Prediction of incident atrial fibrillation with cardiac biomarkers and left atrial volumes

Running title: Cardiac biomarkers, atrial volumes and atrial fibrillation

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ABSTRACT

Objective: Atrial fibrillation is a common arrhythmia associated with risk of stroke, heart failure and death. We aimed to elucidate the associations of cardiac biomarkers, echocardiographic left atrial volumetric indices, and risk of prevalent and incident atrial fibrillation in the general population.

Methods: We assessed cardiac troponin T (cTnT), N-terminal pro-B-type natriuretic peptide (NT-proBNP), growth differentiation factor 15 (GDF-15), maximum ($LAVi_{max}$) and minimum ($LAVi_{min}$) indexed left atrial volumes and left atrial emptying fraction (LAEF) in subjects born in 1950 participating in the prospective observational cohort, Akershus Cardiac Examination 1950 Study. The Cohorts for Heart and Aging Research in Genomic Epidemiology for Atrial Fibrillation (CHARGE-AF) risk score and sex was used to adjust for residual risk of atrial fibrillation.

Results: Out of 3,487 subjects, 157 (4.5%) had prevalent and 123 (3.5%) incident atrial fibrillation. Echocardiographic left atrial volumes and cardiac biomarkers associated with prevalent atrial fibrillation, but GDF-15 was non-significant in adjusted analysis. Incident atrial fibrillation was predicted by $LAVi_{max}$ (adjusted hazard ratio 1.51, 95% CI 1.30-1.75), $LAVi_{min}$ (adjusted hazard ratio 1.52, 95% CI 1.35-1.72), LAEF (adjusted hazard ratio 1.24, 95% CI 1.04-1.48), and NT-proBNP (adjusted hazard ratio 1.57, 95% CI 1.32-1.85). cTnT and NT-proBNP provided incremental prognostic information to echocardiographic left atrial volumes, but GDF-15 demonstrated no prognostic value for incident atrial fibrillation.

Conclusions: In the general population, echocardiographic left atrial volumetric indices and NT-proBNP, but not cTnT and GDF-15, associate with prevalent atrial fibrillation and with long-term risk of incident atrial fibrillation. cTnT and NT-proBNP provide incremental prognostic information to echocardiography.

KEY MESSAGES

What is already known on this topic: Atrial fibrillation prevalence is expected to rise steeply in the future and preventive measures are needed. Cardiac biomarkers and echocardiographic left atrial volumetric indices are established risk prognosticators, but the utility of their combined use remains unclear. In addition, a more novel cardiac biomarker, growth differentiation factor-15 (GDF-15), has been proposed as a marker of atrial fibrillation risk. Some studies have suggested the minimum left atrial volume by echocardiography to be superior to the maximum.

What this study adds: Left atrial maximum volume, minimum volume, and emptying fraction were independently associated with increased risk of incident atrial fibrillation. Cardiac troponin T (cTnT) and N-terminal pro-B-type natriuretic peptide (NT-proBNP) provided incremental prognostic value to left atrial volumes for prediction of atrial fibrillation, while GDF-15 did not associate with increased risk of atrial fibrillation.

How this study might affect research, practice or policy: For atrial fibrillation risk prediction, our data do not support the use of GDF-15 or the use of left atrial minimum instead of maximum volume. cTnT and NT-proBNP concentrations will provide additional information to echocardiography for the prediction of incident atrial fibrillation.

INTRODUCTION

Atrial fibrillation is a common arrhythmia [1, 2] associated with increased risk of stroke, heart failure and death.[3] The prevalence is expected to rise steeply in the future,[4] but risk of atrial fibrillation can be reduced with lifestyle intervention,[5] and the associated poor outcomes of atrial fibrillation are preventable.[6] Yet, many remain at risk or even develop atrial fibrillation without being recognised.[7] Therefore, accurate risk prediction for atrial fibrillation is relevant.

Left atrial dilatation is likely both an upstream and a downstream element in the pathogenesis of atrial fibrillation and was early established as a prognosticator.[8] It is recommended to measure the left atrium volumetrically at its maximum size and most data are on this measure.[9] However, recent evidence suggest that the minimum volume and the emptying fraction (a functional index) may have superior prognostic properties.[10, 11]

Cardiac troponin T (cTnT) and N-terminal pro-B-type natriuretic peptide (NT-proBNP) are established cardiac biomarkers that are routinely used to diagnose acute myocardial infarction and heart failure. Both biomarkers have been found to associate with prognosis of atrial fibrillation,[12] but their incremental prognostic value to echocardiographic left atrial volumetric indices is unknown. Growth differentiation factor 15 (GDF-15) is a biomarker of oxidative stress and inflammation,[13] with prognostic value for major bleeding, stroke and mortality in patients with atrial fibrillation.[14] GDF-15 has been suggested to play a causal role in the development of atrial fibrillation,[15] and may be a risk marker for incident atrial fibrillation.[16] There is, however, limited data from the general population in terms of association with risk of incident atrial fibrillation and associations with left atrial remodelling.

Accordingly, in this study, using a large cohort of subjects recruited from the general population, we hypothesised that established and novel cardiac specific biomarkers and

echocardiographic left atrial volumetric indices would be associated with prevalent and incident atrial fibrillation. We also hypothesised that established and novel cardiac specific biomarkers would provide incremental value to left atrial volumes in predicting incident atrial fibrillation.

METHODS

Study design

The Akershus Cardiac Examination (ACE) 1950 Study is a population-based cohort. All individuals residing in Akershus County, Norway as of 2011 and born in 1950 were invited to the study. Akershus County is one of the more densely populated counties in Norway, surrounding the capital city. The population has a higher education level than the rest of the Norwegian population. Baseline examinations took place in 2012 to 2015 at two study sites, Akershus University Hospital and Bærum Hospital. The study is approved for follow-up until 2050.

Patient and Public Involvement

At the time of start-up, the study had no formal patient or public involvement with regard to study planning and conduction. During the baseline examinations of the study, a random subset of study participants were invited to respond to how they perceived their participation in the study, and if they had any suggestions to improve the study conduct. All the scientific results from the study are communicated to the participants and the general public through local media and www.ace1950.no. All study participants are updated on study progression and dissemination by semi-regular newsletters.

Study variables

At the baseline examinations, study participants filled out a questionnaire providing medical history of atrial fibrillation, heart failure, diabetes, and vascular disease. All participants also underwent clinical examination, blood pressure measurement, echocardiography, blood sampling, and biobanking. Hypertension was defined as having systolic blood pressure above 140 mm Hg, diastolic blood pressure above 90 mm Hg, and/or using antihypertensive medication. History of myocardial infarction, coronary bypass grafting surgery, or percutaneous coronary intervention defined coronary artery disease. If participants reported history of atrial fibrillation diagnosis, this was validated by health records. An electrocardiogram was obtained potentially revealing undiagnosed baseline atrial fibrillation.

Blood sampling and biomarkers

All study participants provided fasting peripheral venous blood samples for biobanking at the baseline examination of the ACE 1950 Study. Blood samples were centrifuged at room temperature and serum was frozen at -80°C. NT-proBNP, cTnT, and GDF-15 were analysed by an electrochemiluminescence immunoassay Elecsys on a Cobas Platform 8000, e801 (Roche Diagnostics, Rotkreuz, Switzerland) between October 2017 and January 2018 at Akershus University Hospital, Norway. The limit of detection (LoD) is 5.0 ng/L and limit of blank (LoB) is 3.0 ng/L for NT-proBNP, the LoD is 3.0 ng/L and LoB is 2.5 ng/L for cTnT, and the LoD is 400ng/L and LoB is 350ng/L for GDF-15. Biomarker concentrations below the LoD were given a concentration of 2.5 ng/L for NT-proBNP, 1.5 ng/L for cTnT, and 200ng/L for GDF-15.

Echocardiography

Echocardiography was performed with a Vivid E9 scanner with M5S probe (GE Healthcare, Horten, Norway). Images were obtained in the parasternal short- and long-axis views as well as in the three apical views. Examiners were instructed to obtain left atrial focused images if

the left atrium was foreshortened in the apical views. The recordings and subsequent analyses using custom software (EchoPAC, GE, Vingmed, Horten, Norway) were done by four trained fellows and two ultrasonographers. Chamber quantifications were performed according to recommendations by the American Society of Echocardiography and the European Association of Cardiovascular Imaging.[9] Left atrial volumes were quantified by the summation of discs method. The mean from tracings in three heart cycles was calculated if the subject was in sinus rhythm during the recording. If the subject was in atrial fibrillation, tracings were done in the five most regular heart cycles, and the mean was calculated. The end-systolic volume was measured at the frame just before mitral valve opening, representing the maximum size (LAV_{max}). The end-diastolic volume was measured at the frame just before mitral valve closure, representing the minimum size (LAV_{min}). Indexing to body surface area, according to the Mosteller formula,[17] yielded $LAVi_{max}$ and $LAVi_{min}$. Left atrial emptying fraction (LAEF) was calculated as $((LAV_{max}-LAV_{min})/LAV_{max}) \times 100\%$.

Follow-up and outcome events

Outcome events data was gathered through a screening for atrial fibrillation substudy and with registry data. The screening substudy was performed in 2015, and participants of ACE 1950 with a CHA_2DS_2-VASc score ≥ 2 for men or ≥ 3 for women and without known atrial fibrillation were invited.[18] Screening was performed in a two-week ambulatory period. Participants were instructed to record a 1-lead ECG with a hand-held ECG device (Zenicor®) twice daily and whenever they experienced symptoms.

The ACE 1950 study received complete data from the Norwegian Patient Registry until December 31, 2020. The registry comprises data from all public hospitals as well as private institutions with a reimbursement agreement with the government. The data included any contact with the national special health services related to the I48 ICD-10 diagnosis, “Atrial fibrillation or atrial flutter”. All I48 diagnoses were validated by ECG in the patient’s

health records. If an ECG confirming atrial fibrillation was absent, otherwise convincing documentation was considered sufficient. The Cause of Death Registry provided data on death from any cause with follow-up until December 31, 2020.

Statistical analyses

Continuous data are presented as median (interquartile range) and categorical data as number (percentages). Differences between groups of individuals with no atrial fibrillation, prevalent atrial fibrillation or incident atrial fibrillation were assessed by Kruskal-Wallis test or Chi-squared test. Post-hoc pairwise comparisons were assessed by the Mann-Whitney U test, or the Chi-squared test, or Fisher's exact test, as appropriate. For the post-hoc comparisons, we used Bonferroni adjustment to account for multiplicity. Categorical data are presented with number and percentages, and were compared with the Chi-squared test. Correlations were assessed with the Spearman's rank correlation coefficient. Multivariable linear regression was used to assess determinants of echocardiographic left atrial indices at baseline.

The Cohorts for Heart and Aging Research in Genomic Epidemiology for Atrial Fibrillation (CHARGE-AF) risk score was calculated to quantify overall risk for atrial fibrillation. This risk score comprises the following differently weighted variables: age, race, height, weight, systolic and diastolic blood pressure, smoking status, antihypertensive treatment, diabetes, heart failure, and myocardial infarction.[19] The CHARGE-AF risk score has been shown to perform better than the CHA_2DS_2-VASc risk score at predicting incident atrial fibrillation.[20] There were 28 individuals with missing values for calculation of CHARGE-AF, and they were excluded from the adjusted analyses. The associations of cardiac biomarkers and echocardiographic left atrial volumetric indices with prevalent atrial fibrillation at baseline were assessed with logistic regression. In the assessment of prognosis for incident atrial fibrillation, subjects with prevalent atrial fibrillation at baseline were excluded. Kaplan-Meier plots were used to visualize the unadjusted risk of incident atrial

fibrillation across quartiles of GDF-15, cTnT, and NT-proBNP concentrations, $LAVi_{max}$, $LAVi_{min}$, and LAEF. The quartiles of LAEF were ordered by decreasing value so that the first quartile represents the highest value. The other variables were ordered in quartiles by increasing value. Comparisons were done with the log-rank test. Cox proportional hazards regression was used to model the risk of incident atrial fibrillation associated with cardiac biomarkers and left atrial volumetric indices as continuous variables. Due to right skewed distributions, cardiac biomarkers were transformed with the natural logarithm (ln) before use in all regression analyses. The hazard ratios are reported per standard deviation increase of the variable of interest, except for LAEF that are reported per standard deviation decrease. The regression models were adjusted for the CHARGE-AF risk score and sex. In the Cox regression analyses, time-to-event was the time from the baseline examination until first atrial fibrillation diagnosis, either in the screening substudy or clinically detected and provided from registry data. Individuals who did not develop atrial fibrillation contributed with time until end of follow-up (December 31, 2020) or until death from any cause. For the prognostic models, we performed additional sensitivity analyses by further adjusting for (1) left ventricular ejection fraction and left ventricular end-diastolic volume or (2) moderate to severe mitral valve regurgitation. C-statistics, net reclassification improvement, and integrated discrimination improvement were assessed to evaluate the incremental prognostic value of cardiac biomarkers to echocardiographic left atrial volumetric indices. A statistical significance level of 0.05 was chosen. Data were analysed with Stata 16 (StataCorp LP, College Station, TX).

Ethics

All participants in the ACE 1950 Study gave their informed consent. The Regional Committee for Medical Health Research Ethics in Norway approved the study (ref: 2011/1475). The study conforms to the ethical guidelines of the Helsinki Declaration.

RESULTS

Baseline characteristics

Out of 5827 eligible individuals, 3706 (64%) were included in the ACE 1950 Study baseline examinations. The rest did not respond or refused to participate. In the current analysis, 3 individuals were excluded due to missing values of biomarkers, and 216 due to missing measurements of left atrial volumes (**Figure 1**). Baseline characteristics according to atrial fibrillation status are shown in **Table 1**.

There were more male participants in the groups with prevalent and incident atrial fibrillation. Subjects with prevalent, and incident atrial fibrillation had increased concentrations of GDF-15 compared with subjects with no atrial fibrillation. Concentrations of cTnT, and NT-proBNP, measurements of $LAVi_{max}$, $LAVi_{min}$, and CHARGE-AF were increased in the group with incident atrial fibrillation and further increased in the group with prevalent atrial fibrillation. LAEF was decreased in subjects with prevalent atrial fibrillation (**Table 1** and **Figure 2**). Determinants of echocardiographic left atrial indices are shown in **Supplementary Table 1.** Prevalent atrial fibrillation was the determinant that most strongly associated with echocardiography.

Table 1. Study population characteristics by atrial fibrillation group

	No atrial fibrillation	Prevalent atrial fibrillation	Incident atrial fibrillation	P			
				Overall test for variance	No AF vs prevalent AF	No AF vs incident AF	Prevalent AF vs incident AF
N	3207	157	123				
Age, years	63.9 (63.5-64.5)	64.0 (63.5-64.5)	63.8 (63.3-64.5)	0.28	0.42	0.19	0.10
Male sex, n (%)	1,586 (49.5%)	117 (74.5%)	82 (66.7%)	<0.001	<0.001	<0.001	0.15
Body mass index, kg/m^2	26.5 (24.1-29.3)	28.4 (24.7-31.6)	27.2 (24.5-30.0)	<0.001	<0.001	0.18	0.05
Current smoker, n (%)	466 (14.6%)	17 (10.8%)	26 (21.1%)	0.05	0.18	0.047*	0.018
Heart rate, beats/min	62 (56-69)	62 (53-72)	60 (54-67)	0.026*	0.92	0.006	0.11
Systolic blood pressure, mmHg	137 (125-149)	132 (121-151)	140 (127-150)	0.08	0.11	0.13	0.029
Diastolic blood pressure, mmHg	77 (70-83)	78 (71-87)	78 (72-86)	0.06	0.048*	0.15	0.80
Myocardial infarction, n (%)	128 (4.0%)	17 (10.8%)	2 (1.6%)	<0.001	<0.001	0.24	0.003
Coronary artery disease, n (%)	213 (6.6%)	28 (17.8%)	7 (5.7%)	<0.001	<0.001	0.68	0.002
Heart failure, n (%)	37 (1.2%)	14 (8.9%)	3 (2.4%)	<0.001	<0.001	0.18	0.025
Diabetes mellitus, n (%)	268 (8.4%)	17 (10.8%)	8 (6.5%)	0.41	0.28	0.46	0.21
Hypertension, n (%)	1,937 (60.4%)	132 (84.1%)	84 (68.3%)	<0.001	<0.001	0.08	0.002
Diuretics, n (%)	84 (2.6%)	12 (7.6%)	4 (3.3%)	0.003	0.001	0.57	0.13
β blockers, n (%)	358 (11.2%)	92 (58.6%)	14 (11.4%)	<0.001	<0.001	0.94	<0.001
Calcium antagonists, n (%)	246 (7.7%)	25 (15.9%)	13 (10.6%)	<0.001	<0.001	0.24	0.19
ACE-I/ARB, n (%)	829 (25.8%)	61 (38.9%)	36 (29.3%)	0.001	<0.001	0.40	0.09
Statins, n (%)	826 (25.8%)	61 (38.9%)	30 (24.4%)	0.001	<0.001	0.73	0.010
CRP ≥ 3 mg/L, n (%)	635 (19.9%)	43 (27.4%)	26 (21.1%)	0.07	0.022*	0.73	0.23
HbA1c, %	5.7 (5.5-5.9)	5.7 (5.5-6.0)	5.7 (5.4-6.0)	0.31	0.14	0.61	0.55
Total cholesterol, mg/dL	5.4 (4.7-6.2)	4.7 (4.0-5.8)	5.2 (4.7-5.9)	<0.001	<0.001	0.10	0.001
HDL cholesterol, mg/dL	1.5 (1.2-1.8)	1.3 (1.1-1.6)	1.5 (1.2-1.8)	<0.001	<0.001	0.51	0.002
Triglycerides, mg/dL	1.2 (0.8-1.6)	1.1 (0.9-1.7)	1.1 (0.8-1.5)	0.27	0.53	0.14	0.14

eGFR, mL/min/1.73m^2	85.2 (75.3-92.6)	81.2 (71.6-92.3)	84.4 (76.0-91.7)	0.09	0.033*	0.73	0.07
GDF-15, ng/L	790.0 (632.0-1019.0)	877.0 (707.0-1215.0)	846.0 (662.0-1107.0)	<0.001	<0.001	0.17	0.07
NT-proBNP, ng/L	52.0 (33.0-89.0)	161.0 (73.0-405.9)	76.0 (42.0-135.0)	<0.001	<0.001	<0.001	<0.001
cTnT, ng/L	6.0 (4.0-8.0)	8.0 (6.0-12.0)	7.0 (6.0-10.0)	<0.001	<0.001	<0.001	0.062
$LAVi_{max}$, mL/m^2	25.5 (21.4-30.3)	34.2 (28.4-42.7)	29.0 (24.5-33.6)	<0.001	<0.001	<0.001	<0.001
$LAVi_{min}$, mL/m^2	13.6 (10.8-16.7)	22.2 (16.4-30.5)	15.6 (11.9-20.4)	<0.001	<0.001	<0.001	<0.001
LAEF, %	46.4 (40.5-52.2)	34.6 (21.7-42.9)	44.5 (36.4-52.0)	<0.001	<0.001	0.018*	<0.001
CHARGE-AF, %	7.3 (5.6-9.7)	9.6 (7.8-13.9)	8.7 (6.7-12.4)	<0.001	<0.001	<0.001	0.05

Values are median (interquartile range) for continuous data and n (%) for categorical data. GDF-15, growth differentiation factor 15; NT-proBNP, N-terminal pro-B-type natriuretic peptide; cTnT, cardiac troponin T; $LAVi_{max}$, left atrial end-systolic volume indexed; $LAVi_{min}$, left atrial end-diastolic volume indexed; LAEF, left atrial emptying fraction; CHARGE-AF risk score indicates Cohorts for Heart and Aging Research in Genomic Epidemiology for Atrial Fibrillation and comprises the following weighted variables: age, race, height, weight, systolic and diastolic blood pressure, smoking status, antihypertensive treatment, diabetes, heart failure, and myocardial infarction.

* not significant after Bonferroni correction ($p < 0.05/3 = 0.017$)

Correlation between cardiac biomarkers and echocardiographic left atrial volumes

Correlations between cardiac biomarkers and left atrial volumetric indices are shown in **Supplementary Table 2**. GDF-15 correlated weakly with $LAVi_{max}$, but not with $LAVi_{min}$ or LAEF. There were no significant correlations when restricting the analyses to subjects with prevalent atrial fibrillation. NT-proBNP and cTnT correlated with $LAVi_{max}$ and $LAVi_{min}$, and inversely with LAEF. When restricting the analyses to subjects with prevalent atrial fibrillation, correlations between cardiac biomarkers and left atrial volumes were stronger.

Association of cardiac biomarkers and echocardiographic left atrial volumes with prevalent atrial fibrillation

In **Table 2**, the associations of cardiac biomarkers, and left atrial volumetric indices with prevalent atrial fibrillation are shown. All six variables were associated with prevalent atrial fibrillation, and only $_{ln}$GDF-15 was non-significant in the adjusted models.

Table 2. Association of cardiac biomarkers and echocardiographic left atrial volumes with prevalent atrial fibrillation

	Odds ratio (95% CI)		
	Unadjusted	Adjusted for CHARGE-AF	Adjusted for CHARGE-AF and sex
$_{ln}$GDF-15	1.36 (1.17 to 1.57)	1.17 (0.997 to 1.36)	1.16 (0.99 to 1.35)
$_{ln}$NT-proBNP	3.43 (2.92 to 4.04)	3.20 (2.72 to 3.78)	3.32 (2.81 to 3.93)
$_{ln}$cTnT	1.75 (1.51 to 2.04)	1.50 (1.27 to 1.76)	1.43 (1.20 to 1.70)
$LAVi_{max}$	2.48 (2.17 to 2.83)	2.34 (2.04 to 2.68)	2.30 (2.01 to 2.63)
$LAVi_{min}$	2.98 (2.59 to 3.43)	2.81 (2.44 to 3.24)	2.78 (2.41 to 3.20)
LAEF	3.61 (3.06 to 4.25)	3.39 (2.87 to 4.02)	3.41 (2.88 to 4.04)

Odds ratio per standard deviation increase of the variable of interest (except LAEF per standard deviation decrease); 95% CI, 95% confidence interval; $_{ln}$GDF-15, growth differentiation factor 15, transformed with the natural logarithm; $_{ln}$NT-proBNP, N-terminal pro-B-type natriuretic peptide, transformed with the natural logarithm; $_{ln}$cTnT, cardiac troponin T, transformed with the natural logarithm; LAVimax, left atrial end-systolic volume indexed; LAVimin, left atrial end-diastolic volume indexed; LAEF, left atrial emptying fraction; CHARGE-AF risk score comprises the following weighted variables: age, race, height, weight, systolic and diastolic blood pressure, smoking status, antihypertensive treatment, diabetes, heart failure, and myocardial infarction.

Prediction of incident atrial fibrillation with cardiac biomarkers and echocardiographic left atrial volumes

Subjects with prevalent atrial fibrillation at baseline (n=157) were excluded from the prognostic analyses (**Figure 1**), leaving a total of 3,330 subjects. During follow-up of median 6.3 (5.9-6.9) years, incident atrial fibrillation occurred in 123 (3.7%) individuals, of whom 13 were detected through screening, and 110 were detected clinically and gathered through national registry data. Censoring due to death from any cause occurred in 89 (2.7%) subjects. The unadjusted associations of incident atrial fibrillation across quartiles of $LAVi_{max}$, $LAVi_{min}$, LAEF, GDF-15, NT-proBNP, and cTnT are visualized in **Figure 3**. In unadjusted analyses, continuous $LAVi_{max}$, $LAVi_{min}$, LAEF, $_{ln}$NT-proBNP, and $_{ln}$cTnT were associated with increased risk of incident atrial fibrillation (**Table 3**). The associations remained statistically significant after adjustment for the CHARGE-AF risk score. After additional

adjustment for sex, the association with $_{ln}$cTnT was attenuated, but $_{ln}$NT-proBNP and left atrial volumes remained significantly associated with outcomes. In the sensitivity analyses, adjustment for left ventricular ejection fraction and left ventricular end-diastolic volume or moderate to severe mitral valve regurgitation did not significantly change the results of the prognostic models (**Supplementary Table 3)**.

Table 3. Risk of incident atrial fibrillation during follow-up

	Hazard ratio (95% CI)		
	Unadjusted	Adjusted for CHARGE-AF	Adjusted for CHARGE-AF and sex
$_{ln}$GDF-15	1.08 (0.91 to 1.29)	0.95 (0.79 to 1.14)	0.95 (0.79 to 1.14)
$_{ln}$NT-proBNP	1.55 (1.30 to 1.84)	1.50 (1.26 to 1.77)	1.57 (1.32 to 1.85)
$_{ln}$cTnT	1.36 (1.17 to 1.59)	1.20 (1.01 to 1.44)	1.16 (0.95 to 1.40)
$LAVi_{max}$	1.60 (1.39 to 1.84)	1.53 (1.32 to 1.77)	1.51 (1.30 to 1.75)
$LAVi_{min}$	1.60 (1.42 to 1.80)	1.54 (1.36 to 1.73)	1.52 (1.35 to 1.72)
LAEF	1.26 (1.06 to 1.50)	1.24 (1.04 to 1.47)	1.24 (1.04 to 1.48)

Hazard ratio per standard deviation increase of the variable of interest (except for LAEF per standard deviation decrease); 95% CI, 95% confidence interval; $_{ln}$GDF-15, growth differentiation factor 15, transformed with the natural logarithm; $_{ln}$NT-proBNP, N-terminal pro-B-type natriuretic peptide, transformed with the natural logarithm; $_{ln}$cTnT, cardiac troponin T, transformed with the natural logarithm; $LAVi_{max}$, left atrial end-systolic volume indexed; $LAVi_{min}$, left atrial end-diastolic volume indexed; LAEF, left atrial emptying fraction; CHARGE-AF risk score comprises the following weighted variables: age, race, height, weight, systolic and diastolic blood pressure, smoking status, antihypertensive treatment, diabetes, heart failure, and myocardial infarction.

The incremental prognostic value of cardiac biomarkers to echocardiographic left atrial volumes are shown in **Table 4**. The C statistics were highest and comparable for $LAVi_{max}$ or $LAVi_{min}$ (p for comparison = 0.43), and cardiac biomarkers did not improve C statistics for these echocardiographic indices. The C statistics for LAEF was lower (p compared to $LAVi_{max}$ = 0.022 and p compared to $LAVi_{min}$ = 0.004) and both NT-proBNP and

cTnT improved the C statistics for LAEF. With regard to Net Reclassification Improvement and Integrated Discrimination Improvement, concentrations of GDF-15 did not improve the prognostic models when added to echocardiography. On the contrary, concentrations of cTnT provided the strongest incremental prognostic information to $LAVi_{max}$, $LAVi_{min}$, and LAEF.

Table 4. Incremental value of cardiac biomarkers to echocardiographic left atrial volumes

	C statistics	Net Reclassification Improvement	Integrated Discrimination Improvement
$LAVi_{max}$	0.65 (0.60 to 0.69)	Reference	Reference
$LAVi_{max}$ + $_{ln}$GDF15	0.65 (0.60 to 0.70)	0.098 (-0.238 to 0.360)	-0.000 (-0.001 to 0.001)
$LAVi_{max}$ + $_{ln}$NT-proBNP	0.66 (0.61 to 0.71)	0.203 (0.001 to 0.363)	0.003 (-0.000 to 0.011)
$LAVi_{max}$ + $_{ln}$cTnT	0.67 (0.62 to 0.71)	0.246 (0.015 to 0.450)	0.002 (-0.000 to 0.006)
$LAVi_{min}$	0.63 (0.58 to 0.69)	Reference	Reference
$LAVi_{min}$ + $_{ln}$GDF15	0.64 (0.58 to 0.69)	0.036 (-0.265 to 0.339)	-0.000 (-0.001 to 0.002)
$LAVi_{min}$ + $_{ln}$NT-proBNP	0.66 (0.60 to 0.71)	0.193 (-0.002 to 0.382)	0.002 (-0.001 to 0.009)
$LAVi_{min}$ + $_{ln}$cTnT	0.66 (0.61 to 0.71)	0.255 (0.034 to 0.447)	0.002 (-0.000 to 0.006)
LAEF	0.58 (0.52 to 0.63)	Reference	Reference
LAEF + $_{ln}$GDF15	0.58 (0.52 to 0.63)	-0.094 (-0.259 to 0.331)	-0.000 (-0.001 to 0.002)
LAEF + $_{ln}$NT-proBNP	0.63 (0.57 to 0.68)*	0.346 (0.175 to 0.532)	0.007 (0.002 to 0.016)
LAEF + $_{ln}$cTnT	0.64 (0.58 to 0.69)**	0.375 (0.201 to 0.529)	0.004 (0.001 to 0.008)

$LAVi_{max}$, left atrial end-systolic volume indexed; $LAVi_{min}$, left atrial end-diastolic volume indexed; LAEF, left atrial emptying fraction; $_{ln}$GDF-15, growth differentiation factor 15, transformed with the natural logarithm; $_{ln}$NT-proBNP, N-terminal pro-B-type natriuretic peptide, transformed with the natural logarithm; $_{ln}$cTnT, cardiac troponin T, transformed with the natural logarithm. *p* compared to basic model: * <0.05, ** <0.01

DISCUSSION

In a large cohort from the general population, we demonstrate that left atrial volumetric indices are associated with prevalent atrial fibrillation and with increased risk of incident atrial fibrillation during follow-up of median 6.3 years (**Figure 4**). The established cardiac biomarkers cTnT and NT-proBNP, reflective of subclinical myocardial injury and myocardial stress, exhibited similar prognostic properties, and provided incremental prognostic

information to echocardiographic left atrial volumes. On the contrary, GDF-15, a more novel biomarker possibly reflecting underlying oxidative stress and inflammation, showed weaker associations with prevalent atrial fibrillation, and no prognostic value for incident atrial fibrillation.

The association with prevalent atrial fibrillation could partly be explained by the more pronounced comorbidities in this group, or by the impact of the atrial fibrillation itself on the myocardium. Our findings for GDF-15 are in line with the Framingham Heart Study, in which GDF-15 was not associated with increased risk of incident atrial fibrillation beyond clinical risk factors.[21] GDF-15 is a strong prognostic biomarker in individuals with established atrial fibrillation,[22, 23] but has limited value to predict incident atrial fibrillation. Most recently in the Atherosclerosis Risk in Communities (ARIC) Study, concentrations of GDF-15 were shown to independently associate with risk of incident atrial fibrillation.[24] In ARIC however, the subjects were included in 1993 to 1995, almost 20 years earlier than the study participants from the current investigation, and GDF-15 concentrations were analysed using an aptamer-based proteomics assay currently not available in clinical practise. Both analytical and temporal differences with the current investigations makes comparison with ARIC challenging, and the question of whether GDF-15 actually associates with risk of incident atrial fibrillation remains unresolved. Cardiac troponins, on the other hand, were independently associated with incident atrial fibrillation in the Framingham Heart Study. In our study, cTnT provided the strongest incremental prognostic value to left atrial volumes but was attenuated after adjustments for CHARGE-AF and sex. In the Framingham Heart Study, they investigated cardiac troponin I in contrast to our investigation of cTnT. Recently, we and other groups have reported distinct differences between cTnT and cardiac troponin I with regard to clinical determinants and prognosis,[25, 26] and these dissimilarities may partly explain the lack of independent association in our study. NT-proBNP was the only cardiac

biomarker associated with incident atrial fibrillation independently of CHARGE-AF and sex in our study. This finding is corroborated by seminal work by Wang et al demonstrating associations of natriuretic peptides with both mortality and incident atrial fibrillation.[27] We extend on these previous findings by demonstrating significant incremental prognostic value of NT-proBNP to both LAEF and $LAVi_{max}$.

The independent associations between all left atrial volumetric indices and incident atrial fibrillation are consistent with another study from the general population in Denmark.[10] This study had a smaller sample size with 1951 participants, but longer follow-up (median 11 years), and more outcome events (n = 184). They demonstrated superior risk classification for $LAVi_{min}$, in contrast to our study where both the C statistics and hazard ratios were more similar for $LAVi_{max}$ and $LAVi_{min}$, and both indices were superior to LAEF. The hypothesis of $LAVi_{min}$ being more clinically relevant is supported by an early invasive study in which the minimum left atrial volume was more strongly associated with left ventricular filling pressure than the maximum volume.[28] Later, it has been shown that NT-proBNP more strongly associates with $LAVi_{min}$ than $LAVi_{max}$.[29] We also found a higher correlation coefficient between NT-proBNP and $LAVi_{min}$ than $LAVi_{max}$. However, as previously shown in our material,[30] $LAVi_{min}$ has both higher intra- and interobserver variability than $LAVi_{max}$. Although this increased variability was not found in the study by Olsen et al,[10] the otherwise lack of normal values and less prospective data do not currently support the superior clinical usefulness of $LAVi_{min}$ compared to $LAVi_{max}$.

Strengths of our study includes the large sample size. Due to the coverage of the Norwegian Patient Registry, we can assume nearly complete follow-up for clinical events, and outcomes were validated in detail. Biobanking was performed in a standardised manner and all biomarkers were analysed at the same time, avoiding possible assay lot-to-lot variability. Participation bias may be present, but the participation rate at 64% is comparable

to other observational cohorts from the general population. There were some missing values for left atrial volumes and the CHARGE-AF risk score, but this was for a minor proportion of study subjects. Our study population is predominantly of Northern European descent with a high level of education, and the results are less generalisable for populations of other ethnic backgrounds or with different degree of education. However, the access to universal health care is uniformly distributed in Norway, diminishing the impacts of socioeconomic bias. The screening sub-study may have induced some bias in the outcome assessment as only those with risk factors for stroke were screened and these may be common risk factors for left atrial enlargement. However, only a small proportion of the outcomes were detected through screening, and it is likely that several would later have become clinically acknowledged.

CONCLUSION

In the general population, echocardiographic left atrial volumes associate with prevalent atrial fibrillation and with long-term risk of incident atrial fibrillation. The established cardiac biomarkers cTnT and NT-proBNP provide incremental prognostic information to echocardiographic volumetric assessment of the left atrium, whereas the novel cardiac biomarker GDF-15 does not.

CONTRIBUTORSHIP STATEMENT

Magnus Nakrem Lyngbakken and Peter Selmer Rønningen contributed equally to this paper. Conception and design – MNL, TB, KS, HR, AT, TO. Acquisition, analysis and interpretation of data – all authors. Drafting of the manuscript – MNL, PSR. Critical revision for important intellectual content – all authors. Final approval of the manuscript – all authors. Guarantors: MNL and PSR.

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COMPETING INTERESTS

T.O. has served on advisory boards for Abbott Diagnostics, Roche Diagnostics and Bayer, and has received research support from Abbott Diagnostics, Novartis, Roche Diagnostics, Singulex and SomaLogic via Akershus University Hospital, and speaker's or consulting honoraria from Roche Diagnostics, Siemes Healthineers and CardioNor. T.B. has received speaker fees from Bayer, Boehringer Ingelheim, BMS and Pfizer (non-related to the submitted work). All other authors have no competing interests.

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FIGURE LEGENDS

Figure 1. Flow chart of study population.

Figure 2. Cardiac biomarker and echocardiographic left atrial volumetric measurement values according to atrial fibrillation status. Data are presented as box (25^{th} percentile, median, 75^{th} percentile) and whisker (maximum of 1.5 × interquartile range from the nearer quartile). No atrial fibrillation (n = 3207), prevalent atrial fibrillation (n = 157), incident atrial fibrillation (n = 123).

Figure 3. Kaplan-Meier plot for incident atrial fibrillation. Subjects stratified according to quartiles of cardiac biomarkers and echocardiographic left atrial volumes.

Figure 4. Graphical abstract summarising the design and main findings of the study.