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RESEARCH ARTICLE

Cardiac myosin-binding protein C in the diagnosis and risk stratification of acute heart failure

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Aims Cardiac myosin-binding protein C (cMyC) seems to be even more sensitive in the quantification of cardiomyocyte injury vs. high-sensitivity cardiac troponin, and may therefore have diagnostic and prognostic utility.

Methods and results In a prospective multicentre diagnostic study, cMyC, high-sensitivity cardiac troponin T (hs-cTnT), and N-terminal pro-B-type natriuretic peptide (NT-proBNP) plasma concentrations were measured in blinded fashion in patients presenting to the emergency department with acute dyspnoea. Two independent cardiologists centrally adjudicated the final diagnosis. Diagnostic accuracy for acute heart failure (AHF) was quantified by the area under the receiver operating characteristic curve (AUC). All-cause mortality within 360 days was the prognostic endpoint. Among 1083 patients eligible for diagnostic analysis, 51% had AHF. cMyC concentrations at presentation were higher among AHF patients vs. patients with other final diagnoses [72 (interquartile range, IQR 39–156) vs. 22 ng/L (IQR 12–42), $P < 0.001$)]. cMyC's AUC was high [0.81, 95% confidence interval (CI) 0.78–0.83], higher than hs-cTnT's (0.79, 95% CI 0.76–0.82, $P = 0.081$) and lower than NT-proBNP's (0.91, 95% CI 0.89–0.93, $P < 0.001$). Among 794 AHF patients eligible for prognostic analysis, 28% died within 360 days; cMyC plasma concentrations above the median indicated increased risk of death (hazard ratio 2.19, 95% CI 1.66–2.89; $P < 0.001$). cMyC's prognostic accuracy was comparable with NT-proBNP's and hs-cTnT's. cMyC did not independently predict all-cause mortality when used in validated multivariable regression models. In novel multivariable regression models including medication, age, left ventricular ejection fraction, and discharge creatinine, cMyC remained an independent predictor of death and had no interactions with medical therapies at discharge.

Conclusion Cardiac myosin-binding protein C may aid physicians in the rapid triage of patients with suspected AHF.

Keywords Cardiac myosin-binding protein C • Acute heart failure • Diagnosis • Prognosis • Therapy guidance

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Introduction

Acute heart failure (AHF) is the most common diagnosis in the emergency department (ED) leading to hospitalization.[1,2] In contrast to the enormous improvements achieved in the management of patients with chronic heart failure, morbidity and mortality remain unacceptably high in patients with AHF. Specifically, the combination of all-cause mortality or recurrent hospitalization at 6 months approaches 50%.[1–3] The dismal outcome of patients with AHF may be at least in part related to diagnostic and prognostic uncertainty in the ED and the associated delay in the identification of patients for early and effective treatments and an incomplete understanding of the pathophysiological mechanisms involved in the AHF syndromes, which may require tailored therapeutic strategies.[4]

Cardiovascular biomarkers incorporated in clinica practice address some of these challenges. First, as quantitative markers of haemodynamic stress and heart failure, natriuretic peptides have substantially improved the rapid detection of AHF among patients presenting with acute dyspnoea.[1,2,5–8] Second, high-sensitivity cardiac troponin (hs-cTn) concentrations allow quantification of cardiomyocyte injury and enhance risk stratification of AHF patients.[6,9,10]

Cardiac myosin-binding protein C (cMyC) (*Figure 1*) is a novel biomarker that is unique to cardiomyocytes and can possibly quantify cardiomyocyte injury even more accurately than hs-cTn.[11–13] In addition, it has shown promising diagnostic and prognostic utility in the management of acute coronary syndromes.[11] cMyC may also have a role in the pathogenesis of AHF as various animal models for hypertrophic cardiomyopathy have shown that homozygous cMyC knock-out mice develop heart failure.[14] Definite pathogenic mutations within cMyC are the most common genetic cause of hypertrophic cardiomyopathy, responsible for 40–50% of new diagnoses.[15] These mutations, and known post-translational modifications of the cMyC protein, occur outside the N-terminal C0 region of the protein which is used for detection by the diagnostic assay.[13,15,16] The fast release kinetics of cMyC quantifying cardiomyocyte injury and its abundance in cardiomyocytes led to the hypothesis that cMyC concentrations may have diagnostic, prognostic, and therapy guidance potential in patients with AHF.[11,12,17]

We, therefore, aimed to test this hypothesis in a large multicentre cohort of patients presenting to the ED with suspected AHF.

Methods

Study population and design

Basics in Acute Shortness of Breath EvaLuation (BASEL V) was a prospective, multicentre diagnostic study aimed at contributing to advancing the early detection and management of patients with AHF (ClinicalTrials.gov, NCT01831115).[18,19] Adult patients presenting with acute dyspnoea to the ED of two university hospitals and three further tertiary care centres in Switzerland were enrolled (Basel, Zurich, Lucerne, St. Gallen, and Aarau). While enrolment was independent of renal function, patients with terminal kidney failure on chronic dialysis were excluded. For this analysis, patients were also excluded if they did not have cMyC plasma concentrations measured from study blood samples at ED presentation, if the final diagnosis remained unclear even after central adjudication, or if the patients were adjudicated as having cardiac dyspnoea due to an acute coronary syndrome or arrhythmia without any other evidence of AHF. For the prognostic analyses, patients with an adjudicated final diagnosis of AHF enrolled in an AHF therapy study were also eligible if study blood was available for the measurement of cMyC.[20]

The study was carried out according to the principles of the Declaration of Helsinki and was approved by the local ethics committees. All patients provided written informed consent. The authors designed the study, gathered, and analysed the data according to the STARD guidelines for studies of diagnostic accuracy and the TRIPOD statement for studies reporting multivariable prediction models for individual prognosis (online supplementary *Table S1*),[21,22] vouched for the data and analysis, wrote the paper, and decided to submit it for publication.

Adjudication of the final diagnosis

The final underlying cause of shortness of breath was centrally adjudicated by two independent cardiologists who had access to all patients' medical records (clinical history, physical examination, 12-lead electrocardiogram, laboratory findings including natriuretic peptide measurements,[1,2] estimated glomerular filtration rate, chest X-ray, echocardiography, lung function testing, computed tomography, response to therapy, and 360-day follow-up, according to current guidelines).[1,2] cMyC measurements were not available for the adjudication of the final diagnosis. In situations of disagreement related to the diagnosis, cases were reviewed and adjudicated in conjunction with a third cardiologist.

Patient follow-up

Patients were contacted after 90 days and at 1 year by telephone calls or in written form by trained researchers, unaware of the patients' cMyC plasma concentrations during the index hospitalization. In case of a possible relevant medical event such as heart failure rehospitalization or all-cause death during the follow-up period, further information was obtained from the hospital records, general physician records, or the national death registry.

Biochemical measurements

At ED presentation, blood samples were collected in tubes containing heparin for the determination of cMyC and high-sensitivity cardiac troponin T (hs-cTnT) concentrations, and potassium ethylenediaminetetraacetic acid for analysis of N-terminal pro-B-type natriuretic peptide (NT-proBNP), respectively. After centrifugation, samples were frozen at −80°C until assayed in a blinded fashion in a dedicated core laboratory. cMyC was measured using an established high-sensitivity assay on the Erenna platform performed by Millipore Sigma.[13] The assay has a limit of detection of 0.4 ng/L and a lower limit of quantification of 1.2 ng/L with a ≤20% coefficient of variation at limits of quantification, and ≤10% coefficient of variation at the 99th centile. Assay precision is not affected by freeze/thaw cycles.[23] The 99th percentile concentration determined previously in patients without obstructive coronary artery disease on invasive angiography is 87 ng/L.[11,13] In this study, the 99th centiles calculated for hs-cTnI/T

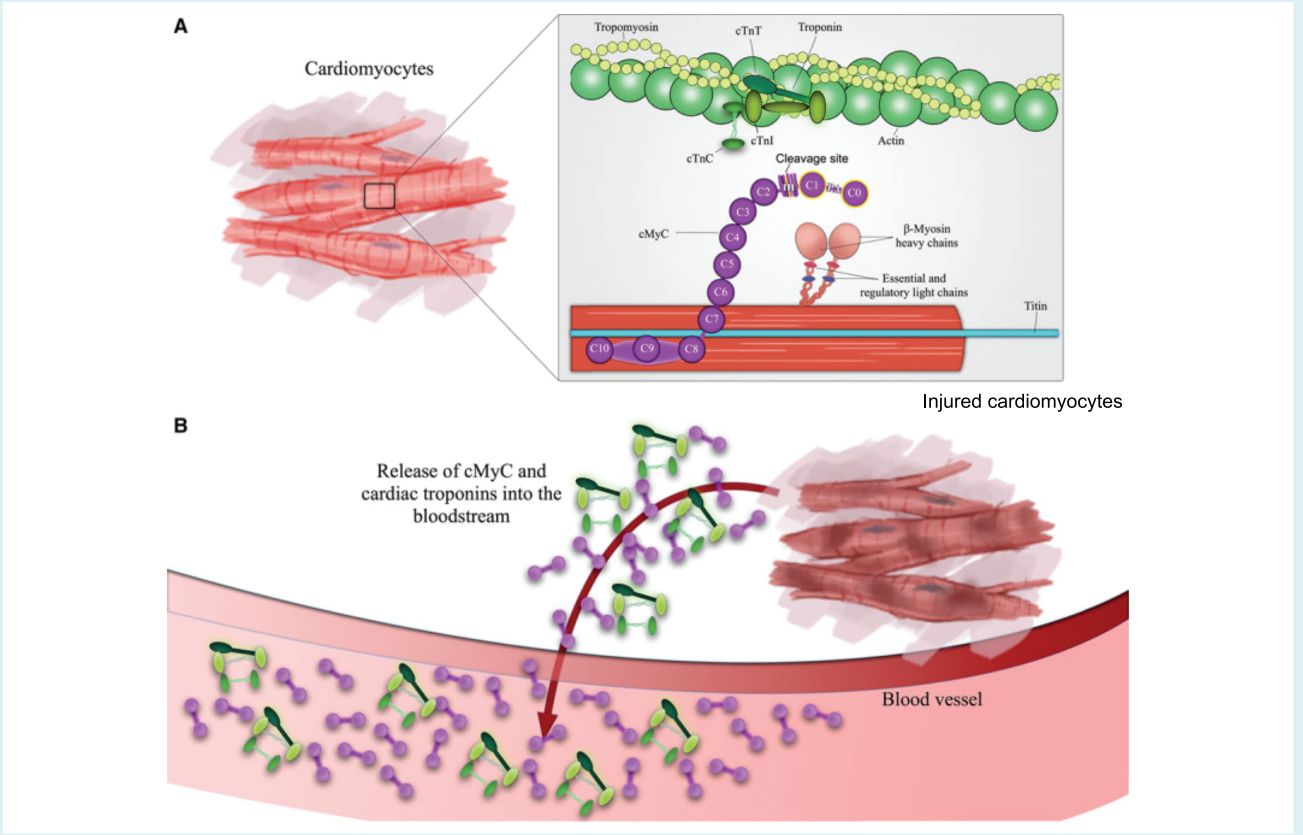

Figure 1 Depiction of cardiac troponin and cardiac myosin-binding protein C (cMyC) release during myocardial injury. Structure of cMyC and cardiac troponins in (*A*) healthy cardiomyocytes and (*B*) during cardiomyocyte injury. The highlighted N-terminal domain C0C1 is the binding site for the previously developed monoclonal antibodies used for detection of the cardiac-specific isoform of cMyC.[11] cTnC, cardiac troponin C; cTnI, cardiac troponin I; cTnT, cardiac troponin T.

matched the established thresholds. The hs-cTnT assay (Elecsys 2010 high-sensitivity troponin T, Roche Diagnostics, Rotkreuz, Switzerland) has a 99th percentile concentration of 14 ng/L.[24] NT-proBNP concentrations were measured using the Elecsys proBNP assay (Elecsys proBNP, Roche Diagnostics AG, Rotkreuz, Switzerland). The intra-assay coefficients of variation ranged from 1.8% to 2.7% and from 2.4% to 3.2% for within-run and total imprecision, respectively.[25] The laboratory technicians who measured cMyC, hs-cTnT, and NT-proBNP were blinded to the clinical data of the patients.

Outcome measures

The diagnostic accuracy for AHF quantified by the area under the receiver operating characteristic curve (AUC) was the primary diagnostic outcome measure. Only patients with confirmed final diagnosis of AHF were eligible for the prognostic analyses. The primary prognostic outcome measure was the utility of cMyC to predict all-cause mortality in AHF patients within 360 days. Secondary outcome measures were the prediction of AHF rehospitalizations; the combination of all-cause mortality or AHF rehospitalizations; the identification of AHF phenotypes according to cMyC plasma concentrations with disproportional benefit or harm in terms of all-cause mortality during the 360-day follow-up in pre-defined subgroups according to their treatment with diuretics, angiotensin-converting enzyme inhibitors (ACE-I) or angiotensin receptor blockers (ARB), beta-blockers, aldosterone antagonists, calcium channel blockers, and digoxin at hospital discharge; the diagnostic and prognostic performance in the subgroup of patients with renal dysfunction.

Assessment of renal function

For this analysis, renal dysfunction was defined as estimated glomerular filtration rate <60 mL/min/1.73 m^2 at presentation to the ED and was calculated by using the Chronic Kidney Disease Epidemiology Collaboration formula.

Statistical methods

The Kolmogorov–Smirnov test and visual inspection of the shape of the distribution of the variables were used to assess their normality. Continuous variables are presented as medians with interquartile range (IQR) as appropriate. Categorical variables are presented as numbers and percentages. Comparisons between groups were made using the Chi-square test, the Mann–Whitney U test, or the Kruskal–Wallis test as appropriate. Comparison of the AUCs of cMyC, hs-cTnT, and NT-proBNP was performed as recommended by DeLong *et al.*[26] The diagnostic accuracy of the combination of cMyC and NT-proBNP was assessed by logistic regression analysis. The optimal cMyC cut-off

concentration for the rapid rule out of AHF was derived from the receiver operating characteristic (ROC) curve. The requirements for this cut-off concentration were imposed by clinical considerations: a minimum sensitivity was set as high as 95% because of the large potential adverse outcome of missing a patient with AHF. The relevant 95% confidence intervals (CIs) of the predictive values were defined by using the Wilson score method without continuity correction. The utility of cMyC to predict all-cause mortality in AHF patients was assessed by comparing the time-dependent ROC curves of cMyC, hs-cTnT, and NT-proBNP.[27] All-cause mortality and AHF rehospitalization as well as the combination of both endpoints during follow-up were plotted in Kaplan–Meier curves, and the log-rank test was used to assess differences between groups. Spearman's rho was used to analyse correlations between cMyC and selected variables. cMyC and pre-defined variables from validated risk models to predict all-cause mortality, or the combination of all-cause mortality or hospitalizations due to AHF were entered in multivariable Cox proportional hazard models.[28] In contrast to the validated risk model for the prediction of the combination of all-cause mortality or hospitalizations due to AHF, high-density lipoprotein plasma concentrations at baseline were not available. Interactions between cMyC plasma concentrations and treatment with diuretics, ACE-I or ARB, beta-blockers, and aldosterone antagonists at hospital discharge were explored. The interaction of *P*-values between biomarker plasma concentrations and the pre-defined AHF subgroups according to their medication on discharge was calculated in bivariable Cox proportional hazards models. These bivariable models were then further adjusted for age, left ventricular ejection fraction (LVEF) and creatinine plasma concentrations at discharge. Hazard ratios are presented with 95% CI. Subgroup analyses were performed in patients with renal dysfunction to compare the diagnostic and prognostic accuracy of cMyC, hs-cTnT, and NT-proBNP. This was an exploratory analysis within a prospective study, and sample size of the overall cohort was not specifically determined for this analysis. No imputation was performed for missing values. Patients without complete 1-year clinical follow-up were censored at the time of the last known contact. Statistical analyses were performed using SPSS/PC Software Package (version 25.0) and R Statistical Software (version 3.5.1), including packages 'pROC', 'timeROC', 'cmprsk', 'survival', 'ggplot', 'tableone', 'haven', 'tibble', and 'predictABLE'.

Results

Patient demographics and characteristics

A total of 1330 patients were enrolled between November 2007 and August 2013. Of these, 1083 were eligible for the diagnostic analysis (online supplementary *Figure S1*). In the overall cohort, the median age was 75 years, and 42% of all patients were women (*Table 1*). Roughly one third (35%) of the patients had a history of AHF, and 41% had known coronary artery disease.

Acute heart failure and cardiac myosin-binding protein C plasma concentrations

Acute heart failure was the adjudicated final diagnosis in 548 patients (51%) included in the diagnostic analysis and in 794 patients (60%) in the overall cohort. cMyC concentrations at presentation were higher among patients with AHF vs. patients with other final diagnosis [72 (IQR 39–156) vs. 22 (IQR 12–42) ng/L, $P < 0.001$]. Among the different AHF phenotypes, acute coronary syndrome with AHF, and pulmonary oedema were associated with even higher cMyC concentrations (online supplementary *Table S2*). cMyC plasma concentrations showed a strong correlation with hs-cTnT (0.792, $P < 0.001$) and NT-proBNP (0.691, $P < 0.001$) concentrations, and a modest correlation with cardiac structure and function as quantified by LVEF (−0.320, all $P < 0.001$; online supplementary *Table S3*).

Among the non-AHF causes of acute dyspnoea, pulmonary embolism [61 (IQR 28–144) ng/L, $n = 56$] had higher cMyC concentrations vs. exacerbated obstructive pulmonary disease [20 (IQR 11–37) ng/L, $n = 161$] and pneumonia (24 (12–44) ng/L, $n = 114$) (online supplementary *Table S4*).

Beyond the presence or absence of AHF, when comparing different cMyC quartiles, patients with higher plasma concentrations were more likely to be older and to have prior history of coronary artery disease. Patients in the top cMyC quartile also had a lower LVEF, and higher NT-proBNP as well as higher creatinine concentrations (online supplementary *Table S5*).

Diagnostic performance

A total of 1083 patients were eligible for diagnostic analysis (online supplementary *Figure S1* and *Table S6*). The diagnostic accuracy of cMyC plasma concentrations to diagnose AHF as quantified by the AUC was high (0.81, 95% CI 0.78–0.83), higher than hs-cTnT's (0.79, 95% CI 0.76–0.82, $P = 0.081$) and lower vs. NT-proBNP's (0.91, 95% CI 0.89–0.93, $P < 0.001$; *Figure 2*). The combination of cMyC and NT-proBNP did not further increase the AUC of NT-proBNP (0.91, 95% CI 0.89–0.93, $P = 0.697$). Subgroup analysis in patients with renal dysfunction demonstrated a reduction of diagnostic accuracy of all the biomarkers in the presence of renal dysfunction; however, the difference between hs-cTnT and cMyC increased (0.69 for hs-cTnT, 95% CI 0.63–0.74 vs. 0.75 for cMyC, 95% CI 0.70–0.80, respectively; $P < 0.001$; online supplementary *Figure S2*).

The pre-defined minimum sensitivity of 95% was achieved at the cMyC cut-off concentration of 16 ng/L. By using this cut-off concentration to exclude the diagnosis of AHF, its sensitivity was 95% (95% CI 93–97%), the negative predictive value was 88% (95% CI 84–92%), and it enabled rule out of 21% (95% CI 18–23%) of the patients. In turn, when using the currently recommended rule-out cut-off for NT-proBNP at 300 pg/mL, the achieved sensitivity and negative predictive value were as high as 98% (95% CI 97–99%) and 97% (95% CI 94–98%), respectively. Furthermore, it allowed to rule out 28% (95% CI 25–31%) of the patients (*Table 2*).

Prognostic implications

Among 1330 patients in the overall cohort, 794 AHF patients were eligible for prognostic analysis (online supplementary *Figure S1*). Of these patients, 790 (99%) had a complete follow-up and a total of 223 (28%) died within the 360-day follow-up period.

Table 3 illustrates the patients' characteristics according to their survival status for the 360-day period. Patients who died within the 360-day follow-up were older, had a lower body mass index, lower blood pressure, and more often had a history of AHF. Notably, their cMyC, hs-cTnT, and NT-proBNP plasma concentrations were substantially higher as compared to survivors.

Patients with AHF and cMyC plasma concentrations above the median were at increased risk of death (hazard ratio 2.19, 95% CI 1.66–2.89; $P < 0.001$; *Figure 3*), which is comparable to hs-cTnT

Table 1 Baseline characteristics of the patients presenting to the emergency department with acute dyspnoea grouped by an adjudicated final diagnosis of acute heart failure

	All patients (n = 1330)	AHF (n = 794)	No AHF (n = 536)	*P*-value
Demographics				
Age (years)	75 (63–83)	79 (70–85)	66 (54–77)	<0.001
Female sex	554 (42)	322 (40.6)	232 (43)	0.350
Weight (kg)	76 (65–88)	76 (65–88)	75 (63–87)	0.163
BMI (kg/m^2)	26 (23–30)	27 (24–30)	26 (22–30)	0.001
Clinical parameters at ED				
SBP (mmHg)	135 (120–153)	135 (120–153)	136 (121–152)	0.658
Heart rate (bpm)	89 (75–105)	87 (72–105)	93 (80–106)	<0.001
Respiratory rate (breaths/min)	22 (18–28)	22 (18–28)	23 (18–28)	0.504
Pulse oximetry (%)	96 (93–98)	96 (93–98)	95 (92–98)	0.330
Temperature (°C)	37.1 (36.6–37.6)	37.0 (36.5–37.4)	37.3 (36.8–37.8)	<0.001
LVEF (%)	50 (33–60)	45 (30–55)	60 (55–62)	<0.001
Medical history				
Hypertension	951 (72)	669 (85)	282 (53)	<0.001
Dyslipidaemia	642 (50)	462 (60)	180 (34)	<0.001
CAD	539 (41)	433 (55)	106 (20)	<0.001
Prior myocardial infarction	319 (24)	260 (34)	59 (11)	<0.001
Prior AHF	455 (35)	395 (50)	60 (11)	<0.001
Atrial fibrillation	432 (33)	378 (48)	54 (10)	<0.001
CKD	446 (34)	367 (46)	79 (15)	<0.001
PAD	155 (12)	123 (16)	32 (6.0)	<0.001
Stroke	156 (12)	120 (15)	36 (6.7)	<0.001
COPD	418 (32)	185 (23)	233 (44)	<0.001
Medication at admission				
ACE inhibitors	431 (33)	322 (42)	109 (21)	<0.001
ARBs	293 (23)	206 (27)	87 (17)	<0.001
Beta-blockers	634 (49)	490 (63)	144 (27)	<0.001
Aldosterone antagonists	112 (8.6)	93 (12)	19 (3.6)	<0.001
CCB	252 (19)	173 (22)	79 (15)	0.001
Digoxin	52 (4.0)	46 (6.0)	6 (1.1)	<0.001
Diuretics	702 (54)	544 (70)	158 (30)	<0.001
Laboratory parameters				
Haemoglobin (g/L)	131 (116–144)	126 (111–139)	139 (126–149)	<0.001
Haematocrit (%)	38 (35–42)	37 (34–41)	40 (37–43)	<0.001
Sodium (mmol/L)	139 (136–141)	139 (137–142)	138 (136–141)	<0.001
Chloride (mmol/L)	101 (98–104)	102 (98–105)	100 (96–103)	<0.001
Potassium (mmol/L)	4.1 (3.8–4.4)	4.2 (3.8–4.5)	4.0 (3.7–4.3)	<0.001
Creatinine (μmol/L)	92 (73–126)	106 (81–147)	79 (63–96)	<0.001
Urea (mmol/L)	8.3 (5.7–12)	9.8 (6.9–14)	6.3 (4.5–8.7)	<0.001
Albumin (g/L)	36 (32–38)	35 (32–38)	36 (33–40)	0.001
NT-proBNP (ng/L)	2473 (374–6628)	5250 (2592–9747)	231(80–835)	<0.001
hs-cTnT (ng/L)	26 (12–50)	37 (21–67)	13 (6–26)	<0.001
cMyC (ng/L)	46 (22–104)	72 (39–156)	22 (12–42)	<0.001

Values are given as median (interquartile range), or *n* (%).
ACE, angiotensin-converting enzyme; AHF, acute heart failure; ARB, angiotensin receptor blocker; BMI, body mass index; CAD, coronary artery disease; CCB, calcium channel-blocker; CKD, chronic kidney disease; cMyC, cardiac myosin-binding protein C; COPD, chronic obstructive pulmonary disease; ED, emergency department; hs-cTnT, high-sensitivity cardiac troponin T; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-B-type natriuretic peptide; PAD, peripheral arterial disease; SBP, systolic blood pressure.

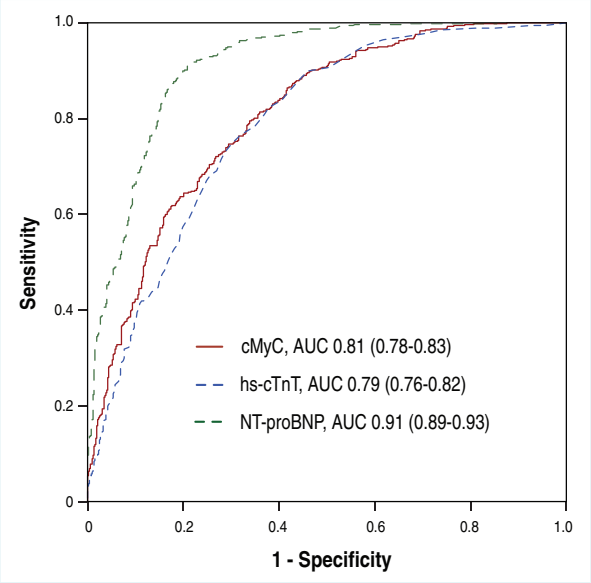

Figure 2 Receiver operating characteristic curve comparison of cardiac myosin-binding protein C (cMyC) with high-sensitivity cardiac troponin T (hs-cTnT) ($P = 0.081$) or N-terminal pro-B-type natriuretic peptide (NT-proBNP) ($P < 0.001$) in patients in the diagnostic cohort with biomarker measurements available ($n = 1060$) for the diagnosis of acute heart failure in the emergency department. AUC, area under the curve.

Table 2 Performance of the cardiac myosin-binding protein C cut-off concentration of 16 ng/L for the rapid exclusion of acute heart failure among patients with acute dyspnoea in the diagnostic analysis

	cMyC ($n = 1083$)	NT-proBNP[a] ($n = 1071$)
Sensitivity, % (95% CI[b])	**95 (93–97)**	**98 (97–99)**
Specificity, % (95% CI)	37 (33–41)	55 (51–59)
NPV, % (95% CI)	**88 (84–92)**	**97 (94–98)**
PPV, % (95% CI)	61 (57–64)	69 (66–73)
Negative likelihood ratio	0.13 (0.09–0.19)	0.03 (0.02–0.06)
Positive likelihood ratio	1.51 (1.41–1.61)	2.19 (1.98–2.40)
Diagnostic odds ratio	11.70 (7.60–18.01)	72.18 (36.54–142.59)
Patients ruled out, *n* (%, 95% CI)	**223 (21, 18–23)**	**289 (28, 25–31)**

CI, confidence interval; cMyC, cardiac myosin-binding protein C; NPV, negative predictive value; NT-proBNP, N-terminal pro-B-type natriuretic peptide; PPV, positive predictive value.
[a]'Rule-out' cut-off at 300 pg/mL.
[b]The method used to calculate the CI for a proportion is the Wilson score method without continuity correction.

(hazard ratio 2.61, 95% CI 1.95–3.51; $P < 0.001$) and NT-proBNP (hazard ratio 2.28, 95% CI 1.72–3.03; $P < 0.001$). Similarly, cMyC above the median was associated with higher rates of all-cause mortality and AHF rehospitalization within 360 days (hazard ratio 1.63, 95% CI 1.31–2.02; $P < 0.001$; online supplementary *Figure S3*).

Cardiac myosin-binding protein C and NT-proBNP plasma concentration provided incremental prognostic information when classifying patients according to below/above the median of each biomarker ($P < 0.001$; online supplementary *Figure S4*). Patients with both cMyC and NT-proBNP concentrations above the median had the highest risk of death, whereas AHF patients with low plasma concentrations of both biomarkers had the lowest risk of death. Similar results were obtained for the combination of cMyC and hs-cTnT (online supplementary *Figure S5*).

The prognostic accuracy of cMyC, hs-cTnT, and NT-proBNP for predicting all-cause mortality, the combination of all-cause mortality and AHF rehospitalization as well as AHF rehospitalizations alone along the 360 days were, overall, comparable using their time-dependent ROC curves (*Figure 4* and online supplementary *Figure S6*). Notably, in the subgroup of patients with renal dysfunction, cMyC showed better prognostic performance for the short-term prognosis as compared to NT-proBNP (AUCs at 15 days 0.826 vs. 0.686, $P = 0.007$; online supplementary *Figure S7*). The combination of NT-proBNP or hs-cTnT with cMyC did not significantly increase their prognostic accuracy for predicting all-cause mortality or its combination with AHF hospitalisations (online supplementary *Figures S8* to *S11*). Variables from validated risk models to predict all-cause mortality or their combination with hospitalizations due to AHF were entered in multivariable regression models (*Tables 4* and *5*). cMyC was not an independent predictor for these outcomes after multivariable adjustment.

Interaction with treatment at hospital discharge

In bivariable Cox proportional hazards models, a significant interaction was found between cMyC plasma concentrations at presentation and treatment with ACE-I at discharge (online supplementary *Table S7A*). After multivariable adjustment including age, LVEF, and creatinine plasma concentrations at discharge, the interactions regarding cMyC plasma concentrations lost their statistical significance (online supplementary *Table S7B*).

Discussion

This secondary analysis within a large prospective diagnostic study aimed to evaluate the clinical utility of cMyC in the diagnosis, risk stratification and therapy guidance of patients with AHF.[1,2] We report six major findings.

First, in line with our hypothesis, cMyC concentrations were higher in patients with an adjudicated final diagnosis of AHF vs. patients with other causes of acute dyspnoea. In the context of the ability of cMyC to precisely quantify cardiac injury, this finding is in full agreement with the previously reported high incidence of elevated hs-cTn concentrations in AHF.[2,9,10] Second, the diagnostic accuracy of cMyC for AHF was high, being slightly higher than hs-cTnT, but lower than the current laboratory gold standard for NT-proBNP. Third, cMyC might help in better AHF phenotyping, as the concentrations were higher in more severe forms such as pulmonary oedema and AHF combined with acute coronary syndrome. Fourth, all-cause mortality was higher in patients with above median cMyC concentrations. Overall, the

prognostic accuracy was moderate-to-high and comparable to that of NT-proBNP and hs-cTnT, with some incremental prognostic value on top of NT-proBNP or hs-cTnT. However, in the fully adjusted multivariable prediction model, cMyC was no longer an independent predictor of death.[28] Fifth, in the bivariable Cox proportional hazards models, a significant interaction between cMyC plasma concentrations at presentation and treatment with ACE-I at discharge was found, indicating that patients with higher cMyC concentrations might derive particular long-term benefits from these drugs. As the interaction was no longer significant

Table 3 Prognostic analyses of patient characteristics according to survival status of acute heart failure patients at 360 days

	Overall (n = 794)	Dead at 360 days (n = 223)	Alive at 360 days (n = 571)	P-value
Demographics				
Age (years)	79 (70–85)	83 (76–87)	77 (67–84)	<0.001
Female sex	322 (41)	103 (46)	219 (38)	0.052
Weight (kg)	76 (65–88)	69 (60–80)	79 (69–91)	<0.001
BMI (kg/m^2)	27 (24–30)	25(22–28)	27 (24–31)	<0.001
Clinical parameters at ED				
SBP (mmHg)	135 (120–153)	126 (111–146)	138 (123–155)	<0.001
Heart rate (bpm)	87 (72–105)	85 (74–104)	87 (71–105)	0.907
Respiratory rate (breaths/min)	22 (18–28)	22 (20–28)	22 (18–28)	0.714
Pulse oximetry (%)	96 (93–98)	96 (92–98)	96 (93–98)	0.325
Temperature (°C)	37.0 (36.5–37.4)	36.9 (36.5–37.5)	37.0 (36.5–37.4)	0.582
LVEF (%)	45 (30–55)	40 (27–55)	45 (30–55)	0.170
Medical history				
Hypertension	669 (85)	188 (85)	481 (85)	0.939
Dyslipidaemia	462 (60)	130 (61)	332 (60)	0.801
CAD	433 (55)	131 (59)	302 (53)	0.138
Prior myocardial infarction	260 (34)	85 (39)	175 (32)	0.050
Prior AHF	395 (50)	128 (58)	267 (47)	0.004
Atrial fibrillation	378 (48)	103 (47)	275 (47)	0.656
CKD	367 (46)	127 (58)	240 (42)	<0.001
PAD	123 (16)	41 (19)	82 (15)	0.144
Stroke	120 (15)	39 (18)	81 (14)	0.242
COPD	185 (23)	55 (25)	130 (23)	0.514
Medication at admission				
ACE inhibitors	322 (42)	94 (44)	228 (41)	0.517
ARBs	206 (27)	49 (23)	157 (28)	0.117
Beta-blockers	490 (63)	132 (61)	358 (64)	0.388
Aldosterone antagonists	93 (12)	36 (17)	57 (10)	0.014
CCB	173 (22)	45 (21)	128 (23)	0.505
Digoxin	46 (6.0)	8 (3.8)	38 (6.8)	0.112
Diuretics	544 (70)	173 (79)	371 (67)	<0.001
Laboratory parameters				
Haemoglobin (g/L)	126 (111–139)	118.5 (109–131)	129 (114–142)	<0.001
Haematocrit (%)	37 (34–41)	36 (33–39)	38 (34–42)	<0.001
Sodium (mmol/L)	139 (137–142)	139 (137–141)	140 (137–142)	0.002
Chloride (mmol/L)	102 (98–105)	100 (97–104)	102 (99–105)	0.001
Potassium (mmol/L)	4.2 (3.8–4.5)	4.3 (3.9–4.7)	4.1 (3.8–4.5)	0.011
Creatinine (μmol/L)	106(81–147)	123(87–172)	100 (80–132)	<0.001
Urea (mmol/L)	9.8 (6.9–13.9)	12.0(8.7–17.2)	9.1 (6.5–12.7)	<0.001
Albumin (g/L)	35 (32–38)	34 (32–37)	36 (33–38)	<0.001
NT-proBNP (ng/L)	5250 (2592–9746)	8096 (4089–18 329)	4491 (2220–8277)	<0.001
hs-cTnT (ng/L)	37 (21–67)	53(33–102)	32 (19–58)	<0.001
cMyC (ng/L)	72 (39–156)	119(52–263)	63 (34–127)	<0.001

Values are given as median (interquartile range), or n (%).
ACE, angiotensin-converting enzyme; AHF, acute heart failure; ARB, angiotensin receptor blocker; BMI, body mass index; CAD, coronary artery disease; cMyC, cardiac myosin-binding protein C; CCB, calcium channel blocker; CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disease; ED, emergency department; hs-cTnT, high-sensitivity cardiac troponin T; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-B-type natriuretic peptide; PAD, peripheral arterial disease; SBP, systolic blood pressure.

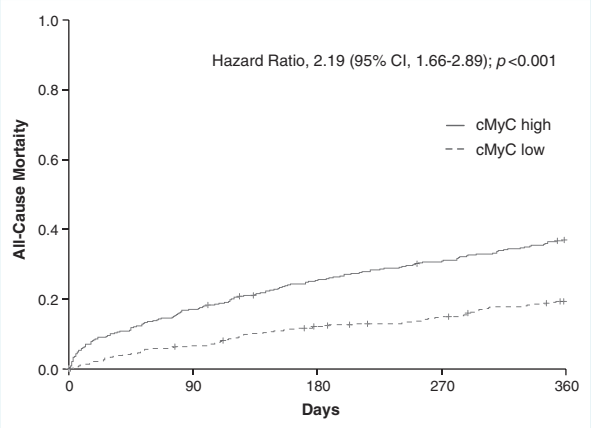

Figure 3 Cumulative mortality in acute heart failure patients according to cardiac myosin-binding protein C (cMyC) plasma concentrations above or below the median (72 ng/L, $n = 794$) plotted in a Kaplan–Meier curve. CI, confidence interval.

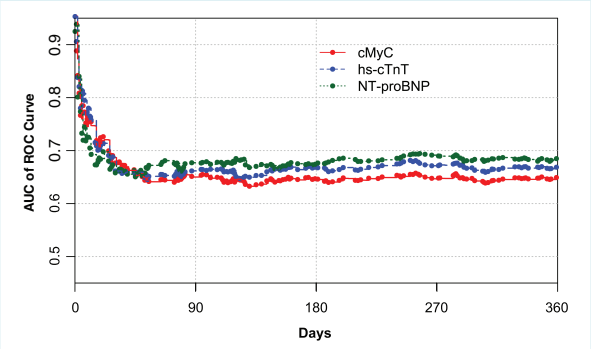

Figure 4 Time-dependent receiver operating characteristic (ROC) curves for all-cause mortality at 360 days for cardiac myosin-binding protein C (cMyC), high-sensitivity cardiac troponin T (hs-cTnT), and N-terminal pro-B-type natriuretic peptide (NT-proBNP) in acute heart failure patients ($n = 748$). There were no significant differences between the areas under the curve (AUC) of cMyC and hs-cTnT at 90, 180 and 360 days ($P = 0.572$, 0.075, and 0.079, respectively) or cMyC and NT-proBNP at 90, 180 and 360 days ($P = 0.340$, 0.234, and 0.090 respectively).

after multivariate adjustment including LVEF, the incremental value of cMyC in the identification of AHF patients deriving long-term benefits from ACE-I remains uncertain. Future studies assessing dynamic changes in cMyC seem warranted, since the change in concentrations may be more relevant for therapy guidance.[29] The dynamic nature of cMyC, with its fast release kinetics and abundance in cardiomyocytes, may provide a particular advantage in this setting.[11,12]

To our knowledge, this is the first large prospective clinical trial to explore the diagnostic and prognostic utility of cMyC for AHF in adults and has demonstrated incremental value to its potential use in the ED. These findings extend and corroborate

Table 4 Multivariable Cox proportional hazards models for mortality at 360 days in acute heart failure patients ($n = 684$)

Variable	Hazard ratio	95% CI	*P*-value
Age (years)	1.038	1.022–1.054	<0.001
Beta-blockers at baseline	0.849	0.629–1.145	0.283
lg BUN (mmol/L)	2.626	1.256–5.492	0.010
Haemoglobin (g/L)	0.998	0.991–1.005	0.542
lg NT-proBNP (ng/L)	2.737	1.812–4.135	<0.001
lg cMyC (ng/L)	**1.290**	**0.983–1.693**	**0.067**

AHF, acute heart failure; BUN, blood urea nitrogen; CI, confidence interval; cMyC, cardiac myosin-binding protein C; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

Table 5 Multivariable Cox proportional hazards models for mortality or acute heart failure (AHF) rehospitalization at 360 days in AHF patients ($n = 697$)

Variable	Hazard ratio	95% CI	*P*-value
Age (years)	1.012	1.001–1.024	0.038
Prior AHF	1.259	0.994–1.594	0.057
Oedema	1.048	0.821–1.338	0.705
Beta-blockers at baseline	0.922	0.722–1.178	0.516
SBP (mmHg)	0.992	0.987–0.997	0.001
Haemoglobin (g/L)	0.999	0.994–1.004	0.619
Sodium (mmol/L)	0.988	0.963–1.013	0.339
lg NT-proBNP (ng/L)	1.956	1.446–2.645	<0.001
lg cMyC (ng/L)	**1.231**	**0.986–1.538**	**0.067**

AHF, acute heart failure; CI, confidence interval; cMyC, cardiac myosin-binding protein C; NT-proBNP, N-terminal pro-B-type natriuretic peptide; SBP, systolic blood pressure.

previous work on the clinical use of cMyC for the diagnosis of AHF in children. In a prospective case-control study that involved 50 children with AHF and 25 healthy children matched for age and sex as a control group, the high diagnostic and prognostic value of cMyC was demonstrated. With the use of a cut-off concentration of 45 ng/mL, the sensitivity reached 100% and its specificity for diagnosing AHF was 96%, while at the cut-off concentration of 152 ng/mL, its sensitivity and specificity for predicting adverse outcomes were as high as 90% and 93%, respectively. The AUC was 0.999 (95% CI 0.997–1.002) for the diagnosis and 0.915 (95% CI 0.796–1.034) for the prognosis of AHF.[30] However, comparisons between the results of the two studies should be assessed with caution, mainly since the aetiology of AHF in children with a mean age < 2 years in this study substantially differed when compared to the elderly adult AHF population as in our study. BASEL V has important methodological strengths including its large sample size, highly representative elderly patient population for acute dyspnoea and AHF,[18,19] and adjudicated final diagnosis by two independent cardiologists/internists according to current guidelines.[1,2]

This study also has several limitations. First, its findings are specific to patients presenting with acute dyspnoea to the ED, and may not apply to the use of cMyC for screening in asymptomatic patients or in patients presenting with very mild symptoms to a general practitioner.[1,2] Further studies are necessary to address uncertainties regarding cut-off concentrations in these settings. Second, despite using a very strong methodology for the central adjudication of the final diagnosis, a very small number of patients may still have been misclassified. This would have led to an underestimation of the diagnostic accuracy of cMyC. Third, in order to maximize the accuracy in the adjudication of the final diagnosis leading to ED presentation with dyspnoea in BASEL V, central adjudication included BNP or NT-proBNP measurements, putting the blinded cMyC measurement at a disadvantage for direct comparison of diagnostic accuracy. Accordingly, the real difference in diagnostic accuracy between cMyC and NT-proBNP may be smaller than found in this study. Fourth, further studies are needed to prospectively validate the cut-offs for the optimal clinical use of cMyC for the diagnosis and prognosis of AHF in the ED, taking into account clinical characteristics that could potentially confound the performance of cMyC. Accordingly, as a biomarker in the evaluation of patients presenting with possible AHF, cMyC should always be used in conjunction with all other clinical information. Fifth, this study required written informed consent. Therefore, as for all studies requiring written informed consent, selection bias towards the enrolment of patients eligible to provide consent was unavoidable. Sixth, while enrolment was independent from renal function, and a substantial number of patients with renal dysfunction were included in this analysis, this study did not include patients with terminal kidney failure on chronic haemodialysis. Accordingly, we cannot comment on the performance of cMyC in this vulnerable patient population.

In conclusion, this large multicentre diagnostic study using central adjudication demonstrated that cMyC plasma concentrations may aid physicians in the rapid triage of patients presenting to the ED with suspected AHF.

Supplementary Information

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

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Conflict of interest: N.K. has received research grants from the Swiss National Science Foundation (P400PM-194 477), Gottfried und Julia Bangerter-Rhyner-Stiftung, and the European Society of Cardiology. T.K. has received speaker honoraria from AstraZeneca. T.N. received speaker/consulting honoraria from Beckman-Coulter, Bayer, Orion Pharma and Ortho Clinical Diagnostics. A.G. received speaker honoraria from Pfizer, Novartis, AstraZeneca, and Amgen. T.B. has received research grants from the Swiss National Science Foundation (PASMP3-134362), the University Hospital Basel, the Department of Internal Medicine, University Hospital Basel, Abbott, and Roche as well as speaker honoraria from Roche. C.M. has received research grants from the Swiss National Science Foundation, the Swiss Heart Foundation, the University Hospital Basel, the Cardiovascular Research Foundation Basel, the KTI, the University of Basel, Abbott, Beckman Coulter, BRAHMS, Ortho Critical Diagnostics, Novartis, Quidel, Roche, Siemens, Singulex, and Sphingotec, as well as speaker/consulting honoraria from Amgen AstraZeneca, BMS, Boehringer Ingelheim, BRAHMS, Bayer, Osler, Novartis, Roche, and Sanofi. M.M. is named as an inventor on a patent held by King's College London for the detection of cMyC as a biomarker of myocardial injury. All other authors have nothing to disclose.

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