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RESEARCH ARTICLE

N-terminal pro-B-type natriuretic peptide and high-sensitivity troponin T hold diagnostic value in cardiac amyloidosis

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Aims Cardiac amyloidosis (CA) is associated with an elevation of natriuretic peptides and troponins, predicting outcome. Nevertheless, the diagnostic yield of these biomarkers has not been extensively investigated. This study aimed to evaluate the diagnostic performance for CA of N-terminal pro-B-type natriuretic peptide (NT-proBNP) and high-sensitivity troponin T (hs-TnT).

Methods and results Patients with suspected CA ($n = 1149$) underwent a diagnostic work-up in three centres in Italy, France ($n = 343$, derivation cohort), and United Kingdom ($n = 806$, validation cohort). Biomarker values with either 100% sensitivity or ≥95% specificity were selected as rule-out/rule-in cut-offs, respectively. In the derivation cohort, 227 patients (66%) had CA, and presented with higher NT-proBNP and hs-TnT. NT-proBNP 180 ng/L and hs-TnT 14 ng/L were selected as rule-out cut-offs, and hs-TnT 86 ng/L as rule-in cut-off. NT-proBNP <180 ng/L or hs-TnT <14 ng/L were found in 7% of patients, and ruled out CA without false negatives. In the validation cohort, 20% of patients (2% false negatives) had NT-proBNP <180 ng/L or hs-TnT <14 ng/L, and 10% showed both biomarkers below cut-offs (0.5% false negatives). These cut-offs refined CA prediction when added to echocardiographic scores in patients with a haematologic disease or an increased wall thickness. In the validation cohort, the 86 ng/L hs-TnT cut-off ruled in 20% of patients (2% false positives). NT-proBNP and hs-TnT cut-offs retained their rule-out and rule-in performance also in cohorts with CA prevalence of 20%, 10%, 5% and 1% derived from the original cohort through bootstrap analysis.

Conclusions Cardiac biomarkers can refine the diagnostic algorithm in patients with suspected CA. NT-proBNP <180 ng/L and hs-TnT <14 ng/L reliably exclude the diagnosis, both in the overall population and subgroups referred for either AL-CA or cardiac (pseudo)hypertrophy.

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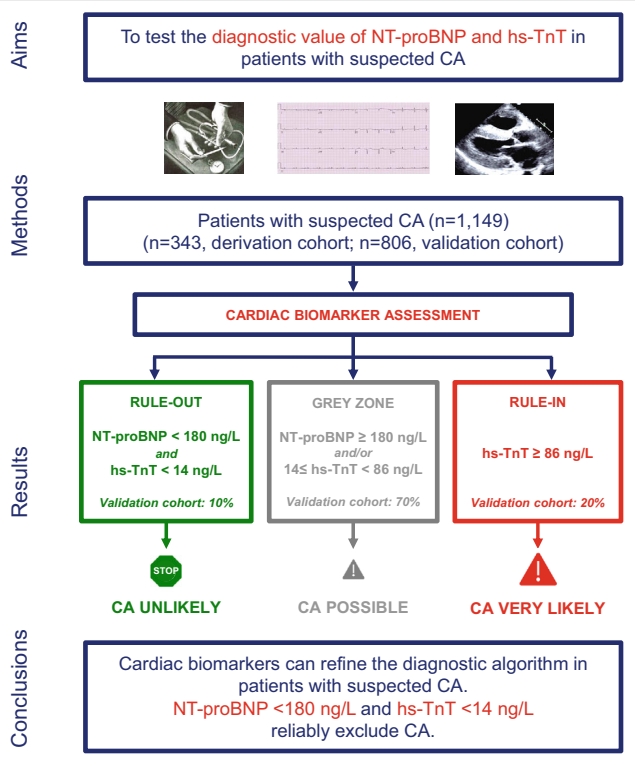
Graphical Abstract



Cardiac biomarkers hold diagnostic value in cardiac amyloidosis (CA). The diagnosis can be reliably excluded when N-terminal pro-B-type natriuretic peptide (NT-proBNP) is <180 ng/L and high-sensitivity troponin T (hs-TnT) is <14 ng/L.

Keywords Biomarkers • NT-proBNP • Troponin • Diagnosis • Cardiac amyloidosis

Introduction

Cardiac amyloidosis (CA) is caused by the accumulation of misfolded proteins into insoluble deposits in the extracellular space of the heart, leading to progressive myocardial damage and heart failure (HF). The two most common types of CA are amyloid light-chain (AL) and transthyretin (ATTR, either wild type [ATTRwt] or variant [ATTRv]) amyloidosis.[1] Median survival from diagnosis is about 2 years for AL-CA (6 months if untreated), 5 years for ATTRwt, and ranges between 1.5 and 6 years for ATTRv, depending on the specific mutation.[1] Early identification of CA is crucial to initiating disease-modifying and supportive therapies.[1,2] Typical signs of CA are left ventricular (LV) pseudohypertrophy, diastolic and then systolic dysfunction, rhythm and conduction disturbances.[1,2] The diagnosis of CA currently relies on a combination of clinical suspicion, electrocardiography, imaging techniques and, in selected cases, tissue biopsy, with cardiac biomarkers playing a minor role.[3] Specifically, the combination of end-diastolic interventricular septal thickness (IVSd) >12 mm in the absence of other causes and N-terminal pro-B-type natriuretic peptide (NT-proBNP) >332 ng/L (in the absence of end-stage renal disease or atrial fibrillation [AF]) has been proposed as a diagnostic criterion for cardiac involvement in systemic AL amyloidosis.[4,5] This approach is very sensitive but poorly specific, casting some doubt on the current recommended use of NT-proBNP to confirm an AL-CA diagnosis.[4,5] No data are available on the diagnostic utility of cardiac biomarkers in ATTR-CA.

NT-proBNP is widely used in patients with acute dyspnoea to rule out HF[6] and high-sensitivity troponins are a cornerstone of the diagnostic algorithm for myocardial infarction.[7] These biomarker-based approaches are accurate and cost-efficient. Similarly, in CA, a biomarker-based approach has the potential to avoid unnecessary, costly, and potentially harmful diagnostic tests in subjects with a low probability of CA, or, conversely, to prompt the early referral to second- and third-line diagnostic exams in selected patients with a high disease probability.

The aim of the study was to evaluate in a large multicentre cohort of patients referred for suspected CA the diagnostic

performance of NT-proBNP and high-sensitivity troponin T (hs-TnT) for CA, and to identify rule-out and rule-in diagnostic cut-offs.

Methods

Study population

The study included 1149 consecutive patients undergoing a diagnostic work-up for suspected CA from 2009 to 2021 at three tertiary referral centres: National Amyloidosis Centre, London, UK (n = 806, 70%); Fondazione Toscana Gabriele Monasterio, Pisa, Italy (n = 252, 22%); and Hôpital Lariboisière, Paris, France (n = 91, 8%). All patients had clinical, electrocardiographic and/or echocardiographic features deemed compatible with CA and underwent a blood sampling for NT-proBNP and hs-TnT. Details on echocardiographic and laboratory exams[8–11] are provided in online supplementary *Appendix S1*. The patient population partially overlapped with that reported in previous studies.[12,13] Patients were divided into a derivation cohort from Italy and France (n = 343, 30%), and a validation cohort from the UK (n = 806, 70%). The diagnosis of CA was ultimately established as detailed below.

Patients from the derivation and validation cohorts were further categorized into two subgroups based on the reason for referral (beyond the clues to CA): (1) 'suspected AL-CA subgroup' (n = 139, 40%, in the derivation cohort; n = 457, 57%, in the validation cohort) including patients with systemic AL amyloidosis, multiple myeloma or smoldering myeloma; (2) 'increased wall thickness (IWT) subgroup' (n = 309, 90%, in the derivation cohort; n = 627, 78%, in the validation cohort) including patients with increased LV wall thickness (IVSd or end-diastolic posterior wall thickness [PWTd] ≥12 mm).

The diagnostic value of NT-proBNP and hs-TnT was also compared to previously proposed echocardiographic scores for the diagnosis of CA: AL score (two points for relative wall thickness [RWT] [2*PWTd/LV end-diastolic diameter] and E/e′ ratio, one point for tricuspid annular plane systolic excursion [TAPSE] and global longitudinal strain [GLS]), and IWT score (three points for RWT and systolic septal longitudinal apex-to-base strain ratio, two points for TAPSE, and one point for E/e′ ratio and GLS).[12] To this aim, only patients with all the variables needed to calculate the AL and IWT scores were examined (n = 352 and n = 575, respectively).

The study protocol conformed to the Declaration of Helsinki and received ethics approval at each participating centre. All patients gave written informed consent.

Diagnosis of cardiac amyloidosis

AL-CA was defined as follows: (1) a combination of typical features on cardiac magnetic resonance (CMR) and histologically proven systemic AL amyloidosis with a non-cardiac biopsy; or (2) an endomyocardial biopsy positive for AL amyloid infiltrate.[12] ATTR-CA was diagnosed in the presence of: (1) an endomyocardial biopsy containing ATTR amyloid; or (2) a combination of typical features on CMR and a histologically proven ATTR amyloidosis with a non-cardiac biopsy; or (3) a grade 2 or 3 cardiac uptake on diphosphonate scintigraphy in the absence of monoclonal gammopathy, after the introduction of the algorithm for the non-invasive diagnosis of ATTR-CA.[14] Histological demonstration of AL or ATTR amyloid deposits required the documentation of Congo Red staining and apple-green birefringence under cross-polarized plus positive immunostaining with anti-light-chain or anti-transthyretin antibodies.[1,3]

Statistical analysis

Statistical analysis was performed using SPSS Statistics version 22, 2013 edition (IBM, Armonk, NY, USA) and R statistical software (http://www.r-project.org, version 3.4.4). Normal distribution was assessed by plotting a histogram and running the Kolmogorov–Smirnov test; variables with normal distribution were presented as mean ± standard deviation, while those with non-normal distribution as median and interquartile range. Comparisons between groups were made through unpaired Student's t-test or Mann–Whitney U test, as appropriate. Categorical variables were compared by the Chi-square test with Yates correction. Area under the curve (AUC) values at receiver operating characteristic (ROC) curve analysis were measured to evaluate the performance of NT-proBNP, hs-TnT and their combination to identify CA. The added prognostic value of biomarkers was assessed through the DeLong's test for correlated ROCs, the net reclassification improvement and the integrated discrimination index. The Hosmer–Lemeshow test was used to calculate the χ^2 value as an index of calibration. P-values <0.05 were considered statistically significant.

Cut-off derivation and validation

Optimal rule-out and rule-in cut-offs were selected in the derivation cohort considering the tradeoff between sensitivity and specificity. Specifically, the NT-proBNP and hs-TnT rule-out cut-offs represented the values with the highest specificity among those with a 100% sensitivity, while the rule-in cut-offs consisted of the biomarker values with the highest sensitivity among those with a ≥95% specificity. The diagnostic performance of rule-out and rule-in cut-offs was evaluated also in terms of negative and positive predictive value (NPV/PPV), negative and positive likelihood ratios (LR−/+). The performance of rule-out and rule-in cut-offs was then assessed in the validation cohort and its subgroups. To further validate the selected biomarker cut-offs, internal validation was performed by bootstrapping. In brief, we randomly sampled patients with and without CA from the validation cohort and generated new datasets of equal size to the original population. Patients were randomly selected with replacement and the ratio of patients with to patients without CA was pre-specified to generate disease prevalence thresholds (1%, 5%, 10%, and 20%). The loop was repeated 1000 times for each pre-specified prevalence of CA. For each loop, the sensitivity, specificity, PPV, NPV of each rule-out and rule-in cut-off were calculated. Results were averaged over the 1000 replicates and presented as median values.

Results

Patient characteristics

Patient characteristics in the derivation cohort are reported in *Table 1*. In the derivation cohort, CA was diagnosed in 227 patients (66%), of whom 102 had AL-CA (45%) and 125 ATTR-CA (55%; ATTRwt, n = 122, 98%; ATTRv, n = 3, 2%), all with the I68L *TTR* mutation. CA was excluded in 116 patients; the alternative diagnoses are reported in online supplementary *Table S1*. Patients with CA were more often male (73%), with similar age and estimated glomerular filtration rate (eGFR), higher New York Heart Association (NYHA) class and lower body mass index and comorbidities (except for AF) than patients without CA. At the time of characterization, 218 (64%) patients presented with clinical HF, prompting

Table 1 Characteristics of the derivation and validation cohorts

	Derivation cohort				Validation cohort			
	All (n = 343)	CA (n = 227)	No CA (n = 116)	p-value	All (n = 806)	CA (n = 495)	No CA (n = 311)	p-value
Men, n (%)	238 (69)	166 (73)	72 (62)	**0.036**	556 (69)	361 (73)	195 (63)	**0.002**
Age (years)	76 (69–83)	76 (69–83)	77 (71–82)	0.958	70 (62–78)	72 (63–79)	68 (60–74)	**<0.001**
BMI (kg/m^2)	26 (24–29)	25 (23–28)	27 (25–29)	**0.011**	27 (24–30)	26 (23–29)	28 (25–32)	**<0.001**
SAP (mmHg)	125 (110–140)	120 (105–130)	140 (125–150)	**<0.001**	124 (112–139)	120 (109–131)	133 (119–151)	**<0.001**
DAP (mmHg)	71 (65–80)	70 (60–80)	80 (70–83)	**<0.001**	75 (69–81)	73 (68–79)	77 (71–84)	**<0.001**
HF at diagnosis, n (%)	218 (64)	148 (65)	70 (60)	0.377	–	–	–	–
NYHA class I/II/III/IV, n (%)	64/156/109/14 (19/45/32/4)	31/108/79/9 (14/47/35/4)	33/48/30/5 (28/42/26/4)	**0.009**	–	–	–	–
Diuretic use, n (%)	196 (57)	137 (60)	59 (51)	0.093	–	–	–	–
Diuretic dose[a] (mg/kg)	0.3 (0.0–0.7)	0.3 (0.0–0.7)	0.0 (0.0–0.4)	**0.005**	–	–	–	–
Hypertension, n (%)	212 (62)	119 (52)	93 (80)	**<0.001**	–	–	–	–
Diabetes, n (%)	64 (19)	33 (15)	31 (27)	**0.006**	–	–	–	–
COPD, n (%)	41 (12)	21 (9)	20 (17)	**0.031**	–	–	–	–
Ischaemic heart disease[b], n (%)	65 (19)	36 (16)	29 (25)	**0.041**	–	–	–	–
Atrial fibrillation, n (%)	109 (32)	74 (33)	35 (30)	0.700	–	–	–	–
Laboratory data								
eGFR (ml/min/1.73 m^2)	53 (37–72)	56 (40–70)	49 (33–75)	0.525	68 (49–92)	59 (43–81)	67 (47–96)	**<0.001**
NT-proBNP (ng/L)	2813 (1174–7282)	3800 (1853–8532)	1319 (479–3455)	**<0.001**	1642 (420–4068)	2849 (1287–5804)	334 (123–1167)	**<0.001**
hs-TnT (ng/L)	49 (32–83)	63 (42–111)	32 (18–43)	**<0.001**	39 (21–72)	59 (36–94)	19 (10–32)	**<0.001**
Echocardiography								
IVSd (mm)	15 (13–18)	16 (13–19)	13 (12–15)	**<0.001**	14 (12–17)	16 (14–18)	12 (11–13)	**<0.001**
PWTd (mm)	13 (12–15)	14 (12–16)	12 (11–13)	**<0.001**	14 (12–17)	16 (14–17)	12 (10–13)	**<0.001**
LVEDD (mm)	46 (42–52)	45 (41–50)	48 (44–53)	**<0.001**	45 (41–50)	44 (40–48)	47 (43–52)	**<0.001**
LVEDVi (ml/m^2)	55 (45–69)	50 (43–64)	65 (52–77)	**<0.001**	38 (30–46)	38 (31–45)	37 (30–47)	0.956
LVESVi (ml/m^2)	25 (19–34)	24 (19–32)	25 (19–36)	0.401	17 (13–24)	18 (13–25)	15 (11–20)	**<0.001**
LVMi (g/m^2)	151 (121–178)	157 (127–187)	135 (109–159)	**<0.001**	133 (101–169)	154 (120–183)	106 (87–129)	**<0.001**
LVEF, n (%)	55 (46–61)	52 (45–60)	60 (52–65)	**<0.001**	57 (48–64)	54 (45–61)	63 (55–67)	**<0.001**
LVEF <40%/40–49%/≥50%, n (%)	47/57/239 (14/16/70)	37/49/141 (16/22/62)	10/8/98 (9/7/84)	**<0.001**	88/121/541 (12/16/72)	70/97/318 (14/20/64)	18/24/223 (7/9/84)	**<0.001**
E/e′ ratio	15 (12–20)	16 (14–21)	12 (9–15)	**<0.001**	10 (8–17)	14 (10–19)	8 (7–10)	**<0.001**
LA area (cm^2)	29 (24–33)	29 (24–33)	28 (23–32)	0.203	23 (18–28)	24 (21–29)	20 (16–23)	**<0.001**
TAPSE (mm)	17 (14–21)	16 (13–19)	20 (17–24)	**<0.001**	19 (14–23)	17 (13–21)	22 (20–26)	**<0.001**

BMI, body mass index; CA, cardiac amyloidosis; COPD, chronic obstructive pulmonary disease; DAP, diastolic arterial pressure; eGFR, estimated glomerular filtration rate; HF, heart failure; hs-TnT, high-sensitivity troponin T; IVSd, end-diastolic interventricular septum; LA, left atrial; LVEDD, left ventricular end-diastolic diameter; LVEDVi, left ventricular end-diastolic volume index; LVEF, left ventricular ejection fraction; LVESVi, left ventricular end-systolic volume index; LVMi, left ventricular mass index; NT-proBNP, N-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association; PWTd, end-diastolic posterior wall thickness; SAP, systolic arterial pressure; TAPSE, tricuspid annular plane systolic excursion.

[a]Diuretic dose: daily loop diuretic dose converted to furosemide equivalence normalized by body weight, with standard conversion factors of bumetanide 1 mg oral = torsemide 20 mg oral = furosemide 40 mg oral.

[b]Ischaemic heart disease: history of acute coronary syndrome, previous percutaneous coronary intervention or coronary artery bypass graft, or documentation of >50% stenosis in at least one epicardial vessel.

a diagnostic work-up to establish the underlying aetiology; the remaining patients were referred for other reasons (incidental finding of increased LV wall thickness, peripheral neuropathy, haematologic disease without HF, etc.). Prevalence of HF at diagnosis was similar in CA patients and controls. NT-proBNP (3800 ng/L [1853–8532] vs. 1319 [479–3455] $p < 0.001$) and hs-TnT (63 ng/L [42–111] vs. 32 [18–43], $p < 0.001$) were higher in patients with CA than in those without.

In the validation cohort, CA was diagnosed in 495 patients (61%; AL-CA, $n = 263$, 53%; ATTR-CA, $n = 232$, 47%; ATTRwt: $n = 167$, 72%; ATTRv: $n = 65$, 28%). Other nine patients had ATTRv amyloidosis without cardiac involvement (*Table 1* and online supplementary *Table S1*). Among the 74 patients with ATTRv amyloidosis, the majority had the T60A ($n = 19$, 26%) or the V122I ($n = 45$, 61%) TTR variant, while the remaining had other pathogenic mutations (V30M [$n = 3$], A56P, A120S, F44L, I107V, S70R, S77Y, V20I). CA patients were older, with lower body mass index and eGFR, and higher NT-proBNP (2849 ng/L [1287–5804] vs. 334 [123–1167], $p < 0.001$) and hs-TnT (59 ng/L [36–94] vs. 19 [10–32], $p < 0.001$) than those without CA.

A comparison between the derivation and validation cohorts is provided in online supplementary *Table S2*.

NT-proBNP and hs-TnT to diagnose cardiac amyloidosis: derivation cohort

Both NT-proBNP and hs-TnT displayed a good diagnostic performance for CA (AUC 0.721 and 0.810, respectively; *Figure 1*). The two biomarker combination (AUC 0.821) improved discrimination over NT-proBNP ($p = 0.002$), but not over hs-TnT ($p = 0.149$).

Rule-out cut-offs

A 180 ng/L NT-proBNP of showed a 100% sensitivity (100% NPV, LR− 1.13, true negatives = 13 [4%], false negatives = 0); hs-TnT of 14 ng/L also had a 100% sensitivity (100% NPV, LR− 0.00, true negatives = 13 [4%], false negatives = 0). Twenty-three patients without CA (7% of the entire cohort) had at least one biomarker value under these cut-offs and were ruled out; 1% of patients had both biomarkers below these cut-offs. Conversely, no patient with CA fell below these cut-off values (*Table 2* and *Figure 2*).

Rule-in cut-offs

A hs-TnT of 86 ng/L showed a 96% specificity (94% PPV, LR+ 7.97, true positives = 78 [23%], false positives = 5 [1%]), and was selected as a rule-in cut-off (*Table 2* and *Figure 2*). NT-proBNP was not enough specific for CA, hence no reliable rule-in cut-off could be established: a NT-proBNP value of 24 469 ng/L had a 96% specificity (PPV 67%, LR+ 1.02, true positives = 10 [3%], false positives = 5 [1%]).

NT-proBNP and hs-TnT to diagnose cardiac amyloidosis: validation cohort

In the validation cohort, AUC values for NT-proBNP and hs-TnT were 0.830 and 0.841, respectively, and 0.843 for their combination (*Figure 1*). The rule-out and rule-in cut-offs from the derivation cohort performed well in the validation cohort.

Rule-out cut-offs

The 180 ng/L NT-proBNP cut-off showed a 98% sensitivity (92% NPV, LR− 0.05, true negatives = 109 [14%], false negatives = 9

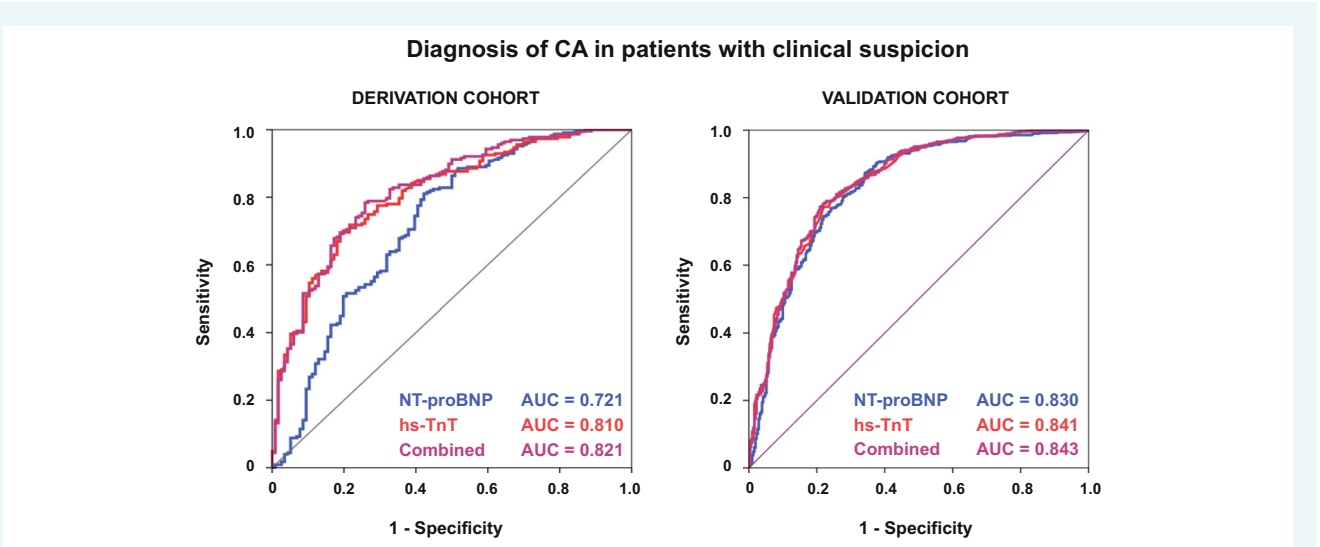


Figure 1 Diagnostic performance of N-terminal pro-B-type natriuretic peptide (NT-proBNP) and high-sensitivity troponin T (hs-TnT) for cardiac amyloidosis. NT-proBNP and hs-TnT had high areas under the curve (AUC) at the receiver operating characteristic curve analysis for the diagnosis of cardiac amyloidosis (CA) in both the derivation and the validation cohorts. In the derivation cohort, the combination of the two biomarkers improved discrimination over NT-proBNP, but not over hs-TnT.

Table 2 NT-proBNP and hs-TnT rule-out and rule-in cut-offs for cardiac amyloidosis in the derivation cohort

Rule-out cut-offs

Biomarker	Optimal cut-off (ng/L)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	LR+	LR−	TN, *n* (%)	FN, *n* (%)
NT-proBNP	180	100 (98–100)	11 (6–18)	69 (63–74)	100 (75–100)	1.13	0.00	13 (4)	0 (0)
hs-TnT	14	100 (98–100)	11 (6–18)	69 (63–74)	100 (75–100)	1.13	0.00	13 (4)	0 (0)
Either NT-proBNP <180 ng/L or hs-TnT <14 ng/L								23 (7)	0 (0)
Combined NT-proBNP <180 ng/L and hs-TnT <14 ng/L								3 (1)	0 (0)

Rule-in cut-off

Biomarker	Optimal cut-off (ng/L)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	LR+	LR−	TP, *n* (%)	FP, *n* (%)
hs-TnT	86	34 (28–41)	96 (90–99)	94 (87–98)	43 (37–49)	7.97	0.69	78 (23)	5 (1)

CI, confidence interval; FN, false negative; FP, false positive; hs-TnT, high-sensitivity troponin T; LR, likelihood ratio; NPV, negative predictive value; NT-proBNP, N-terminal pro-B-type natriuretic peptide; PPV, positive predictive value; TN, true negative; TP, true positive.

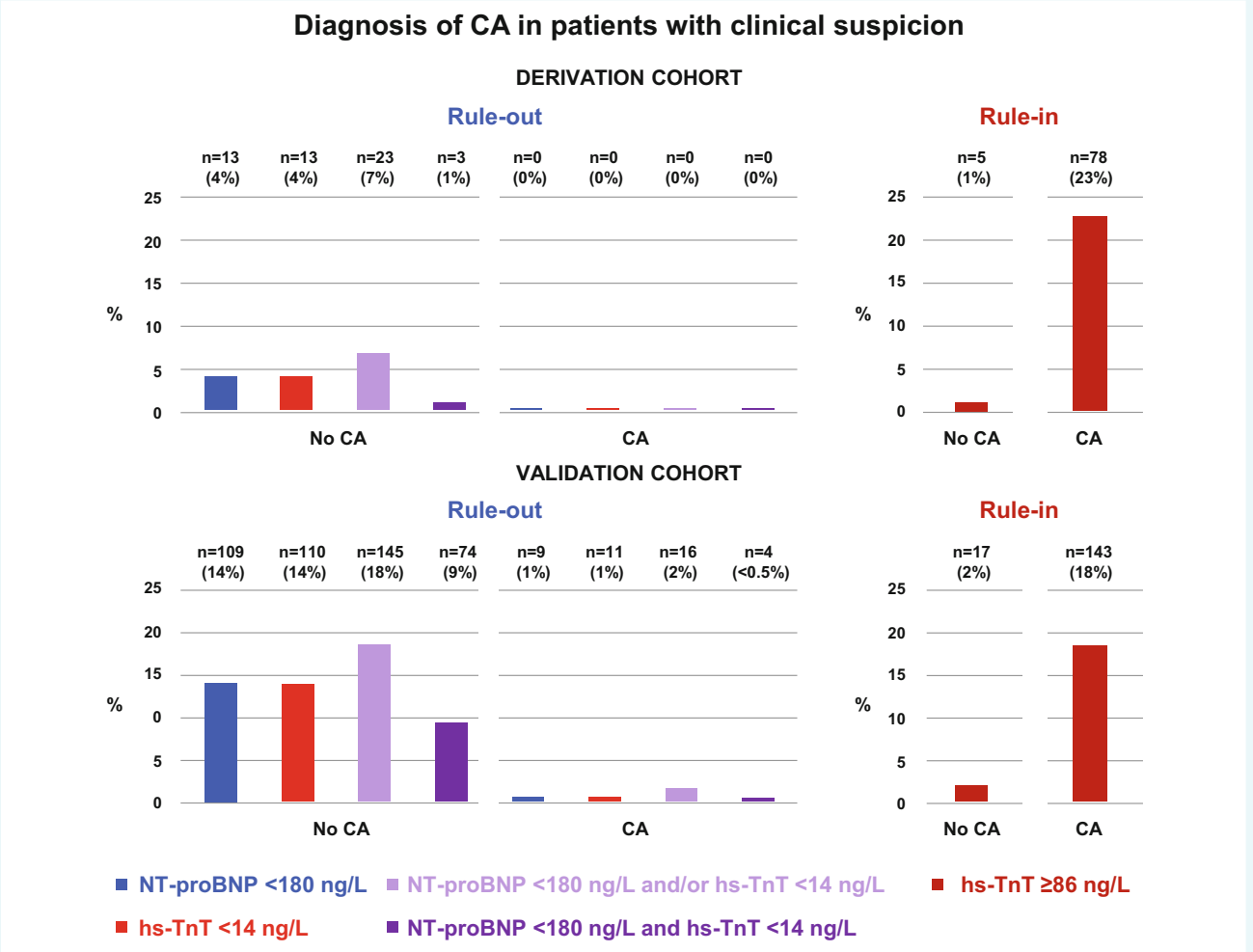


Figure 2 N-terminal pro-B-type natriuretic peptide (NT-proBNP) and high-sensitivity troponin T (hs-TnT) rule-out and rule-in cut-offs for cardiac amyloidosis. In the derivation cohort, a NT-proBNP value <180 ng/L and a hs-TnT value <14 ng/L emerged as effective rule-out cut-offs; a hs-TnT value ≥86 ng/L was optimal to rule in cardiac amyloidosis (CA). The rule-out and rule-in cut-offs identified in the derivation cohort performed well also in the validation cohort.

Table 3 NT-proBNP and hs-TnT rule-out and rule-in cut-offs for cardiac amyloidosis in the validation cohort

Biomarker	Optimal cut-off (ng/L)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	LR+	LR−	TN, *n* (%)	FN, *n* (%)
Validation cohort									
Rule-out cut-offs									
NT-proBNP	180	98 (97–99)	35 (30–41)	71 (67–74)	92 (86–96)	1.51	0.05	109 (14)	9 (1)
hs-TnT	14	98 (96–99)	35 (30–41)	71 (67–74)	91 (84–95)	1.51	0.06	110 (14)	11 (1)
Either NT-proBNP <180 ng/L or hs-TnT <14 ng/L								145 (18)	16 (2)
Combined NT-proBNP <180 ng/L and hs-TnT <14 ng/L								74 (9)	4 (0.5)
Rule-in cut-off									
hs-TnT	86	29 (25–33)	95 (91–97)	89 (84–94)	46 (42–49)	5.29	0.75	143 (18)	17 (2)
Suspected AL-CA subgroup									
Rule-out cut-offs									
NT-proBNP	180	97 (95–99)	41 (34–48)	69 (64–74)	92 (84–97)	1.64	0.07	79 (17)	7 (2)
hs-TnT	14	97 (94–98)	38 (31–45)	68 (63–72)	89 (80–95)	1.55	0.09	73 (16)	9 (2)
Either NT-proBNP <180 ng/L or hs-TnT <14 ng/L								98 (21)	13 (3)
Combined NT-proBNP <180 ng/L and hs-TnT <14 ng/L								54 (12)	3 (1)
Rule-in cut-off									
hs-TnT	86	38 (32–44)	96 (92–98)	93 (86–97)	53 (48–58)	9.13	0.65	99 (22)	8 (2)
IWT subgroup									
Rule-out cut-offs									
NT-proBNP	180	99 (97–100)	25 (19–33)	78 (75–82)	90 (77–97)	1.33	0.04	43 (7)	5 (1)
hs-TnT	14	98 (97–99)	25 (19–33)	78 (75–81)	86 (73–94)	1.32	0.06	43 (7)	7 (1)
Either NT-proBNP <180 ng/L or hs-TnT <14 ng/L								61 (10)	10 (2)
Combined NT-proBNP <180 ng/L and hs-TnT <14 ng/L								25 (4)	2 (<0.5)
Rule-in cut-off									
hs-TnT	86	29 (25–34)	92 (87–96)	91 (85–95)	33 (28–37)	3.83	0.76	135 (22)	13 (2)

AL-CA, light-chain cardiac amyloidosis; CI, confidence interval; FN, false negative; FP, false positive; hs-TnT, high-sensitivity troponin T; IWT, increased wall thickness; LR, likelihood ratio; NPV, negative predictive value; NT-proBNP, N-terminal pro-B-type natriuretic peptide; PPV, positive predictive value; TN, true negative; TP, true positive.

[1%]); similarly, a hs-TnT of 14 ng/L had a 98% sensitivity (91% NPV, LR− 0.06, true negatives = 110 [14%], false negatives = 11 [1%]). Twenty percent of patients (*n* = 161) had NT-proBNP or hs-TnT below cut-offs. Among the 16 false negatives (2%), 13 patients had systemic AL amyloidosis (12 of whom with initial signs of cardiac involvement at echocardiography and/or CMR), two were ATTRv cases with minimal cardiac involvement at echocardiography, and one was an octogenarian with ATTRwt-CA. Notably, 10% patients

had both biomarkers below the respective cut-offs (NT-proBNP <180 ng/L and hs-TnT <14 ng/L), and false negatives decreased to 4 (0.5%) (*Table 3* and *Figure 2*).

Rule-in cut-offs

A hs-TnT ≥86 ng/L ruled in 20% of patients (n = 160): 143 CA (18%) and 17 non-CA (2% false positives) (*Table 3* and *Figure 2*).

Cut-off performance in cohorts with different prevalence of the disease

The rule-out and rule-in cut-offs performed well even in cohorts with a lower prevalence of CA, namely 1%, 5%, 10% and 20%. Median values (with interquartile range) of sensitivity, specificity, PPV, and NPV of each rule-out and rule-in cut-off across the 1000 bootstrap samples for each CA prevalence cohort are reported in online supplementary *Table S4*. In the pooled data from all 1000 bootstrap samples, the 180 ng/L NT-proBNP cut-off had 100%, 98%, 99%, 98% median sensitivity in cohorts with CA prevalence of 1%, 5%, 10% and 20%, respectively; the 14 ng/L hs-TnT cut-off had 100%, 98%, 98%, and 98% median sensitivity in cohorts with CA prevalence of 1%, 5%, 10% and 20%, respectively; the 86 ng/L hs-TnT cut-off had 94%, 95%, 94%, and 95% median specificity in cohorts with CA prevalence of 1%, 5%, 10% and 20%, respectively.

NT-proBNP and hs-TnT to diagnose cardiac amyloidosis: subgroup analysis

In the validation cohort, 457 patients were included in the suspected AL-CA subgroup, and CA was diagnosed in 194 patients (42%). The IWT subgroup consisted of 627 patients, and CA was finally diagnosed in 458 (73%) (online supplementary *Table S5*).

Cut-off performance in the suspected AL-CA subgroup and the increased wall thickness subgroup

In the suspected AL-CA subgroup of the validation cohort, the rule-out (NT-proBNP <180 ng/L, hs-TnT <14 ng/L) and rule-in (hs-TnT ≥86 ng/L) cut-offs yielded similar results than in the whole validation cohort: 98 non-CA (21%) and 13 CA patients (3%) had at least one biomarker below the respective cut-off; notably, 54 patients without CA (12%) and only three CA (all with an established diagnosis of systemic AL amyloidosis and with signs of early CA at CMR) had both biomarkers below the respective cut-off (*Table 3* and online supplementary *Figure S1*).

In the IWT subgroup, either of the two biomarkers below the respective rule-out cut-off allowed to exclude 61 patients without CA (10%) and 10 CA patients (2%), while the combination of both below the respective cut-off allowed to rule out 25 (4%) non-CA and two CA patients (0.3%) (*Table 3* and online supplementary *Figure S2*).

Cut-off performance versus haematologic consensus criteria

We further compared the diagnostic performance of the hs-TnT rule-in cut-off and the haematologic consensus criteria based on the combination of NT-proBNP >332 ng/L and IVSd >12 mm (AUC 0.877 vs. 0.817, p = 0.013) in a subset of patients with suspected AL-CA and without AF or end-stage renal disease (n = 257). In this cohort, a hs-TnT ≥86 ng/L allowed to rule in 44 CA patients (17%), with only two false positives (specificity 98%), whereas the haematologic consensus criteria showed lower specificity (85%), since they ruled in a greater proportion of CA patients (n = 109, 42%) at the cost of a higher percentage of false positives (n = 17, 7%) (online supplementary *Table S6*).

Cut-off performance according to sex, renal function, atrial fibrillation, left ventricular ejection fraction, left ventricular hypertrophy

The performance of biomarker rule-out (NT-proBNP <180 ng/L, hs-TnT <14 ng/L) and rule-in (hs-TnT ≥86 ng/L) cut-offs was also tested in other subgroups of the validation cohort (online supplementary *Table S7*): men versus women; preserved (eGFR ≥60 ml/min/1.73 m^2) versus decreased (eGFR <60 ml/min/1.73 m^2, <45 ml/min/1.73 m^2, <30 ml/min/1.73 m^2) renal function; AF absent versus AF present; preserved (≥50%) versus reduced (<50%) LV ejection fraction; no/mild hypertrophy (both IVSd and PWTd ≤13 mm) versus moderate/severe hypertrophy (either IVSd or PWTd >13 mm). Rule-out and rule-in cut-offs retained a good performance in most subgroups, with few exceptions: rule-out cut-offs were less useful in patients with moderately-to-severely reduced renal function (<45 ml/min/1.73 m^2 or <30 ml/min/1.73 m^2) or with AF; conversely, the rule-in cut-off performed well also in these subgroups.

Referral-specific cut-offs

As a further analysis, we calculated referral-specific rule-out and rule-in cut-off values in the suspected AL-CA and IWT subgroups of the derivation cohort (online supplementary *Table S3*) and tested them in the corresponding subsets of the validation cohort. The rule-out (NT-proBNP <278 ng/L, hs-TnT <14 ng/L) and rule-in cut-offs (hs-TnT ≥102 ng/L) in the suspected AL-CA subgroup of the derivation cohort partly differed from those calculated in the whole cohort, although this did not affect the diagnostic performance. Conversely, similar cut-offs were observed in the IWT subgroups, with NT-proBNP <180 ng/L and hs-TnT <14 ng/L as rule-out cut-offs, and hs-TnT ≥79 ng/L as rule-in cut-off (online supplementary *Table S8*).

Amyloidosis subtype-specific cut-offs

Finally, we stratified our population based on the diagnostic suspect of either AL-CA or ATTR-CA at the time of enrolment: (1) the 'suspected AL-CA subgroup', which was the same as the one collected based on referral; (2) the 'suspected ATTR-CA subgroup' (n = 188, 55%, in the derivation cohort, n = 331, 41%, in the validation cohort) including patients with increased LV wall thickness (interventricular septal or posterior wall thickness ≥12 mm) in the absence of plasma cell disorders. Baseline characteristics of the two cohorts are presented in online supplementary *Tables S9* and *S10*. The performance of the proposed cut-off for the rule out

(NT-proBNP <180 ng/L, hs-TnT <14 ng/L) and the rule in (hs-TnT ≥86 ng/L) of CA was overall similar to that observed in the general population, although the number of ruled-in and ruled-out patients was slightly higher in the suspected AL-CA than in the suspected ATTR-CA subgroup (online supplementary *Table S11*). Biomarker rule-out and rule-in cut-off were also calculated separately in the two subgroups (suspected AL-CA and suspected ATTR-CA) from the derivation cohort and tested in the corresponding subsets of the validation cohort. Rule-out and rule-in cut-offs were similar to those from the whole cohort (for suspected AL-CA: rule-out NT-proBNP <278 ng/L and hs-TnT <14 ng/L, rule-in hs-TnT ≥102 ng/L; for suspected AL-CA: rule-out NT-proBNP <180 ng/L and hs-TnT <15 ng/L, rule-in hs-TnT ≥79 ng/L), and yielded similar diagnostic performance compared to the originally calculated cut-offs (online supplementary *Table S12*).

Biomarkers and echocardiographic scores

In patients with suspected AL-CA, the AUC of the echocardiographic AL score (0.841) was comparable to that of NT-proBNP (0.881, $p = 0.084$) and hs-TnT (0.867, $p = 0.276$), but lower than the combination of the two biomarkers (0.887, $p = 0.034$) (online supplementary *Figure S3*). The inclusion of NT-proBNP and hs-TnT, either alone or in combination, significantly improved discrimination and reclassification when added to the AL score ($p < 0.001$; *Table 4* and online supplementary *Figure S4*). An AL score of two had the best NPV (77%), which resulted in a similar rule-out performance compared to NT-proBNP <180 ng/L or hs-TnT <14 ng/L (online supplementary *Table S13*).

In the IWT subgroup, the IWT score showed a higher AUC than NT-proBNP (0.859 vs. 0.767, $p < 0.001$), but not different to hs-TnT (0.819, $p = 0.102$) or the combination of NT-proBNP and hs-TnT (0.817, $p = 0.090$) (online supplementary *Figure S3*). The addition of either hsTnT or the combination of two biomarkers improved both reclassification and discrimination over IWT alone (*Table 4* and online supplementary *Figure S5*). An IWT score of two had the best NPV (77%), allowing to rule out a similar proportion of non-CA patients compared to the combination of either NT-proBNP <180 ng/L or hs-TnT <14 ng/L (online supplementary *Table S13*).

Discussion

The diagnostic value of NT-proBNP and hs-TnT in CA was evaluated for the first time in a large, multicentre study, allowing to

Table 4 Performance metrics of the combination of echocardiographic scores and biomarkers

Index	Model 1: AL score	Model 2: AL score + NT-proBNP	Model 3: AL score + hs-TnT	Model 4: AL score + NT-proBNP + hs-TnT
Discrimination				
AUC	0.841 (95% CI 0.800–0.883) [Reference]	0.893 (95% CI 0.858–0.928), $p < 0.001$	0.891 (95% CI 0.855–0.926), $p < 0.001$	0.900 (95% CI 0.867–0.933), $p < 0.001$
Calibration				
HL	χ^2 1.99, $p = 0.981$	χ^2 71.19, $p < 0.001$	χ^2 17.00, $p = 0.030$	χ^2 71.37, $p < 0.001$
Reclassification				
IDI	Reference	0.098 (95% CI 0.070–0.126), $p < 0.001$	0.102 (95% CI 0.073–0.131), $p < 0.001$	0.117 (95% CI 0.086–0.148), $p < 0.001$
NRI	Reference	0.909 (95% CI 0.728–1.09), $p < 0.001$	0.849 (95% CI 0.667–1.032), $p < 0.001$	1.028 (95% CI 0.856–1.200), $p < 0.001$
Index	**Model 1: IWT score**	**Model 2: IWT score + NT-proBNP**	**Model 3: IWT score + hs-TnT**	**Model 4: IWT score + NT-proBNP + hs-TnT**
Discrimination				
AUC	0.859 (95% CI 0.828–0.890) [Reference]	0.868 (95% CI 0.838–0.899), $p = 0.139$	0.892 (95% CI 0.864–0.920), $p = 0.001$	0.893 (95% CI 0.865–0.921), $p = 0.001$
Calibration				
HL	χ^2 5.80, $p = 0.670$	χ^2 11.08, $p = 0.197$	χ^2 17.81, $p = 0.023$	χ^2 14.20, $p = 0.077$
Reclassification				
IDI	Reference	0.019 (95% CI 0.008–0.029), $p < 0.001$	0.075 (95% CI 0.053–0.097), $p < 0.001$	0.076 (95% CI 0.053–0.098), $p < 0.001$
NRI	Reference	0.458 (95% CI, 0.316–0.599), $p < 0.001$	0.731 (95% CI 0.580–0.883), $p < 0.001$	0.728 (95% CI 0.575–0.881), $p < 0.001$

AL, amyloid light-chain; AUC, area under the curve; CI, confidence interval; HL, Hosmer–Lemeshow; hs-TnT, high-sensitivity troponin T; IDI, integrated discrimination improvement; IWT, increased wall thickness; NRI, net reclassification improvement; NT-proBNP, N-terminal fraction of pro-B-type natriuretic peptide.

identify rule-out (NT-proBNP <180 ng/L, hs-TnT <14 ng/L) and rule-in (hs-TnT ≥86 ng/L) cut-offs for CA.

Early and accurate identification of CA is crucial but challenging.[2] The current diagnostic flow charts for CA[1,3,15] include a time-consuming, multi-step, multi-modal approach involving expensive or invasive techniques (including endomyocardial biopsy), some of which are available only in a few experienced centres.[1] Reliable and readily available diagnostic tools to discard or strengthen the suspicion of CA are therefore needed.

Circulating biomarkers are low-cost, quantitative parameters largely studied as tools for screening, diagnosis, risk stratification, and guide to treatment in HF and cardiomyopathies.[16] Natriuretic peptides and troponins increase in CA due to volume overload, cardiac remodelling following amyloid infiltration, and direct cytotoxicity of amyloid precursors.[17] Natriuretic peptides and troponins have been incorporated into several risk stratification scores such as the Mayo staging systems for AL-CA or the National Amyloidosis Centre staging system for ATTR-CA.[17] In AL-CA, natriuretic peptides are also used to guide treatment choices and monitor the response to therapy.[17] While the elevation of cardiac biomarkers is currently considered a 'red flag' for the suspicion of CA,[2] they are not currently considered a mandatory first-step diagnostic tool, with the sole exception of NT-proBNP in patients with systemic AL amyloidosis.[4,5]

Previous studies have investigated the role of NT-proBNP to detect cardiac involvement in patients with either systemic AL amyloidosis or a plasma cell dyscrasia, with an AUC ranging from 0.85 to 0.97,[12,18–20] which is in line with the AUC (0.86) in patients with suspected AL-CA in our validation cohort. Current haematologic consensus criteria indicate an IVSd >12 mm and a NT-proBNP >332 ng/L for the diagnosis of cardiac involvement in AL amyloidosis.[4,5] Nevertheless, the 332 ng/L threshold was validated mainly for prognostic purposes, and it has been reported to lack specificity for AL-CA, as further demonstrated in our analysis.[20] In the present study, given the high percentage of non-CA patients with elevated NT-proBNP, we could not identify a reliable rule-in cut-off for CA, suggesting that NT-proBNP is not specific enough to detect AL-CA. The diagnostic value of NT-proBNP has been less studied in ATTR amyloidosis or in unselected cohorts of patients with clinical suspicion of CA. In a small study involving 36 patients with familial amyloid polyneuropathy and no cardiac symptoms, NT-proBNP had a 0.92 AUC for echocardiography-detected cardiac involvement, and an 82 ng/L value was proposed as a diagnostic cut-off (92% sensitivity, 90% specificity).[21] In another study including 978 patients with suspected CA and LV hypertrophy, NT-proBNP showed a 0.74 AUC for CA.[12]

Most studies on biomarkers in amyloidosis have employed conventional troponin assays, and there are a few data on high-sensitivity assays.[17] Nicol *et al.*[20] reported a fair diagnostic performance of hs-TnT (AUC 0.87, best cut-off 35 ng/L, 84% sensitivity, 87% specificity) for the identification of cardiac involvement in systemic AL amyloidosis, and included hs-TnT in a diagnostic score (together with GLS and the ratio of the apical longitudinal/sum of base and mid-longitudinal strain), outperforming the haematologic consensus criteria based on IVSd >12 mm and NT-proBNP >332 ng/L. Takashio *et al.*[22] enrolled 187 subjects with increased LV wall thickness (51% of whom with biopsy-proven CA, either AL or ATTR), reporting an AUC of 0.79 for hs-TnT for the identification of CA, with a cut-off value of 31 ng/L showing 74% sensitivity and 76% specificity.

In the present study, a NT-proBNP of 180 ng/L and a hs-TnT of 14 ng/L emerged as optimal rule-out cut-offs, whereas a hs-TnT value of 86 ng/L resulted as a good rule-in cut-off. Seven percent of patients (all without CA) had at least one biomarker below the respective rule-out cut-off, while 1% had both biomarkers below the respective rule-out cut-off; the hs-TnT rule-in cut-off correctly identified CA in 23% of cases, with only 1% of false positives. Hence, a biomarker-based approach allowed to exclude or confirm the diagnosis of CA in about one quarter of patients undergoing a diagnostic screening for the disease. The biomarker-based cut-offs performed well also in the validation cohort. A NT-proBNP <180 ng/L or a hs-TnT <14 ng/L alone ruled out 14% of non-CA patients, with just 1% of false negatives. Eighteen percent of non-CA cases and 2% of CA had at least one biomarker below the respective cut-off. By using a more stringent criterion for the combination of the two biomarkers (i.e. both below the respective cut-off), fewer non-CA patients were ruled out (9%), but the percentage of false negatives was reduced drastically (0.5%), for a total of around 10% of cases being ruled out. Notably, all false negatives were patients with high clinical suspicion of the disease (mostly subjects with systemic AL amyloidosis or ATTRv amyloidosis with minimal cardiac involvement detected by CMR), deserving close clinical and instrumental follow-up anyway. In the validation cohort, a hs-TnT ≥86 ng/L ruled in 20% of cases, namely 18% of CA patients and just 2% of non-CA subjects. Again, in the validation cohort, NT-proBNP and hs-TnT were confirmed to reliably exclude or establish the diagnosis of CA in nearly one third of patients (*Graphical Abstract*).

In clinical practice, the two most common reasons for patient referral for the suspicion of CA are the presence of a plasma cell dyscrasia suggesting possible underlying AL-CA, or unexplained LV hypertrophy. The diagnostic performance of NT-proBNP and hs-TnT cut-offs (NT-proBNP <180 ng/L, hs-TnT <14 ng/L, hs-TnT ≥86 ng/L) was fair in both referral subgroups. We also calculated referral-specific rule-out and rule-in values in the suspected AL-CA and IWT subgroups. By using this approach, biomarker cut-offs slightly differed from those derived from the whole cohort, but their rule-out and rule-in performance did not change significantly.

Additionally, the diagnostic performance of the proposed rule-in and rule-out cut-offs was similar in subgroups selected based on the initial clinical suspicion of either AL-CA or ATTR-CA, although none of them can be usually excluded a priori in clinical practice.

Echocardiography is a gatekeeper in patients referred for clinical suspicion of CA. A few studies have investigated the possibility of using echocardiography-based scores to guide the referral to second-level diagnostic tests.[12,13] Boldrini *et al.*[12] introduced the AL score and the IWT score to support the diagnosis of CA among subjects with either systemic AL amyloidosis or unexplained LV hypertrophy, respectively. These approaches, though effective, require reading by experienced operators and deformation analysis, limiting their applicability in everyday clinical practice.

Herein, we have shown that the diagnostic yield of NT-proBNP and hs-TnT, as assessed by ROC analysis, is similar to echocardiographic scores. Moreover, cardiac biomarkers improved metrics of discrimination and reclassification for the diagnosis when added to either the AL score in the subgroup with suspected AL-CA, or the IWT score in the subgroup with LV hypertrophy. Biomarkers may therefore prove as simple, cost-effective, and widely available tools to select populations with the lowest and highest probability of CA, thus warranting different diagnostic approaches. A biomarker-integrated diagnostic approach is not intended to replace guideline-recommended diagnostic algorithms for CA, but rather to improve them (online supplementary *Figure S6*). Indeed, in the real-world setting, the ruling-out of CA in 10% of patients with suspected disease by the use of two biomarkers (i.e. with NT-proBNP <180 ng/L and hs-TnT <14 ng/L) may avoid useless non-invasive or, in selected cases, invasive examination, and potentially guide the physician toward alternative diagnosis. The global awareness about CA is expected to increase further in the next years, and so will the overall number of patients with expected disease. The availability of simple, widely available, and objective tools to exclude the diagnosis of CA may have a significant clinical impact, especially in non-tertiary centres, where the availability of experienced personnel and of advanced imaging tools may be limited.

Study limitations

This is a study conducted in three tertiary centres for CA, therefore a referral bias leading to a higher prevalence of CA among subjects with a suspected disease cannot be excluded. Nonetheless, the percentage of CA in our cohort is in line with that reported in similar studies.[22] Some differences could be observed between the derivation and validation cohorts, since the validation cohort included more patients with less advanced disease stage and/or milder phenotypes in both CA and non-CA subgroups, as well as more patients with ATTRv or non-AL non-ATTR amyloidosis subtypes. Furthermore, most patients in the suspected AL-CA subgroup in the UK had systemic AL amyloidosis, as opposed to a minority of patients from Italy and France. These differences reflect heterogeneous referral policies among centres but increase the robustness of the results by showing that their validity is maintained even in cohorts with slightly different characteristics.

Biomarker diagnostic performance can be influenced by the disease prevalence in the population in which they are tested. To obviate this, rule-out and rule-in cut-offs were chosen based on the optimal tradeoff between sensitivity and specificity, which should not be affected by disease prevalence. Furthermore, we also performed bootstrap validation in hypothetical cohorts with CA prevalence as low as 20%, 10%, 5% and 1%, showing that the selected cut-offs for NT-proBNP and hs-TnT retained their rule-out and rule-in performance. These results increase the reliability of our results and their translatability to clinical settings outside that of tertiary referral centres, where CA prevalence is inevitably expected to be higher.

Notably, we provide a detailed clinical, biohumoral and echocardiographic characterization of patients with suspected CA in this study, which also allowed us to perform several subgroup analyses to further confirm our results. Unfortunately, we were unable to retrieve complete data on some comorbidities, HF history, NYHA class and diuretic use in the validation cohort, which nonetheless are not deemed instrumental for the generalizability of our results.

We demonstrate that NT-proBNP and hs-TnT rule-out and rule-in cut-offs are still effective in several subgroups, although the rule-out cut-offs are less performant in smaller populations of patients with moderately-to-severely reduced renal function (<45 ml/min/1.73 m^2 or <30 ml/min/1.73 m^2) or with AF at the time of diagnosis.

We propose specific hs-TnT cut-offs for the diagnosis of CA, however, we acknowledge that different hospitals adopt various high-sensitivity troponin assays, whose values are not directly correlated.[23] Therefore, future studies should be dedicated to validating the diagnostic performance of other high-sensitivity troponins in CA.

Conclusions

Cardiac biomarkers hold relevant diagnostic value in CA and might be used early in the patient work-up to inform clinical decision making. The diagnosis can be reliably excluded when NT-proBNP is <180 ng/L and hs-TnT <14 ng/L, both in the whole population and in subgroups with suspected AL-CA or LV hypertrophy.

Supplementary Information

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

Conflict of interest: none declared.

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