eclampsia, but the mechanism underlying this, and its extent, is not well understood [70]. These data [70] confirm those previously reported by Furenäs et al. [48], suggesting that elevated levels of cTnI and cTnT in pregnant women call for greater attention to cardiac symptoms and renal function.

A more recent study by Minhas et al. [72] analyzed data on 2,358 women (1,736 non-pregnant and 622 pregnant; age range 18–40 years) from the US Centers for Disease Control and Prevention (National Health and Nutrition Examination Survey) [77, 78]. Less than 5 % of both non-pregnant and pregnant women had diabetes, and less than 10 %, hypertension. Measurement of hs-cTnT and hs-cTnI values was made using three different methods (Architect Abbott Laboratories, Siemens Healthcare Diagnostics, VITROS Ortho Clinical Diagnostics) on samples collected from 1999 to 2004 [72]. The hs-cTnI and hs-cTnT of women with uncomplicated pregnancies were comparable to those of non-pregnant women, both before and after statistical adjustment for all parameters, including demographics and cardiovascular risk factors (age, race, ethnicity, diabetes, blood pressure value, glomerular filtration rate value) [72]. Therefore, all currently available evidence [70–76] indicates that pregnant women with an uncomplicated delivery present substantially stable hs-cTnI and hs-cTnT levels that are similar to those of the pre-partum period.

Several studies have evaluated the clinical relevance of cardiac troponins methods in women with pregnancies complicated by diabetes, hypertension, eclampsia or cardiomyopathy [70–76, 79]. Overall, there appears to be a significant association between elevated levels of cardiac troponins in pregnancy and a higher risk of cardiovascular complications, dystocia and fetal distress [43, 70, 71, 73–76, 80].

Clinical relevance of combined assay of both cardiac specific biomarkers in pregnancy

The combined measurement of natriuretic peptides and cardiac troponins provides complementary (rather than redundant) pathophysiological and clinical information in patients with cardiovascular diseases [12, 81, 82]. Increased BNP or NT-proBNP can be caused by physiological activities and conditions (e.g., change in posture, water immersion, physical exercise, pregnancy and partum) or, alternatively, by stress conditions (e.g., trauma, sepsis, renal failure, and cardiovascular diseases) [12, 15–19, 81]. If the stressor is very powerful and/or long standing, it can lead to myocardial tissue damage, inducing an increase in hs-cTnI and hs-cTnT circulating levels above the 99th percentile URL values, thus demonstrating the presence of myocardial injury, according to the Fourth Universal Definition of Myocardial Infarction [25].

Few studies have measured both the cardiac specific biomarkers in women with normal or complicated pregnancies with the aim of evaluating whether the combined assay of NPs and hs-cTn can improve the diagnosis, risk stratification and/or management of pregnant women with preexisting cardiac disease or with a pregnancy affected by a cardiovascular complication [44, 48, 76]. In 2020, Furenäs et al. [48] enrolled 196 healthy pregnant women from maternal outpatient clinics in order to describe the intraindividual changes in cardiac biomarker levels during and after pregnancy, and to verify existing cut-off levels for heart failure and myocardial ischemia in pregnant women. Inclusion criteria were pre-pregnancy women classified as healthy on the basis of cardiac echography, age 18–44 years, without heart, lung or kidney disease, and under no regular medication. The Authors measured NT-proBNP and hs-cTnT using the Elecsys immunometric methods (Roche Diagnostics, Mannheim, Germany) on four occasions: at 10–12, 20–25 gestational weeks of pregnancy, after delivery, and 6 months postpartum [48]. None of the women had hypertensive disease or other peripartum or postpartum cardiovascular complications, but one had fever during delivery. NT-proBNP values were significantly higher ($p<0.05$) at 10–12 gestational weeks (mean 67 ng/L, SD 45 ng/L, n=196) with respect to both 20–25 gestational weeks (mean 58 ng/L, SD 45 ng/L, n=163) and post-partum (mean 62 ng/L, SD 44, n=150). Conversely, hs-cTnT values were broadly stable during pregnancy showing only a significant mean increment in the peri-partum compared with the other measurements, including three asymptomatic women with hs-cTnT values above the cut-off value of 14 ng/L (sample at 0–25 gestational weeks: mean 5.02 ng/L, SD 0.13 ng/L, n=196; sample at peri-partum: 6.67 ng/L, SD 3.32 ng/L, n=119; $p<0.05$) [48]. The Authors concluded that NT-proBNP values of <300 ng/L can be used to exclude the diagnosis of heart failure, while hs-cTnT values of <14 ng/L can be used to exclude the presence of myocardial damage in pregnant and/or peripartum women [48].

In 2022, Chang et al. [76] enrolled 307 pregnant women with pre-existing cardiac disease (45 % with congenital heart disease) to test the clinical utility of temporal trends of NT-proBNP and hs-cTnI throughout pregnancy in women with preexisting cardiac disease, and to assess the association between NT-proBNP and hs-cTnI levels with pregnancy outcomes. In this study [76], NT-proBNP values remained steady over the course of pregnancy with a transient increase during labor and delivery with higher levels in subgroups of stable cardiac patients. NT-proBNP, adjusted for age and CARPREG II risk score, was significantly associated with combined heart failure and preeclampsia (adjusted odds ratio per log NT-proBNP increase: 2.14; 95 % CI: 1.48–3.10; $p<0.001$). The Authors suggest the use of an NT-proBNP cut-off level of 200 ng/L for diagnosing heart failure/preeclampsia in the pregnant cardiac population [76]. They also suggest that clinicians can expect women with reduced left ventricular ejection fraction or increasing cardiac structural complexity to have higher, but stable, NT-proBNP levels throughout pregnancy. Furthermore, the presence of NT-pro BNP levels above 200 ng/L in pregnant women, independently associated with heart failure and preeclampsia, should prompt clinicians to closely monitor women throughout pregnancy and peri-partum. Conversely, on measuring hs-cTnI in 191 pregnant women; only 19 (about 10 %) presented biomarker values above the hs-cTnI cut-off value (i.e., 15 ng/L), thus suggesting the presence of a myocardial injury [76]. Of these women with increased hs-cTn values, four had supraventricular tachycardia, two HF, one preeclampsia, and one myocarditis, while in the remaining cases, the causes of increased hs-cTnI were not ascertained. The Authors were unable to establish a consistent relationship between hs-cTnI and adverse outcomes, such as preeclampsia and heart failure [76]. This negative result might be due to the smaller number of pregnant women (n=191) with measured cardiac troponin compared to those with NT-proBNP (n=307), and especially to the extremely small number of pregnant women with higher hs-cTnI values enrolled in this study (only 19/191, about 10 %) [76].

In order to clinically interpret the relative changes in circulating levels of cardiac-specific biomarkers (i.e., cardiac natriuretic hormones and cardiac troponins) in pregnant women, some general pathophysiological considerations should be made [81–88]. The cardiac natriuretic system, including two active peptide hormones (i.e., ANP and BNP), shares some potent physiological actions (including diuretic, natriuretic, vascular muscle-relaxing, anti-inflammatory and anti-growth effects) related to several complex interactions with hormonal, immunological and nervous systems by way of specific receptors located in the membranes of cardiomyocytes (12, 15–19, 56–59, 81–83). Of particular relevance in pregnancy are the relations between the cardiac natriuretic system and sex steroids, Renin-Angiotensin-Aldosterone system, glycemic and lipid regulation, and renal function (12, 17, 43, 44, 56–61, 81–88). In particular, the higher BNP/NT-proBNP levels measured during the first trimester in healthy pregnant women of NPs are probably due a physiological NPs system's response to an increase in steroid

estrogen production by the placenta, activation of Renin-Angiotensin-Aldosterone system, and blood volume expansion [17, 44, 48–50, 85–88]. Likewise, the rapid increase in BNP/NT-proBNP in the peri-partum period is due to the physiological response of NPs system to stress during labor and delivery [44, 48, 52, 53, 62].

Unlike NPs, hs-cTnI and hs-cTnT levels in "healthy" pregnant women are not significantly different from those in non-pregnant healthy women, but the biomarker levels can significantly increase in the peri-partum period, even if these values are usually lower than the recommended cut-off levels (i.e., the 99th percentile URL specific for females) for myocardial injury [44, 48, 62, 72]. This increase in hs-cTnI and hs-cTnT in the peri-partum period of women without APO is characterized by a rapid upward and downward trend (lasting only few days) similar to that found after strenuous physical exercise in healthy individuals and athletes [89–92]. Indeed, cardiac troponins are predominantly located in the cardiac sarcomere bound to myofibrils, while only approximately 4–9 % of cTnI and cTnT are present in the cytosol of cardiomyocytes as unbound-forms showing a lower molecular mass than sarcomeric forms of biomarkers [91–95]. Experimental studies suggest several possible mechanisms underlying the extrusion of cytosol unbound- or bound-forms (microparticles) of biomarkers from cardiomyocytes in the absence of cell death, including transient increases in cell permeability due to cell damage or formation of membranous blebs [91–95]. Currently no experimental data are available to explain the mechanisms underlying the release of hs-cTnI and hs-cTnT in pregnant women during peri-partum.

Several recent studies have reported that pregnant women with APO (including hypertension, pre-eclampsia, diabetes, and congenital or acquired cardiomyopathies) present increased levels of cardiac troponins more frequently than pregnant women without APO [70, 71, 73–76, 79]. It has also been reported that the frequency of elevated levels of cardiac troponins is greater in pregnant women with cardiovascular diseases or diabetes, associated with dystocia and fetal distress [71–76, 79, 80]. Accordingly, it is conceivable that hs-cTnI and hs-cTnT concentrations ≥99th percentile URL value are associated with a higher cardiovascular risk with progression to symptomatic heart and renal failure, as occurs in apparently healthy women in the reference population [13, 14, 26–34, 41, 96]. It should be borne in mind that the presence of a value (specific for female sex and assay method) [24, 39, 41, 68, 69] above the 99th percentile URL of the reference population always indicates the presence of myocardial injury in accordance with the Fourth Universal Definition of Myocardial Infarction [25].

Discussion

Since pregnancy acts as a physiologic cardiovascular stress test by virtue of the hemodynamic changes that occur [3, 84, 85], it may exacerbate underlying cardiac disease or lead to *de novo* cardiomyopathy or peripartum cardiomyopathy [1–3].

As reported by Hameed et al. [3], the incidence of maternal mortality is increasing in the United States, every year approximately 17 per 100,000 women dying of pregnancy-related causes. These Authors [3] recently reported that in the majority of women who died in US of cardiovascular complications during pregnancy or postpartum, the presence of cardiac diseases was frequently not diagnosed before death. In this US study [3], cardiac conditions account for approximately one third of all pregnancy-related deaths, with a disproportionate number of deaths among non-Hispanic Black people, suggesting that these women could suffer from insufficient healthcare before and during pregnancy [97]. On the other hand, aortic dissection is one of the most common causes of cardiac death in pregnancy, accounting for 10 out of 28 cardiac deaths in a cohort of 1.5 million live births enrolled in Nordic countries [98], which have the lowest maternal mortality rate in the world [99].

Furthermore, hypertensive disorders are the most frequent cardiovascular complications of pregnancy (i.e., preeclampsia complicates 4–5 % of pregnancies) [6–8]. The presence of hypertension in pregnant women is of particular clinical concern given the fact that it carries a higher risk of maternal and fetal morbidity and mortality, as well as a higher lifetime risk of cardiovascular diseases in women with hypertension [6–8]. In view of this evidence, the most recent available guidelines recommend that a more accurate evaluation of cardiovascular risk be made in early pregnancy, particularly in women with known cardiac diseases; examinations conducted should include echocardiography and laboratory biomarkers [6–11]. However, only the 2018 ESC Guidelines for the management of cardiovascular diseases during pregnancy recommend that ECG and troponin levels measurement should be performed in pregnant woman with chest pain [35].

The results of several studies on women with a normal or complicated pregnancy indicate that the measurement of cardiac-specific biomarkers may be useful in predicting cardiovascular risk, delivery outcome, and/or fetal and newborn health [48, 50, 51, 64–66, 70–76, 79, 80]. Unfortunately, the few studies available on this issue (the use of hs-cTn methods, in particular) are heterogeneous in terms of the number of women enrolled, presence of a control

group with normal pregnancy, type of complication (e.g., hypertension, eclampsia, diabetes, cardiomyopathies), biomarkers studied, and analytical performance of methods used. In particular, only a few studies have been performed with the specific aim of evaluating the cut-off or reference values of cardiac biomarkers in pregnant women [48, 49].

Despite the above limitations, some pathophysiological and clinical considerations can guide clinicians toward a more appropriate use of cardiac biomarkers in the assessment of cardiovascular risk in pregnant women. In line with the Fourth Universal Definition of Myocardial Infarction [25], we believe a value above the 99th URL percentile specific for each hs-cTnI and hs-cTnT method, should be sufficient for a diagnosis of myocardial injury in a pregnant woman. Moreover, we suggest that the best possible clinical practice is to measure both the cardiac specific biomarkers (i.e., BNP or NT-proBNP and hs-cTnI or hs-cTnT) during the first trimester of pregnancy (basal sample), particularly in pregnant women with a previous diagnosis of cardiovascular disease and/or those with high BMI, smoking habit, and/or increased blood pressure. This proposal is supported by several experimental and clinical evidences [43, 48, 50, 64–66, 70, 71, 73–76, 79, 80].

On considering NPs assay it is important to bear in mind that the biomarker trend during uncomplicated pregnancy is characterized by an increase in biomarker values in the first trimester with a progressive decline up to the third trimester, followed by a sharp rise in the peri-partum period [42, 44, 49, 60–62]. Due to the diversity of concentration values in healthy pregnant and non-pregnant women during pregnancy and peri-partum periods, the wide intra- and inter-individual variations of these biomarkers (both 40–60 %), and the systematic difference among the assay methods BNP and NT-proBNP [12, 56, 81, 100], it is difficult to indicate reliable cut-off values for these biomarkers [48, 49].

Currently, the assay of cardiac specific biomarkers is not specifically recommended in expert documents and guidelines [6–11] for the screening or primary prevention of hypertensive and other cardiovascular disorders in the pre- or early pregnancy period, even if an accurate clinical evaluation before pregnancy is recommended in some guidelines for women with established chronic hypertension or risk factors for preeclampsia [6–8, 11]. Only the consensus document published by Hameed et al. [3] in 2023 states that pregnant or post-partum women presenting with cardiac symptoms should be evaluated for peripartum cardiomyopathy and significant cardiac conditions using cardiac testing, such as an ECG, cardiac natriuretic peptides assay, ECG, or a combination of these, along with a cardiology consultation.

Recent data reported by Hauspurg et al. [66] indicate that the loss of the physiological upward and downward trend during pregnancy, which is replaced by stable low or high BNP or NT-proBNP concentrations, suggests the presence of cardiovascular complications (e.g., heart failure and pre-eclampsia) and is associated with a dystocic delivery and neonatal stress.

Considering the hs-cTnI and hs-TnT assay, the data reported in Figure 1B demonstrate that on average the age trend of hs-cTnI levels (measured with the ARCHITECT method) of healthy women during the fertile period (17–50 years) are relatively stable around the LoD value of the assay method (about 1–2 ng/L) [39, 68]. In particular, Ravichandran et al. [71] reported that hs-cTnI could be measured (ARCHITECT method) with a median of 1 ng/L (range 0–783 ng/L) in 546 (62 %) pregnant women (total population 880, mean age 20.1 years, SD=5.1 years), among whom 28 (3 %) were classified as having pregnancy-induced hypertension and 10 (1 %), preeclampsia. Furthermore, hs-cTnI was above the 99th percentile URL value in only 19 (2 %) women [71]. The more recent studies report no significant difference between the hs-cTnI and hs-cTnT levels of healthy pregnant women and those of healthy non-pregnant women [70, 71]. It is therefore reasonable to assume that hs-cTnI and hs-cTnT values above the 99th percentile URL value are always associated with APO, in particular gestational hypertension, preeclampsia and ischemic heart disease [70, 71, 73–76, 79]. Moreover, these data indicate that hs-cTnI and hs-cTnT should be considered a reliable marker of cardiovascular risk, also in pregnant women.

Circulating hs-cTnI and hs-cTnT levels are considered a very reliable index of myocardial tissue turnover in healthy individuals, corresponding to the renewal of ≤10 mg of cardiac tissue in healthy adult subjects [22–24, 93, 94, 100, 101]. The physiological renewal rate of myocardial tissue is proportional to cardiac mass, which could explain why biomarker values are on average higher in men (mean heart mass 300 g; range 280–340 g) than in women (average cardiac mass 250 g; range 230–280 g; Figure 1B). Furthermore, these data explain why circulating hs-cTnI and hs-cTnT levels are stable in healthy individuals of both sexes after adolescence (≥18 years) up to 55 years; Figure 1B, and more closely correlated with body height than BMI [22–24, 41, 69, 93, 94, 100, 101].

Umazume et al. [44] measured mean hs-cTnI concentrations and mean left ventricular (LV) mass (evaluated by echocardiography) values in 51 healthy pregnant women during five different periods of pregnancy: first trimester (Tr 1), second trimester (Tr 2), third trimester (Tr 3), post-partum (days 2–6, PP1) and post-post-partum (days 24–39 days, PP2). If the original data reported by

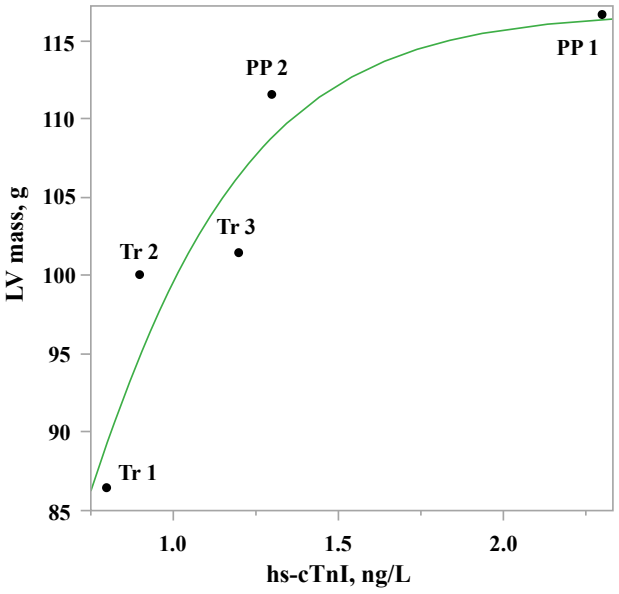

Figure 2: Relationship between hs-cTnI concentrations and LV mass values in healthy pregnant women during different periods of pregnancy. The Figure reports the results of a logistic 3P regression analysis using the original data reported by Umazume et al. [44], concerning the mean hs-cTnI concentrations (X axis) and the mean left ventricular (LV) mass (evaluated by ECG) values (Y axis) measured in 51 healthy pregnant women during five periods of pregnancy: first trimester (Tr 1), second trimester (Tr 2), third trimester (Tr 3), post-partum (days 2–6, PP1) and post-partum (days 24–39 days, PP2). The regression analysis was calculated using the original data reported by Umazume et al. in Table 2 for mean LV mass and mean hs-cTnI values [44]. This analysis was performed by the Authors of the present review article using the JMP 17.2.1 statistical program (SAS Institute Inc.) for the statistical analysis of the original data [44].

Umazume et al. (i.e., ref. [44], Table 2) are evaluated by means of a logistic 3P regression analysis, a highly significant curvilinear association (chi squared test: $p<0.0001$, $R=0.93643$) is found between the means for hs-cTnI concentrations (X axis) and mean LV mass (Y axis) evaluated by Umazume et al. [44], as reported in Figure 2. These data indicate that there is also a close association between cTnI concentration and cardiac mass in women during pregnancy, not only in healthy men and women in the general population [24]. Moreover, these data are in agreement with available data indicating that cTnI values in healthy pregnant women tend to increase in relation to cardiac output peaks in the early third trimester, following a non-linear adaptation pattern of cardiovascular function, as suggested by Meah et al. [84].

Several recent studies have demonstrated that a variation of ≥30 % in hs-cTnI and hs-cTnT concentration values between two samples collected from the same individual at different time intervals should be considered a clinically relevant variation, suggesting a significant increase in biomarker release from cardiomyocytes [24, 37, 41, 69, 100, 102]. Even in the absence of studies specifically designed to evaluate the clinical relevance of variations in hs-cTnI and hs-cTnT throughout the course of normal and complicated pregnancies, a progressive increase ≥30 % of hs-cTnI and hs-cTnT found in some serial samples collected from pregnant women should lead clinicians to suspect (the presence of a) pathological remodeling of myocardial tissue (in pregnant women).

Conclusions

Currently, the clinical use of cardiac-specific biomarkers in pregnancy is severely limited because few studies have been performed with the specific aim of evaluating the cut-off or reference values of cardiac-specific biomarkers in pregnant women [48, 49]. The assay of cardiac specific biomarkers (i.e., cardiac natriuretic peptides and/or cardiac troponins) is not specifically recommended in expert documents and guidelines [6–11] for screening or primary prevention in women with hypertensive and other cardiovascular disorders in pre- or early pregnancy. Only the consensus document by Hameed et al. [3] recommends that pregnant or post-partum women presenting with cardiac symptoms should be evaluated for peripartum cardiomyopathy and significant cardiac conditions using cardiac natriuretic peptides, combined with ECG or echocardiogram, or a combination of these.

According to the Fourth Universal Definition of Myocardial Infarction [25], it is conceivable that pregnant women with hs-cTnI and hs-cTnT values ≥99th reference URL (i.e., the cut-off value for the diagnosis of myocardial injury) should be considered at high cardiovascular risk. There is therefore an urgent need for clinical studies designed to confirm this hypothesis.

As a practical clinical consideration, all biomarker assays should be performed in the same laboratory using the same method in order to maximize the accuracy of biomarker variability estimation in all pregnant women with respect to a reliable baseline testing value, because the measured values of both cardiac-specific biomarkers are method-dependent [37–41, 55, 56, 69, 81, 100, 102].

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References

1. Knight M. The findings of the MBRRACE-UK confidential enquiry into maternal deaths and morbidity. Obstet Gynaecol Reprod Med 2019; 29:21–3.
2. Kotit S, Yacoub M. Cardiovascular adverse events in pregnancy: a global perspective. Glob Cardiol Sci Pract 2021;5:e202105.
3. Hameed AB, Haddock A, Wolfe DS, Florio K, Drummond N, Allen C, et al. Alliance for innovation on maternal health: consensus bundle on cardiac conditions in obstetric care. Obstet Gynecol 2023;141:253–63.
4. Markovitz AR, Stuart JJ, Horn J, Williams PL, Rimm EB, Missmer SA, et al. Does pregnancy complication history improve cardiovascular disease risk prediction? Findings from the HUNT study in Norway. Eur Heart J 2019;40:113–20.
5. Grandi SM, Filion KB, Yoon S, Ayele HT, Doyle CM, Hutcheon JA, et al. Cardiovascular disease-related morbidity and mortality in women with a history of pregnancy complications. Circulation 2019;139:1069–79.
6. Brown MA, Magee LA, Kenny LC, Karumanchi SA, McCarthy FP, Saito S, et al. Hypertensive disorders of pregnancy: ISSHP classification, diagnosis, and management recommendations for international practice. Hypertension 2018;72:24–43.
7. Poon LC, Shennan A, Hyett JA, Kapur A, Hadar E, Divakar H, et al. The International Federation of Gynecology and Obstetrics (FIGO) initiative on pre-eclampsia: a pragmatic guide for first-trimester screening and prevention. Int J Gynaecol Obstet 2019;145(Suppl 1):1–33.
8. Cameron NA, Everitt IK, Lee KA, Yee LM, Khan SS. Chronic hypertension in pregnancy: a lens into cardiovascular disease risk and prevention. Hypertension 2023;80:1162–70.
9. Graves M, Howse K, Puwell J, Smith GN. Pregnancy-related cardiovascular risk indicators. Primary care approach to postpartum management and prevention of future disease. Can Fam Physician 2019;65:883–9.
10. Cho L, Davis M, Elgendy I, Epps K, Lindley KJ, Mehta PK, et al. Summary of updated recommendations for primary prevention of cardiovascular disease in women. J Am Coll Cardiol 2020;75:2602–18.
11. Garovic VD, Dechend R, Easterling T, Karumanchi SA, McMurtry Baird S, Magee LA, et al. Hypertension in pregnancy: diagnosis, blood pressure goals, and pharmacotherapy: a scientific statement from the American Heart Association. Hypertension 2022;79:e21–41.
12. Vittorini S, Clerico A. Cardiovascular biomarkers: increasing impact of laboratory medicine in cardiology practice. Clin Chem Lab Med 2008; 46:748–63.
13. Farmakis D, Mueller C, Apple FS. High-sensitivity cardiac troponin assays for cardiovascular risk stratification in the general population. Eur Heart J 2020;41:4050–6.
14. Clerico A, Zaninotto M, Passino C, Aspromonte N, Piepoli MF, Migliardi M, et al. Evidence on clinical relevance of cardiovascular risk evaluation in the general population using cardio-specific biomarkers. Clin Chem Lab Med 2021;59:79–90.
15. De Bold AJ, Borenstein HB, Veress AT, Sonnenberg H. A rapid and important natriuretic response to intravenous injection of atrial myocardial extracts in rats. Life Sci 1981;28:89–94.
16. Goetze JP. Biosynthesis of cardiac natriuretic peptides. Results Probl Cell Differ 2010;50:97–112.
17. Clerico A, Giannoni A, Vittorini S, Passino C. Thirty years of the heart as an endocrine organ: physiological role and clinical utility of cardiac natriuretic hormones. Am J Physiol Heart Circ Physiol 2011;301: H12–20.
18. Goetze JP, Bruneau BG, Ramos HR, Ogawa T, de Bold MK, de Bold AJ. Cardiac natriuretic peptides. Nat Rev Cardiol 2020;17:698–717.
19. Emdin M, Clerico A, Clemenza F, Galvani M, Latini R, Masson S, et al. Recommendations for the clinical use of natriuretic peptides. Ital Heart J 2005;6:430–46.
20. McDonagh TA, Metra M, Adamo M, Gardner RS, Baumbach A, Böhm M, et al. 2021 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: developed by the Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC) with the special contribution of the Heart Failure Association (HFA) of the ESC. Eur Heart J 2021;42: 3599–726.
21. Heidenreich PA, Bozkurt B, Aguilar D, Allen LA, Byun JJ, Colvin MM, et al. 2022 AHA/ACC/HFSA guideline for the management of heart failure: a report of the American College of Cardiology/American Heart Association Joint Committee on Clinical Practice Guidelines. Circulation 2022;145:e895–1032.
22. Buja LM, Vela D. Cardiomyocyte death and renewal in the normal and diseased heart. Cardiovasc Pathol 2008;17:349–74.
23. Bergmann O, Bhardwaj RD, Bernard S, Zdunek S, Barnabé-Heider F, Walsh S, et al. Evidence for cardiomyocyte renewal in humans. Science 2009;324:98–102.
24. Giannoni A, Giovannini S, Clerico A. Measurement of circulating concentrations of cardiac troponin I and T in healthy subjects: a tool for monitoring myocardial tissue renewal? Clin Chem Lab Med 2009; 47:1167–77.
25. Thygesen K, Alpert JS, Jaffe AS, Chaitman BR, Bax JJ, Morrow DA, et al. Fourth universal definition of myocardial infarction (2018). Eur Heart J 2019;40:237–69.
26. Sze J, Mooney J, Barzi F, Hillis GS, Chow CK. Cardiac troponin and its relationship to cardiovascular outcomes in community populations – a systematic review and meta-analysis. Heart Lung Circ 2016;25: 217–28.
27. Van der Linden N, Klinkenberg LJJ, Bekers O, Loon LJCV, Dieijen-Visser MPV, Zeegers MP, et al. Prognostic value of basal high-sensitive cardiac troponin levels on mortality in the general population: a meta-analysis. Medicine 2016;95:e5703.
28. Willeit P, Welsh P, Evans JDW, Tschiderer L, Boachie C, Jukema JW, et al. High-sensitivity cardiac troponin concentration and risk of first-ever cardiovascular outcomes in 154,052 participants. J Am Coll Cardiol 2017;70:558–68.
29. Hughes MF, Ojeda F, Saarela O, Jørgensen T, Zeller T, Palosaari T, et al. Association of repeatedly measured high-sensitivity-assayed troponin I with cardiovascular disease events in a general population from the MORGAM/BiomarCaRE Study. Clin Chem 2017;63:334–42.
30. Sigurdardottir FD, Lynbakken MN, Holmen OL, Dalen H, Hveem K, Røsjø H, et al. Relative prognostic value of cardiac troponin I and C-reactive protein in the general population (from the North-Trøndelag Health [HUNT] Study). Am J Cardiol 2018;121:949–55.
31. Welsh P, Preiss D, Shah ASV, McAllister D, Briggs A, Boachie C, et al. Comparison between high-sensitivity cardiac troponin T and cardiac troponin I in a large general population cohort. Clin Chem 2018;64: 1607–16.
32. Lam CSP, Castillo R, Ho DT, Kasliwal RR, Khurana R, Naik S, et al. High-sensitivity troponin I for cardiovascular risk stratification in the general asymptomatic population: perspectives from Asia-Pacific. Int J Cardiol 2019;282:93–8.

33. Lippi G, Cervellin G, Sanchis-Gomar F. Predicting mortality with cardiac troponins: recent insights from meta-analyses. Diagnosis 2019;8:37–49.
34. Welsh P, Preiss D, Hayward C, Shah ASV, McAllister D, Briggs A, et al. Cardiac troponin T and troponin I in the general population. Comparing and contrasting their genetic determinants and associations with outcomes. Circulation 2019;139:2754–64.
35. Regitz-Zagrosek V, Roos-Hesselink JW, Bauersachs J, Blomström-Lundqvist C, Cífková R, De Bonis M, et al. 2018 ESC Guidelines for the management of cardiovascular diseases during pregnancy. Eur Heart J 2018;39:3165–241.
36. Hess G, Runkel S, Zdunek D, Hitzler WE. Reference interval determination for N-terminal-B-type natriuretic peptide (NT-proBNP): a study in blood donors. Clin Chim Acta 2005;360:187–93.
37. Clerico A, Zaninotto M, Aimo A, Cardinale DM, Dittadi R, Sandri MT, et al. Variability of cardiac troponin levels in normal subjects and in patients with cardiovascular diseases: analytical considerations and clinical relevance. Clin Chem Lab Med 2023;61:1209–29.
38. Franzini M, Lorenzoni V, Masotti S, Prontera C, Chiappino D, Latta DD, et al. The calculation of the cardiac troponin T 99th percentile of the reference population is affected by age, gender, and population selection: a multicenter study in Italy. Clin Chim Acta 2015;438:376–81.
39. Clerico A, Ripoli A, Masotti S, Musetti V, Aloe R, Dipalo M, et al. Evaluation of 99th percentile and reference change values of a high sensitivity cTnI method: a multicenter study. Clin Chim Acta 2019;493: 156–61.
40. Clerico A, Ripoli A, Zaninotto M, Masotti S, Musetti V, Ciaccio M, et al. Head-to-head comparison of plasma cTnI concentration values measured with three high-sensitivity methods in a large Italian population of healthy volunteers and patients admitted to emergency department with acute coronary syndrome: a multi-center study. Clin Chim Acta 2019;496:25–34.
41. Clerico A, Padoan A, Zaninotto M, Passino C, Plebani M. Clinical relevance of biological variation of cardiac troponins. Clin Chem Lab Med 2020;59:641–52.
42. Franz MB, Andreas M, Schiessl B, Zeisler H, Neubauer A, Kastl SP, et al. NT-proBNP is increased in healthy pregnancies compared to non-pregnant controls. Acta Obstet Gynecol Scand 2009;88:234–7.
43. Kampman MA, Balci A, van Veldhuisen DJ, van Dijk AP, Roos-Hesselink JW, Sollie-Szarynska KM, et al. N-terminal pro-B-type natriuretic peptide predicts cardiovascular complications in pregnant women with congenital heart disease. Eur Heart J 2014;35: 708–15.
44. Umazume T, Yamada T, Yamada S, Ishikawa S, Furuta I, Iwano H, et al. Morpho-functional cardiac changes in pregnant women: associations with biomarkers. Open Heart 2018;5:e000850.
45. Vaught AJ, Kovell LC, Szymanski LM, Mayer SA, Seifert SM, Vaidya D, et al. Acute cardiac effects of severe pre-eclampsia. J Am Coll Cardiol 2018;72:1–11.
46. Kumari M, Kovach T, Sheehy B, Zabell A, Morales R, Moodley SJ, et al. Circulating NT-proBNP but not soluble corin levels were associated with preeclampsia in pregnancy-associated hypertension. Clin Biochem 2019;67:12–15.
47. Yu L, Zhou Q, Peng Q, Zeng S, Yang Z. Velocity vector imaging echocardiography and NT-proBNP study of fetal cardiac function in pregnancy-induced maternal hypertension. J Clin Ultrasound 2019;47: 285–91.
48. Furenäs E, Eriksson P, Wennerholm UB, Dellborg M. Pregnancy in a healthy population: dynamics of NTproBNP and hs-cTroponin T. Open Heart 2020;7:e001293.
49. Dockree S, Brook J, Shine B, James T, Vatish M. Pregnancy-specific reference intervals for BNP and NT-pro BNP-changes in natriuretic peptides related to pregnancy. J Endocr Soc 2021;5:bvab091.
50. Denoble AE, Moyett JM, Goldstein SA, Ward CC, Truong T, Erkanli A, et al. Prospective observational study of N-terminal Pro-Brain Natriuretic Peptide levels in obese and nonobese women during pregnancy. Am J Perinatol 2022;40:467–74.
51. Oliveros-Ruiz ML, Vallejo M, Lerma C, Murata C, Navarro Robles J, Lara JG, et al. Association between brain natriuretic peptide and cardiac dysfunction in hypertensive pregnancy disorders. Pregnancy Hypertens 2022;27:117–22.
52. Burlingame JM, Yamasato K, Ahn HJ, Seto T, Tang WHW. B-type natriuretic peptide and echocardiography reflect volume changes during pregnancy. J Perinat Med 2017;45:577–83.
53. Yoshimura T, Yoshimura M, Yasue H, Ito M, Okamura H, Mukoyama M, et al. Plasma concentration of atrial natriuretic peptide and brain natriuretic peptide during normal human pregnancy and the postpartum period. J Endocrinol 1994;140:393–7.
54. Minhas AS, Rooney MR, Fang M, Zhang S, Ndumele CE, Tang O, et al. Prevalence and correlates of elevated NT-proBNP in pregnant women in the general U.S. population. J Am Coll Cardiol 2023;2:100265.
55. Clerico A, Zaninotto M, Prontera C, Giovannini S, Ndreu R, Franzini M, et al. State of the art of BNP and NT-proBNP immunoassays: the CardioOrmoCheck study. Clin Chim Acta 2012;414:112–9.
56. Clerico A, Passino C, Franzini M, Emdin M. Cardiac biomarker testing in the clinical laboratory: where do we stand? General overview of the methodology with special emphasis on natriuretic peptides. Clin Chim Acta 2015;443:17–24.
57. Kuroski de Bold ML. Estrogen, natriuretic peptides and the renin-angiotensin system. Cardiovasc Res 1999;41:524–31.
58. Maffei S, Del Ry S, Prontera C, Clerico A. Increase in circulating levels of cardiac natriuretic peptides after hormone replacement therapy in postmenopausal women. Clin Sci (Lond) 2001;101:447–53.
59. Chang AY, Abdullah SM, Jain T, Stanek HG, Das SR, McGuire DK, et al. Associations among androgens, estrogens, and natriuretic peptides in young women: observations from the Dallas Heart Study. J Am Coll Cardiol 2007;49:109–16.
60. Morton A, Teasdale S. Physiological changes in pregnancy and their influence on the endocrine investigation. Clin Endocrinol 2022;96: 3–11.
61. Soma-Pillay P, Nelson-Piercy C, Tolppanen H, Mebazaa A. Physiological changes in pregnancy. Cardiovasc J Afr 2016;27:89–94.
62. Kimura Y, Kato T, Miyata H, Sasaki I, Minamino-Muta E, Nagasawa Y, et al. Factors associated with increased levels of brain natriuretic peptide and cardiac troponin I during the peripartum period. PLoS One 2019;14:e0211982.
63. Greene DN, Schmidt RL, Christenson RH, Rongitsch J, Imborek KL, Rebuck H, et al. Distribution of high-sensitivity cardiac troponin and N-terminal pro-Brain Natriuretic Peptide in healthy transgender people. JAMA Cardiol 2022;7:1170–4.
64. Sheikh M, Ostadrahimi P, Salarzaei M, Parooie F. Cardiac complications in pregnancy: a systematic review and meta-analysis of diagnostic accuracy of BNP and N-Terminal Pro-BNP. Cardiol Ther 2021;10:501–14.
65. Esbrand FD, Zafar S, Panthangi V, Cyril Kurupp AR, Raju A, Luthra G, et al. Utility of N-terminal (NT)-Brain Natriuretic Peptide (proBNP) in the diagnosis and prognosis of pregnancy associated cardiovascular conditions: a systematic review. Cureus 2022;14:e32848.
66. Hauspurg A, Marsh DJ, McNeil RB, Bairey Merz CN, Greenland P, Straub AC, et al. Association of N-Terminal Pro-Brain Natriuretic

Peptide concentration in early pregnancy with development of hypertensive disorders of pregnancy and future hypertension. JAMA Cardiol 2022;7:268–76.
67. Gupta DK, de Lemos JA, Ayers CR, Berry JD, Wang TJ. Racial differences in natriuretic peptide levels: the Dallas Heart study. JACC Heart Fail 2015;3:513–19.
68. Wu AHB, Christenson RH, Greene DN, Jaffe AS, Kavsak PA, Ordonez-Lianos J, et al. Clinical laboratory practice recommendations for the use of cardiac troponin in acute coronary syndrome: expert opinion from the Academy of the American Association for Clinical Chemistry and the Task Force on Clinical Applications of Cardiac Bio-Markers of the International Federation of Clinical Chemistry and Laboratory Medicine. Clin Chem 2018;64:645–55.
69. Clerico A, Zaninotto M, Padoan A, Masotti S, Musetti V, Prontera C, et al. Evaluation of analytical performance of immunoassay methods for cTnI and cTnT: from theory to practice. Adv Clin Chem 2019;93: 239–62.
70. Dockree S, Brook J, Shine B, James T, Green L, Vatish M. Cardiac-specific troponins in uncomplicated pregnancy and pre-eclampsia: a systematic review. PLoS One 2021;16:e0247946.
71. Ravichandran J, Woon SY, Quek YS, Lim YC, Noor EM, Suresh K, et al. High-sensitivity Cardiac Troponin I levels in normal and hypertensive pregnancy. Am J Med 2019;132:362–6.
72. Minhas AS, Echouffo-Tcheugui JB, Zhang S, Ndumele CE, McEvoy JW, Christenson R, et al. High-sensitivity Troponin T and I among pregnant women in the US-The National Health and Nutrition Examination Survey, 1999–2004. JAMA Cardiol 2023;8:406–8.
73. Pergialiotis V, Prodromidou A, Frountzas M, Perrea DN, Papantoniou N. Maternal cardiac troponin levels in pre-eclampsia: a systematic review. J Matern Fetal Neonatal Med 2016;29:3386–90.
74. Fleming SM, O'Gorman T, Finn J, Grimes H, Daly K, Morrison JJ. Cardiac Troponin I in pre-eclampsia and gestational hypertension. BJOG 2000; 107:1417–20.
75. Morton A, Morton A. High sensitivity cardiac troponin I levels in preeclampsia. Pregnancy Hypertens 2018;13:79–82.
76. Chang SA, Khakh P, Janzen M, Lee T, Kiess M, Rychel V, et al. Trending cardiac biomarkers during pregnancy in women with cardiovascular disease. Circ Heart Fail 2022;15:e009018.
77. Centers for Disease Control and Prevention. National Health and Nutrition Examination Survey: 1999–2004 data documentation, codebook, and frequencies. https://wwwn.cdc.gov/Nchs/Nhanes/ 1999-2000/SSTROP_A.htm [Accessed 6 October 2022].
78. Johnson CL, Paulose-Ram R, Ogden CL, Carroll MD, Kruszon-Moran D, Dohrmann SM, et al. National health and nutrition examination survey: analytic guidelines, 1999–2010. Vital Health Stat 2013;161:1–24.
79. Jacobsen DP, Røysland R, Strand H, Moe K, Sugulle M, Omland T, et al. Cardiovascular biomarkers in pregnancy with diabetes and associations to glucose control. Acta Diabetol 2022;59:1229–36.
80. Sarma AA, Hsu S, Januzzi JL, Goldfarb IT, Thadhani R, Wood MJ, et al. First trimester cardiac biomarkers among women with peripartum cardiomyopathy: are there early clues to this late-pregnancy phenomenon? Am J Perinatol 2023;40:137–40.
81. Perrone AM, Zaninotto M, Masotti S, Musetti V, Padoan A, Prontera C, et al. The combined measurement of high-sensitivity cardiac troponins and natriuretic peptides: a useful tool for clinicians? J Cardiovasc Med 2020;21:953–63.
82. Morfino P, Aimo A, Castiglione V, Vergaro G, Emdin M, Clerico A. Biomarkers of HFpEF: natriuretic peptides, high-sensitivity toponins and beyond. J Cradiovasc Dev Dis 2022;9:256.
83. Clerico A, Giannoni A, Vittorini S, Emdin M. The paradox of low BNP levels in obesity. Hart Fail Rev 2012;17:81–96.
84. Meah VL, Cockcroft JR, Backx K, Shave R, Stöhr EJ. Cardiac output and related haemodynamics during pregnancy: a series of meta-analyses. Heart 2016;102:518–26.
85. Abbas AE, Lester SJ, Connolly H. Pregnancy and the cardiovascular system. Int J Cardiol 2005;98:179–89.
86. Chatuphonprasert W, Jarukamjorn K, Ellinger I. Physiology and pathophysiology of steroid biosynthesis, transport and metabolism in the human placenta. Front Pharmacol 2018;9:1027.
87. Pritchard JA. Changes in blood volume during pregnancy. Anesthesiology 1965;26:393–9.
88. Sanghavi M, Rutherford JD. Cardiovascular physiology of pregnancy. Circulation 2014;130:1003–8.
89. Middleton N, George K, Whyte G, Gaze D, Collinson P, Shave R. Cardiac troponin T release is stimulated by endurance exercise in healthy humans. J Am Coll Cardiol 2008;52:1813–14.
90. Aakre KM, Omland T. Physical activity, exercise and cardiac troponins: clinical implications. Prog Cardiovasc Dis 2019;62:108–15.
91. Baker P, Leckie T, Harrington D, Richardson A. Exercise-induced cardiac troponin elevation: an update on the evidence, mechanism and implications. Int J Cardiol Heart Vasc 2019;22:181–6.
92. Perrone MA, Passino C, Vassalle C, Masotti S, Romeo F, Guccione P, et al. Early evaluation of myocardial injury by means of high-sensitivity methods for cardiac troponins after strenuous and prolonged exercise. J Sport Med Fitness 2020;60:1297–305.
93. Marjot J, Kaier TE, Martin ED, Reji SS, Copeland O, Iqbal M, et al. Quantifying the release of biomarkers of myocardial necrosis from cardiac myocytes and intact myocardium. Clin Chem 2017;63: 990–6.
94. Mair J, Lindahl B, Hammarsten O, Müller C, Giannitsis E, Huber K, et al. How is cardiac troponin released from injured myocardium? Eur Heart J Acute Cardiovasc Care 2018;7:553–60.
95. Hickman PE, Potter JM, Aroney C, Koerbin G, Southcott E, Wu AH, et al. Cardiac troponin may be released by ischemia alone, without necrosis. Clin Chim Acta 2010;411:318–23.
96. Chesnaye NC, Szummer K, Bárány P, Heimbürger O, Magin H, Almquist T, et al. Association between renal function and troponin T over time in stable chronic kidney disease patients. J Am Heart Assoc 2019;8:e013091.
97. Davis NL, Smoots AN, Goodman DA. Pregnancy-related deaths: data from 14 U.S. maternal mortality review committees, 2008–2017. [Accessed 17 November 2021].
98. Vangen S, Bødker B, Ellingsen L, Saltvedt S, Gissler M, Geirsson RT, et al. Maternal deaths in the Nordic countries. Acta Obstet Gynecol Scand 2017;96:1112–9.
99. Trends in maternal mortality. 1990–2015: estimates by WHO, UNICEF, UNFPA, World Bank Group, and the United Nation Population Division. Geneva: World Health Organization; 2015.
100. Clerico A, Zaninotto M, Aimo A, Cardinale DM, Dittadi R, Sandri MT, et al. Variability of cardiac troponin levels in normal subjects and in patients with cardiovascular diseases: analytical considerations and clinical relevance. Clin Chem Lab Med 2023;61:335–46.
101. Bergmann O, Zdunek S, Felker A, Salhpoor M, Alkass K, Bernard S, et al. Dynamics of cell generation and turnover in the human heart. Cell 2015;161:1566–75.
102. Clerico A, Zaninotto M, Passino C, Padoan A, Migliardi M, Plebani M. High-sensitivity methods for cardiac troponins: the mission is not over yet. Adv Clin Chem 2021;103:215–52.