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NT-ProBNP and high-sensitivity troponin T as screening tests for subclinical chronic heart failure in a general population

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Abstract

Aims   The aim of this study was to establish age-specific and sex-specific cut-off values for N-terminal pro-brain natriuretic peptide (NT-proBNP) and high-sensitivity troponin T (hs-troponin T) in healthy subjects and assess cardiac biomarkers as screening tools for subclinical heart failure (HF) in a general population.

Methods and results   Altogether, 1936 participants were randomly selected from the general population Tromsø 7 study in Northern Norway. Diagnostic accuracy (sensitivity, specificity, and negative and positive predictive value) of cardiac markers for echocardiographically defined subclinical HF was evaluated. The receiver-operating characteristic analysis showed that areas under the curve were relatively low (under 0.75) for both NT-proBNP and hs-troponin T, suggesting that the diagnostic accuracy of these biomarkers for subclinical HF was not excellent, especially for mild forms of HF and younger age group 40–49 years. Sex-specific and age-specific cut-offs for hs-troponin T (99th percentiles) and NT-proBNP (97.5th percentiles) were established in healthy subjects from the same general population. The sex-specific and age-specific cut-offs for NT-proBNP had higher specificity for subclinical HF compared with the previously established single cut-off 125 pg/mL. Age-specific cut-off for hs-troponin T (18 ng/L) for men ≥60 years had also higher specificity than the single cut-off 14 ng/L. These cut-offs had high specificity, but low sensitivity, that makes hs-troponin T and NT-proBNP good biomarkers to rule in HF in case of a positive test, but not good enough to rule out all unrecognized HF due to false negative results.

Conclusions   N-terminal pro-brain natriuretic peptide and hs-troponin T are suboptimal screening tools for subclinical HF in a general population due to low sensitivity.

Keywords   Cardiac markers; Heart failure; Reference intervals; Epidemiology studies

Introduction

Chronic heart failure (HF) is a heterogeneous disease that comprises HF with left ventricular systolic dysfunction and reduced ejection fraction (HFrEF) and HF with preserved left ventricular ejection fraction (HFpEF) also known as diastolic HF.[1] HFpEF is an emerging public health problem as its prevalence is increasing worldwide.[2] Diagnostic workup of HFpEF is a challenge for health care systems; the accurate diagnosis requires a comprehensive echocardiographic examination or cardiac magnetic resonance imaging. Routine screening with echocardiography is not recommended due to the lack of benefit, as well as limited availability and high cost.[3] In a general population, many HFpEF patients with early stages of the disease are easily missed. Population-based echocardiographic studies have shown that over 50% of the population with cardiac dysfunction had no clinical symptoms of HF.[4,5] Subclinical heart dysfunction has been associated with increased mortality and increased risk of sudden cardiac death.[6,7] Early detection of subclinical HF at the population level could promote prevention strategies and possibly open up for targeted treatment of early stages of the disease.[8]

Biomarker-guided approach may provide a solution to this problem. The established biomarkers such as brain natriuretic peptide (BNP) and N-terminal pro-brain natriuretic peptide (NT-proBNP) are widely used for the diagnosis of HF.[9] These biomarkers were validated mostly for patients with symptomatic HFrEF in case–control designed studies.[10,11] Studies of diagnostic accuracy of the cardiac biomarkers to identify subclinical HF at a general population level are few.[12,13] NT-proBNP concentration below the established cut-offs for HF has predicted the risk of cardiovascular events and death in a general population.[14] Some studies indicate the NT-proBNP cut-offs used for the diagnosis of the HF need to be revised especially for elderly patients.[15] Other established cardiac markers such as troponins T and I measured by highly sensitive methods (hs-troponin) have prognostic value in patients with chronic HF independent of NT-proBNP levels.[16] The European Society of Cardiology and the American College of Cardiology (ESC/ACC) recommend to use the 99th percentile derived from a reference population as a cut-off value for cardiac troponins.[17] The cut-offs widely used for hs-troponin are usually not age and sex specific. Several studies suggest that using age-specific and sex-specific cut-offs may have both diagnostic and prognostic implications and should be considered for further validation.[18,19] Diagnostic accuracy of cardiac markers at a general population level remains to be evaluated in large population studies representative for all age and sex groups.

The aim of this study was to evaluate diagnostic accuracy of cardiac biomarkers for subclinical HF in a general population.

Methods

This study is a sub-project of the seventh health survey in the Tromsø study. The Tromsø study is a population-based, prospective study in the municipality of Tromsø, Norway. The study comprises seven screenings of the general population, the first in 1974 and the last in 2015–16 (Tromsø 7). All Tromsø residents 40–99 years of age (n = 32 591) were invited by post to participate in the Tromsø study 7 in 2015. The attendance rate was 65%. Altogether, 21 083 participants aged 40 years or older attended phase I of the Tromsø study 7. All participants completed a questionnaire with questions on health conditions, lifestyle, physical activity, alcohol consumption, use of medicines, and education level. The questionnaires were checked by trained personnel. The following separate questions about different diseases were used: 'Have you ever had, or do you have heart failure/atrial fibrillation/angina pectoris/diabetes mellitus/stroke/heart attack?' (no; yes now, yes, previously). The participants who answered 'no' to these questions were defined as not having the recognized diseases. Blood pressure (BP), pulse, height, weight, and waist and hip circumference were measured by the standard procedure.

BP was measured three times on the participant's right upper arm with an oscillometric digital automatic device Dinamap ProCare 300 monitor (GE Healthcare, Oslo, Norway), measurements being separated by a 1 min interval. The average of the second and the third BP measurements was used. A random sample of the participants in the phase I of the Tromsø study 7 was invited to a second visit (phase II; Supporting Information, *Figure S1*). Of those, 2340 aged 40–89 years attended the phase II of the Tromsø 7 study with echocardiographic examination (echo examination). A total of 485 participants had uncomplete echo examination due to low echogenicity, and 404 had missing blood tests or questionnaire data on diseases and medicines. Excluding the participants due to missing and indeterminate data did not substantially change the age and sex distribution, mean body mass index (BMI), BP, or kidney function of the study population.

A total of 1936 participants with complete questionnaire data underwent the extensive clinical examination, electrocardiogram (ECG), and provided blood samples for measurements of the cardiac biomarkers high-sensitivity troponin T (hs-troponin T) and NT-proBNP. Of them, 1750 participants were examined with complete echo examination according to a standardized protocol performed at the Cardiology Department, University Hospital of North Norway (Supporting Information, *Figure S1*). Echo examination was performed by an experienced echo technician using the GE Vivid E9 scanner (GE Medical, Horten, Norway).

Blood tests were performed at the Department of Laboratory Medicine, University Hospital of North Norway, which is a clinical laboratory accredited according to the ISO 15189 standard. Hs-troponin T, NT-proBNP, and creatinine were analysed by the Cobas 8000 instrument. Hs-troponin T and NT-proBNP were measured by the electrochemiluminescence immunoassays 'ECLIA'. Coefficient of variation (CV) for hs-troponin T was <4% at a concentration of 12.7 ng/L, and CV for NT-proBNP was <2.5% at concentrations of 80 and 201 pg/mL. Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration formula with creatinine.[20]

Previously established upper reference limits (cut-offs)

The established cut-off for this hs-troponin T method is 14 ng/L.[21]

The established cut-off for NT-proBNP is 125 ng/L for ruling out chronic HF.[1]

Statistical analyses

Statistical analyses were performed by using SPSS program (IBM Corp, Released 2013, IBM SPSS Statistics for Windows,

Version 22.0, Armonk, NY, USA). For establishing upper reference limits for cardiac markers, a clinically healthy population with preserved kidney function was selected based on the following selection criteria:

- absence of self-reported heart diseases (myocardial infarction/heart attack, angina pectoris, atrial fibrillation, and HF), stroke, diabetes mellitus, and hypertension;
- absence of symptoms of acute heart disease and BP < 140/90 mm/Hg;
- without antihypertensive medication;
- absence of extreme obesity (BMI 18.5–39.9 kg/m^2);
- without moderate/severe anaemia (haemoglobin < 10 g/dL); and
- with eGFR > 60 mL/min/1.73 m^2.

A separate analysis was performed for the subgroup with no signs of heart disease on echo examination (echo-healthy). This group was selected by the following criteria from the healthy population subgroup: no systolic dysfunction; no left ventricular hypertrophy (left ventricular mass/height$^{2.7}$ < 50 g/height$^{2.7}$ in men and <47 g/height$^{2.7}$ in women); no left atrium enlargement (left atrial volume/body surface area ≤ 34 mL/m^2); no clinically significant valvular heart disease (no aortic stenosis with aortic valve mean gradient < 20 mmHg and no mitral stenosis with aortic or mitral insufficiency ≤ grade 2); and no diastolic dysfunction.

Systolic dysfunction was defined as left ventricular ejection fraction (EF) < 50%.[1]

Diastolic dysfunction was defined as two or more factors according to the ESC Guidelines[22]: average E/e/ > 14; septal e/ velocity < 7 cm/s or lateral e/ velocity < 10 cm/s; tricuspid regurgitation velocity > 2.8 m/s; and left atrial volume index > 34 mL/m^2.

Upper reference limits were established as 99th percentiles for hs-troponin T and 97.5th percentiles for NT-proBNP.[17,23] Outliers were defined using QQ-plots and boxplots and were removed before the final calculations of percentiles. Altogether, seven outliers were removed.

Study of diagnostic accuracy

The study of diagnostic accuracy followed the STARD initiative criteria.[24] All the participants were randomly recruited from a general population sample. The data collection was planned before the blood tests and echocardiography were performed. All the participants received both index test (cardiac biomarkers hs-troponin T and NT-proBNP) and the reference standard examination (echo examination) at the same day with no complications. The investigator responsible for echo examination was blind to the results of the laboratory tests and vice versa. There was no clinical intervention between the index test and the reference standard. To study the diagnostic accuracy of cardiac markers for subclinical HF, the participants with known HF were excluded from the sample (n = 60). Systolic and diastolic dysfunction was defined by the echo examination as mentioned above. Diagnostic accuracy was assessed by the receiver-operating characteristic (ROC) curve analysis. All analyses were sex specific. The results were presented as area under the ROC curve (AUC) with 95% confidence interval (95% CI). Further, sex-specific and age-specific cut-offs for hs-troponin T and NT-proBNP were evaluated for defining subclinical systolic and diastolic dysfunction with sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) with 95% CI.

Ethics

The Tromsø study is the general population health study approved by the Data Directorate Norway (07/00886-22/EOL). The biobank registration number for the Tromsø study is 277. 'The challenging spectrum of HF in a general population. Tromsø Study 7' is a part of the Tromsø 7 study that was approved by the Regional Ethics Committee North Norway (REK 2014/940). Evaluation of the cardiac biomarkers was a part of this project.

Results

The study population represents a general population without symptoms of acute coronary disease and acute HF. The participants attended the examination without cardiac pain or ECG signs of acute coronary disease. However, the study population was not entirely heart and kidney healthy. General characteristics of the study population are presented in *Table 1*. Approximately 7.1% of the study population had reduced kidney function with eGFR < 60 mL/min/1.73 m^2. Only 52.8% of the study population were defined as clinically healthy (without hypertension, extreme obesity, earlier myocardial infarction/heart attack, earlier angina pectoris, atrial fibrillation, earlier stroke, known HF, and known diabetes mellitus) and with eGFR > 60 mL/min/1.73 m^2. Altogether, 40.7% of the study population had echo defined chronic HF (systolic and/or diastolic dysfunction) (*Table 1*). However, only 3% of the study population have reported that they had HF, so these conditions were mainly unrecognized. Compared with the participants without chronic HF, the participants with echo defined chronic HF were significantly older [mean age 68.1 (SD 10.0) years vs. 60.9 (SD 10.7) years], had higher BMI [mean BMI 28.1 (SD 4.5) vs. 26.4 (SD 4.1)] kg/m^2, and had more hypertension (45.9% vs. 30.0%) and diabetes mellitus (7.8% vs. 3.4%).

Further results presented in *Table 2* show sex-specific and age-specific upper reference limits for hs-troponin T and

Table 1 General characteristics of the study population: the Tromsø study 7

	Men $n = 913$	Women $n = 1023$	All $n = 1936$
Mean age (SD), years	64.0 (10.6)	62.9 (11.2)	63.4 (10.9)
Mean BMI, kg/m^2	27.7 (3.7)	26.6 (4.9)	27.1 (4.4)
Hs-troponin T, median (99‰), ng/L	7.0 (49.0)	4.0 (24.8)	5.0 (33.6)
NT-proBNP, median (97.5‰), pg/mL	53.0 (961.2)	70.0 (694.0)	63.0 (852.0)
BMI ≥ 40 kg/m^2, %	0.7	1.8	1.2
BMI ≥ 30 kg/m^2, %	22.7	20.4	21.5
BMI < 18.5 kg/m^2, %	0.0	1.6	0.8
eGFR < 60 mL/min/1.73 m^2, %	6.9	7.2	7.1
Hypertension, %	36.5	33.0	33.9
Antihypertensive medicines, %	37.4	29.9	33.4
Earlier heart attack, %	8.0	3.5	5.6
Recognized heart failure, %	4.8	1.4	3.0
Atrial fibrillation, %	10.4	5.3	7.7
Angina pectoris, %	5.3	2.1	3.5
Earlier stroke, %	4.6	2.2	3.3
Known diabetes mellitus, %	7.1	4.8	5.9
Clinically healthy, %[a]	47.4	57.6	52.8
All echo defined heart failure[b]	44.5	37.4	40.7
Isolated systolic dysfunction	18.8	12.0	15.2
Isolated diastolic dysfunction	17.7	19.8	18.8
Combined heart dysfunction	8.0	5.6	6.7
Moderate to severe heart failure[c]	8.8	6.2	7.4

BMI, body mass index; eGFR, estimated glomerular filtration rate; hs-troponin T, high-sensitivity troponin T; NT-proBNP, N-terminal pro-brain natriuretic peptide.

[a]Without cardiovascular disease (one or several of the following conditions: known diabetes mellitus, earlier heart attack, earlier stroke, atrial fibrillation, known angina pectoris, hypertension, and antihypertensive medicines), with BMI 18–39.9 kg/m^2, and with eGFR > 60 mL/min/1.73 m^2.

[b]Systolic dysfunction (ejection fraction < 50%) and/or diastolic dysfunction (defined by two or more echo criteria).

[c]Systolic dysfunction (ejection fraction < 40%) and/or diastolic dysfunction (defined by three or more echo criteria).

Table 2 Serum hs-troponin T (ng/L) and NT-proBNP (pg/mL) in the healthy[a] sample of the population from Northern Norway

	Hs-troponin T							
	Men				Women			
Age, years	N	Median	97.5‰	99‰	N	Median	97.5‰	99‰
40–49	84	3.9	9.9	13.9	119	2.9	10.0	10.0
50–59	97	5.0	15.8	18.9*	144	2.9	10.0	14.6*
60–69	172	6.0	16.0	19.9**	224	3.0	10.0	17.0**
≥70	80	9.0	20.0	22.8**	101	5.0	18.0	21.9**
All	433	5.0	17.0	19.0	589	2.9	12.0	18.0
	NT-proBNP							
	Men				Women			
Age, years	N	Median	97.5 ‰	99‰	N	Median	97.5 ‰	99‰
40–49	84	24.5	109.3	138.4	117	53.0	155.1	194.0
50–59	98	31.0	137.6	170.1*	145	51.5	199.3	219.4
60–69	172	43.0	190.1	265.9**	224	65.5	251.1	293.3*
≥70	79	82.0	285.0	292.7**	100	89.0	329.9	358.0**
All	433	40.0	214.3	257.9	589	62.0	244.0	288.7

Hs-troponin T, high-sensitivity troponin T; NT-proBNP, N-terminal pro-brain natriuretic peptide.

[a]Healthy population was defined as individuals without the following conditions: known coronary heart disease/myocardial infarction, stroke, diabetes mellitus, hypertension, and antihypertensive medicine; and with estimated glomerular filtration rate (Chronic Kidney Disease Epidemiology Collaboration formula with s-creatinine) > 60 mL/min/1.73 m^2 and with BMI 18.5–39.9 kg/m^2.

*$P < 0.05$ difference by the age groups (independent sample Kruskal–Wallis test; reference is the first group).

**$P < 0.001$ difference by the age groups (independent sample Kruskal–Wallis test; reference is the first group).

NT-proBNP in the selected clinically healthy sample of the study population. The established cut-offs 14 ng/L for hs-troponin T corresponded with 99th percentile only in men under the age of 50 years. Women under the age of 50 years had lower 99th percentile (10 ng/L). For the entire healthy male population, the 99th percentile for hs-troponin T was

19 ng/L. The 99th percentile for all healthy women was not very different from men, estimated to be around 18 ng/L. Further, we selected a subsample of the population that was not only clinically healthy but also had normal echocardiography (echo-healthy; *Table 3*). The 99th percentiles for hs-troponin T in men and women under the age of 60 years were lower than 14 ng/L. The 99th percentiles for hs-troponin T in men and women over 60 years of age were the same, 18 ng/L. The established cut-off 125 pg/mL for NT-proBNP corresponded with 97.5th percentile only in echo-healthy men under the age of 60 years. Other age groups of echo-healthy men and women had higher 97.5th percentiles of NT-proBNP.

Table 4 shows the results of sex-specific ROC curve analyses with diagnostic accuracy of hs-troponin T and NT-proBNP for unrecognized echo defined HF. AUC for NT-proBNP was not statistically significantly different from 0.50 for unrecognized HF in women and men younger than 60 years. Both NT-proBNP and hs-troponin T were better markers for moderate to severe unrecognized HF than for HF overall (*Table 4*). Using hs-troponin T and NT-proBNP together to define unrecognized HF did not substantially increase AUC in any of the specific age or sex groups (Supporting Information, *Table S2*).

Different cut-offs for hs-troponin T and NT-proBNP were evaluated by ROC analyses stratified by sex and age and

Table 3 Serum hs-troponin T (ng/L) and NT-proBNP (pg/mL) in the echo-healthy[a] sample of the population from Northern Norway

Age, years	Hs-troponin T							
	Men				Women			
	N	Median	97.5‰	99‰	N	Median	97.5‰	99‰
40–59	69	4.0	10.0	13.0	116	2.9	7.0	7.0
≥60	71	6.0	17.2	18.0	93	4.0	16.3	18.0
All	140	4.0	15.0	17.6	209	2.9	12.0	17.5

Age, years	NT-proBNP							
	Men				Women			
	N	Median	97.5 ‰	99‰	N	Median	97.5 ‰	99‰
40–59	69	24.0	126.3	139.0	117	47.0	192.1	222.8
≥60	71	49.0	228.2	284.5	93	59.0	285.6	308.3
All	140	31.5	189.3	255.9	210	54.0	249.2	287.2

Hs-troponin T, high-sensitivity troponin T; NT-proBNP, N-terminal pro-brain natriuretic peptide.
[a]Healthy population was defined as individuals without known myocardial infarction, hypertension, stroke, atrial fibrillation, diabetes, and antihypertensive medicine, with estimated glomerular filtration rate (Chronic Kidney Disease Epidemiology Collaboration formula with s-creatinine) > 60 mL/min/1.73 m^2, with BMI 18.5–39.9 kg/m^2, and with normal echocardiographic examination of the heart: with ejection fraction > 50%, without left ventricular hypertrophy, without left atrial enlargement, without diastolic dysfunction, and without valve dysfunction.

Table 4 Receiver-operating characteristic analyses: diagnostic accuracy of serum hs-troponin T and NT-proBNP for prediction of unrecognized[a] heart failure in a general Norwegian population

Echo-based diagnosis	Age, years	Biomarker	Men (n = 798)		Women (n = 915)	
			Pos/neg	AUC, mean (95% CI)	Pos/neg	AUC, mean (95% CI)
All unrecognized heart dysfunction[b]	40–59	Hs-troponin T	73/164	0.62 (0.53–0.70)*	52/264	0.55 (0.47–0.64)[NS]
		NT-proBNP		0.53 (0.45–0.61)[NS]		0.44 (0.35–0.52)[NS]
	≥60	Hs-troponin T	282/279	0.62 (0.58–0.67)**	290/309	0.62 (0.57–0.66)**
		NT-proBNP		0.62 (0.57–0.66)**		0.62 (0.58–0.67)**
	40–89	Hs-troponin T	355/443	0.66 (0.62–0.69)**	342/573	0.67 (0.63–0.70)**
		NT-proBNP		0.63 (0.59–0.67)**		0.63 (0.59–0.67)**
Moderate to severe unrecognized heart failure[c]	≥60	Hs-troponin T	58/503	0.69 (0.61–0.76)**	53/546	0.68 (0.61–0.76)**
		NT-proBNP		0.78 (0.71–0.86)**		0.72 (0.63–0.80)**
	40–89	Hs-troponin T	70/728	0.68 (0.61–0.75)**	57/858	0.72 (0.65–0.79)**
		NT-proBNP		0.74 (0.66–0.81)**		0.71 (0.63–0.79)**

AUC, area under the curve; CI, confidence interval; hs-troponin T, high-sensitivity troponin T; NT-proBNP, N-terminal pro-brain natriuretic peptide.
[a]No known diagnosis of heart failure.
[b]Systolic dysfunction (reduced ejection fraction < 50%) and/or ≥2 echo criteria for diastolic dysfunction.
[c]Systolic dysfunction (reduced ejection fraction < 40%) and/or ≥3 echo criteria for diastolic dysfunction.
[NS]Not significant from 0.50.
*$P < 0.05$.
**$P < 0.001$.

compared with the previously established single cut-offs for all ages (*Tables 5* and *6*). The previously established single cut-offs for all ages 14 ng/L for hs-troponin T and 125 pg/mL for NT-proBNP had relatively high specificity (87.8–99.2%), but very low sensitivity (<35%) and relatively low NPV (<75%) for unrecognized HF in men and women of all ages. For women under the age of 60 years, the sex-specific and

age-specific cut-off 192 pg/mL (97.5th percentile in echo-healthy women of this age) had higher specificity (97.3%) for unrecognized HF than the single cut-off 125 pg/mL (*Table 5*). For participants 60 years and older, the age-specific cut-offs 228 pg/mL for men and 285 pg/mL for women had higher specificity for unrecognized HF than the single cut-off 125 pg/mL. The hs-troponin T cut-off 18 ng/L had higher

Table 5 Age-stratified evaluation of different cut-offs of hs-troponin T (ng/L) and NT-proBNP (pg/mL) for all unrecognized heart failure in a sample of general population

	Cut-offs	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)
Men, age 40–59 years					
Hs-troponin T	10	17.8 (9.0–26.6)	95.7 (92.6–98.8)	65.0 (44.4–85.9)	72.4 (66.4–78.3)
	14	9.6 (2.8–16.3)	98.8 (97.1–100.0)	77.8 (50.6–100.0)	71.1 (65.2–76.9)
NT-proBNP	125	4.1 (0.0–8.7)	97.6 (95.2–99.9)	42.9 (6.2–79.5)	69.6 (63.6–75.5)
Men, age ≥ 60 years					
Hs-troponin T	14	21.6 (16.8–26.4)	87.8 (84.0–91.7)	64.2 (54.6–73.9)	52.6 (48.0–57.1)
Hs-troponin T	18	14.2 (10.1–18.3)	95.7 (93.3–98.1)	76.9 (65.5–88.4)	52.5 (48.1–56.8)
NT-proBNP	125	35.1 (29.5–40.7)	82.4 (78.0–86.9)	66.9 (59.3–74.5)	55.7 (50.9–60.5)
NT-proBNP	228	16.7 (12.3–21.0)	93.2 (90.2–96.1)	71.2 (60.3–82.1)	52.5 (48.1–56.9)
Men, all ages					
Hs-troponin T	14	19.2 (15.1–23.2)	91.9 (89.3–94.4)	65.4 (56.2–74.5)	58.6 (55.0–62.3)
NT-proBNP	125	28.7 (24.0–33.4)	88.0 (85.0–91.1)	65.8 (58.3–73.3)	60.7 (56.9–64.4)
Women, age 40–59 years					
Hs-troponin T	7	7.7 (0.5–14.9)	97.0 (94.9–99.0)	33.3 (6.7–60.0)	84.2 (80.1–88.3)
	14	0.0 (0.0–0.0)	99.2 (98.2–100.0)	0.0 (0.0–0.0)	83.4 (79.3–87.6)
NT-proBNP	125	5.6 (0.6–12.1)	90.5 (87.0–94.1)	10.7 (0.7–22.2)	83.0 (78.6–87.3)
NT-proBNP	192	0.0 (0.0–0.0)	97.3 (95.4–99.3)	0.0 (0.0–0.0)	83.2 (79.0–87.3)
Women, age ≥ 60 years					
Hs-troponin T	14	9.0 (5.7–12.2)	97.4 (95.6–99.2)	76.5 (62.2–90.7)	53.3 (49.2–57.3)
Hs-troponin T	18	5.2 (2.6–7.7)	98.1 (96.5–99.6)	71.4 (52.1–90.8)	52.4 (48.4–56.5)
NT-proBNP	125	37.6 (32.0–43.2)	80.9 (76.5–85.3)	64.9 (57.7–72.1)	58.0 (53.3–62.7)
NT-proBNP	285	13.8 (9.8–17.8)	96.4 (94.4–98.5)	78.4 (67.1–89.7)	54.4 (50.2–58.6)
Women, all ages					
Hs-troponin T	14	7.6 (4.8–10.4)	98.3 (97.2–99.3)	72.2 (57.6–86.9)	64.1 (60.9–67.2)
NT-proBNP	125	32.7 (27.8–37.7)	85.3 (82.4–88.2)	57.1 (50.2–64.1)	68.0 (64.6–71.4)

CI, confidence interval; hs-troponin T, high-sensitivity troponin T; NPV, negative predictive value; NT-proBNP, N-terminal pro-brain natriuretic peptide; PPV, positive predictive value.

Table 6 Age-stratified evaluation of different cut-offs of hs-troponin T (ng/L) and NT-proBNP (pg/mL) for unrecognized moderate to severe heart failure in a sample of general population

	Cut-offs	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)
Men, age ≥ 60 years					
Hs-troponin T	14	40.3 (28.1–52.5)	87.5 (83.7–91.3)	41.0 (28.6–53.3)	87.2 (83.3–91.0)
Hs-troponin T	18	30.6 (19.2–42.1)	95.5 (93.1–97.9)	59.4 (42.4–76.4)	86.5 (82.7–90.2)
NT-proBNP	125	67.7 (56.1–79.4)	82.6 (78.3–87.0)	45.7 (35.5–55.8)	92.2 (89.0–95.5)
NT-proBNP	228	53.2 (40.8–65.6)	93.1 (90.1–96.0)	62.3 (49.2–75.3)	90.2 (86.9–93.6)
Men, all ages					
Hs-troponin T	14	33.7 (23.0–44.6)	91.6 (89.1–94.2)	39.7 (27.6–51.8)	89.5 (86.7–92.3)
NT-proBNP	125	58.1 (46.9–69.3)	88.1 (85.1–91.1)	44.3 (34.4–54.2)	92.8 (90.4–95.2)
Women, age ≥ 60 years					
Hs-troponin T	14	14.7 (5.9–23.7)	97.5 (95.8–99.2)	52.9 (29.2–76.7)	85.8 (82.3–89.4)
Hs-troponin T	18	9.8 (2.4–17.3)	98.1 (96.7–99.6)	50.0 (21.7–78.3)	85.2 (81.6–88.8)
NT-proBNP	125	60.7 (48.4–72.9)	80.8 (76.5–85.1)	37.4 (27.8–46.9)	91.6 (88.4–94.8)
NT-proBNP	285	26.3 (15.2–37.3)	96.6 (94.6–98.6)	59.3 (40.7–77.8)	87.4 (84.0–90.8)
Women, all ages					
Hs-troponin T	14	13.6 (5.4–21.9)	98.3 (97.3–99.3)	47.4 (24.9–69.8)	91.1 (88.9–93.3)
NT-proBNP	125	56.1 (44.1–68.0)	85.1 (82.2–88.0)	29.6 (26.6–37.6)	94.5 (92.6–96.5)

CI, confidence interval; hs-troponin T, high-sensitivity troponin T; NPV, negative predictive value; NT-proBNP, N-terminal pro-brain natriuretic peptide; PPV, positive predictive value.

specificity for unrecognized HF than cut-off 14 ng/L for men over 60 years of age. However, the sensitivity of all age-specific and sex-specific 99th and 97.5th percentiles for hs-troponin T and NT-proBNP as cut-offs for unrecognized HF was low (<20%). The sensitivity of the single NT-proBNP cut-off 125 pg/mL was also low (<40% in all age and sex groups). The sensitivity of age-specific and sex-specific cut-offs for NT-proBNP and hs-troponin T was higher for moderate to severe forms of unrecognized HF, but it still remained lower than 70% (*Table 6*).

The diagnostic accuracy of NT-proBNP was slightly better in women for subclinical diastolic dysfunction than for systolic dysfunction; however, the sensitivity remained relatively low for both forms of subclinical chronic HF (Supporting Information, *Tables S3* and *S4*).

The ROC curve analyses showed that there was no optimal cut-off with both high sensitivity and specificity (>80%). A sensitivity of 90% for hs-troponin T for unrecognized HF was achieved at the cut-off 3 ng/L (the detection limit for the method). For NT-proBNP, a sensitivity over 90% to detect unrecognized HF was achieved at a cut-off of 20 pg/mL in men and 30 pg/mL in women, which is below the median (data not shown). However, the specificity of these cut-offs was low (<35%); therefore, it is difficult to use these cut-offs in general practice due to low specificity and many false positive results.

There were few patients with known diagnosis of HF in this population (60 of 1936 participants). AUC for known stable HF was 0.81 (95% CI 0.74–0.88) for NT-proBNP and 0.79 (95% CI 0.74–0.85) for hs-troponin T for both sexes, which is higher than the results for unrecognized HF presented in *Table 4*. The sensitivity of the established cut-off 125 pg/mL for NT-proBNP for known HF was 74.1% (95% CI 62.9–85.4), the specificity was 79.0% (95% CI 77.2–80.9), PPV was 9.8% (95% CI 7.0–12.6), and NPV was 99.0% (95% CI 98.5–99.5) for both sexes. The sensitivity of the established cut-off 14 ng/L for hs-troponin T for known HF was 39.7% (95% CI 27.1–52.2), specificity was 91.7% (95% CI 90.5–93.0), PPV was 12.9% (95% CI 8.0–17.8), and NPV was 98.0% (95% CI 97.4–98.7) for both sexes.

Discussion

The International Federation of Clinical Chemistry (IFCC) committee on Clinical applications of Cardiac biomarkers (C-CB) recommended in 2019 to study cardiac biomarkers, particularly natriuretic peptides in a variety of heterogeneous cohorts, and to stratify upper reference limits by age and sex.[23] Accordingly, this study presents sex-specific and age-specific upper reference limits for NT-proBNP and hs-troponin T derived from a healthy general population sample after excluding cardiovascular disease comorbidities, dia-

betes mellitus, and kidney failure. Our results correspond well with the results from other population studies in other countries that showed the 99th percentile for hs-troponin T to be between 9 and 11 ng/L for women younger than 60 years.[21,25] Both echo-healthy men and women over the age of 60 years had the same 99th percentile for hs-troponin T (around 18 ng/L), which is over the established cut-off 14 ng/L. Our results show that the sex difference in 99th percentiles is only significant for the ages younger than 60 years and diminishes in the elderly age groups of healthy individuals. The cut-offs for NT-proBNP were also different from the established cut-offs. The 97.5th percentiles for all age groups were much lower in healthy men and women compared with the recommended upper reference limits from the method manufacturer Roche based on a previously published study of 4266 heart healthy individuals.[26] Lower NT-proBNP upper limits in our study might be caused by exclusion of participants with reduced kidney function.

Age-specific and sex-specific 99th percentiles of hs-troponin T and 97.5th percentiles of NT-proBNP had high specificity, but low sensitivity to define subclinical HF. That makes it difficult to use it as rule-out screening tests for subclinical HF due to many false negative results. AUC was relatively low (under 0.75), suggesting that the diagnostic performance of these biomarkers in subclinical general population was not excellent. Some previous studies reported better diagnostic accuracy of NT-proBNP for subclinical chronic HF in selected high-risk groups of general population such as patients with hypertension and diabetes mellitus or the elderly over 65 years of age.[12,13] Our findings for moderate to severe chronic HF for the group over 60 years of age were similar to the Italian study of the elderly.[13]

According to the existing literature, the diagnostic accuracy of NT-proBNP is better in patients with clinical signs of HF.[27–30] For example, the study of Verdu *et al*. showed that NT-proBNP had AUC 0.94 (95% CI 0.91–0.97) for clinically suspected HF in primary health care patients.[28] In our study, the diagnostic accuracy of NT-proBNP for recognized HF was also much higher than that for subclinical HF in a general population.

A cut-off with high specificity can be used to rule in a disease due to low false positive rate. Our results showed that it is better to use age-specific and sex-specific cut-offs for NT-proBNP to rule in unrecognized HF: 125 pg/mL for men and 192 pg/mL for women under the age of 60 years and 228 pg/mL for men and 285 pg/mL for women over 60 years. The previously established NT-proBNP cut-off 125 pg/mL for all ages to rule out chronic HF had relatively low sensitivity for subclinical HF to be considered a good rule-out cut-off due to high false negative rate. However, in the subgroup with recognized HF, the NT-proBNP cut-off 125 pg/mL had much better diagnostic performance with sensitivity and specificity over 70% and NPV 99%. That indicates that NT-proBNP is a valuable marker in clinically suspected HF

but is not a good screening test for subclinical HF in a general population.

The main limitation of this study is its cross-sectional design and self-reported data about diseases and medications. Data about non-cardiac diseases were unfortunately unavailable for us. We have excluded all outliers for hs-troponin T and NT-proBNP measurements using the established statistical methods before calculating the 99th and 97.5th percentiles. Thus, we have excluded all unknown reasons for extreme hs-troponin T and NT-proBNP values due to unknown diseases. The strengths of the study include a relatively large sample randomly recruited from a general population with possibility to select a healthy sample with high percentage of healthy elderly participants and women. Other strengths of the study are standardized echo measurements and standardized serum cardiac markers measurements at the ISO 15189 certified clinical laboratory. The investigation of diagnostic accuracy of hs-troponin T and NT-proBNP in this general population sample followed the STARD initiative criteria to assure the quality of the study.[24]

Conclusions and clinical implications

This study established sex-specific and age-specific upper reference limits (cut-offs) for NT-proBNP and hs-troponin T in both clinically healthy and echo-healthy population randomly recruited from a general population sample. Age-specific and sex-specific upper reference limits of NT-proBNP and hs-troponin T as cut-offs could be used to rule in subclinical HF in a general population. However, hs-troponin T and NT-proBNP were not sufficient enough as screening tests to rule out subclinical HF in a general population due to low sensitivity.

Acknowledgements

Conflict of interest

Funding

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Flow chart of the study population.
Table S2. ROC analyses: diagnostic accuracy of combined serum hs-troponin T and NT-proBNP for prediction of unrecognised[a] heart failure in a general Norwegian population.
Table S3. ROC analyses: diagnostic accuracy of serum hs-troponin T and NT-proBNP for prediction of subclinical[a] systolic and diastolic dysfunction in a general Norwegian population.
Table S4. Age-stratified evaluation of different cut-offs of hs-troponin T and NT-proBNP for subclinical chronic systolic and diastolic dysfunction in a sample of general population.

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