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Cardiac troponin T concentrations and patient-specific risk of myocardial infarction using the novel PALfx parameter

Damiano Ognissanti[a], Christian Bjurman[b], Martin J. Holzmann[c,f], Elvar Theodorsson[d], Max Petzold[e], Marija Cvijovic[a,*], Ola Hammarsten[b,*]

[a] Department of Mathematical Sciences, Chalmers University of Technology and the University of Gothenburg, SE-412 96 Gothenburg, Sweden
[b] Department of Clinical Chemistry and Transfusion Medicine, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, SE-41345 Gothenburg, Sweden
[c] Department of Emergency Medicine, Karolinska University Hospital, Huddinge, Stockholm, Sweden
[d] Department of Clinical Chemistry and Department of Clinical and Experimental Medicine, Linköping University, Linköping, Sweden
[e] Centre for Applied Biostatistics, Sahlgrenska Academy, University of Gothenburg, SE-41345 Gothenburg, Sweden
[f] Functional Area of Emergency Medicine, Karolinska University Hospital, Stockholm, Sweden

A B S T R A C T

Background: Myocardial infarction (MI) is more likely if the heart damage biomarker cardiac troponin T (cTnT) is elevated in a blood sample from a patient with chest pain. There is no conventional method to estimate the risk of MI at a specific cTnT concentration. The purpose of this study was to evaluate the performance of a novel method that converts cTnT concentrations to patient-specific risks of MI.

Methods: Admission cTnT measurements in 15,425 ED patients from three hospitals with a primary complaint of chest pain, with or without a clinical diagnosis of MI, were Box-Cox-transformed to normality density functions to calculate the percentage with MI among patients with a given cTnT concentration, the parametric predictive value among lookalikes (PALfx). The ability of the PALfx to generate stable risk estimates of MI was examined by bootstrapping and expressed as the coefficient of variation (CV).

Results: Four age and sex-specific subgroups above or below 60 years of age with distinct cTnT distributions were identified among patients without MI. The cTnT distributions across subgroups with MI were similar, allowing us to use all admissions with MI to calculate the PALfx in the four subgroups. For instance, at a baseline cTnT concentration of 7 ng/L, a female patient < 60 years would have a 0.5% risk of MI whereas a male patient > 60 years would have a 1.9% risk of MI. To assess the stability of the PALfx method we bootstrapped smaller and smaller subsets of the 15,422 ED visits. We found that 1950 patients without MI and 50 patients with MI were sufficient to limit the variation of the PALfx with a CV of 0.8–5.4%, close to the CV using the entire dataset. The MI risk estimates were similar when data from the three hospitals were used separately to derive the PALfx equations.

Conclusions: The PALfx can be used to estimate the risk of MI at patient-specific cTnT concentrations with acceptable margins of error. The patient-specific risk of disease using the PALfx could complement decision limits.

1. Introduction

Cardiac-specific troponin T (cTnT) is elevated in blood following cardiac injury and one of the preferred diagnostic biomarkers when myocardial infarction (MI) is suspected [1,2]. Unfortunately, 25% of emergency department (ED) patients over 65 years of age without MI have cTnT concentrations above the current decision limit [3]. In addition, patients with heart failure or kidney disease without MI often have stable cTnT elevations that rival the concentrations found in patients with MI, resulting in a significant overlap among patients with or without MI [3,4]. Therefore, methods to convert cTnT concentrations to patient-specific risks of MI would likely facilitate everyday diagnostic and treatment decisions.

To evaluate the risk of MI in relation to cTnT concentrations we often use empirical methods, including the positive predictive value (PPV) (Fig. 1). In PPV, we include all patients with cTnT concentrations above the decision limit and determine the fraction of patients with MI among them [5]. The PPV result is the risk of MI among all patients above a certain cTnT decision limit. The problem with the PPV is that it is unable to estimate the risk at the patient's cTnT concentration. As all patients with a cTnT level above the decision limit are included—many with higher cTnT concentrations than the actual patient—the PPV often overestimates the actual patient risk of MI. This problem also applies to common logistic regression techniques [6].

A way to circumvent this problem is to determine the fraction of patients with MI within a cTnT range covering the actual patient's cTnT concentration; the empirical predictive value among lookalikes (ePAL) [7]. The ePAL includes patients within a range covering the actual

* Corresponding authors.
E-mail addresses: marija.cvijovic@gu.se (M. Cvijovic), ola.hammarsten@clinchem.gu.se (O. Hammarsten).

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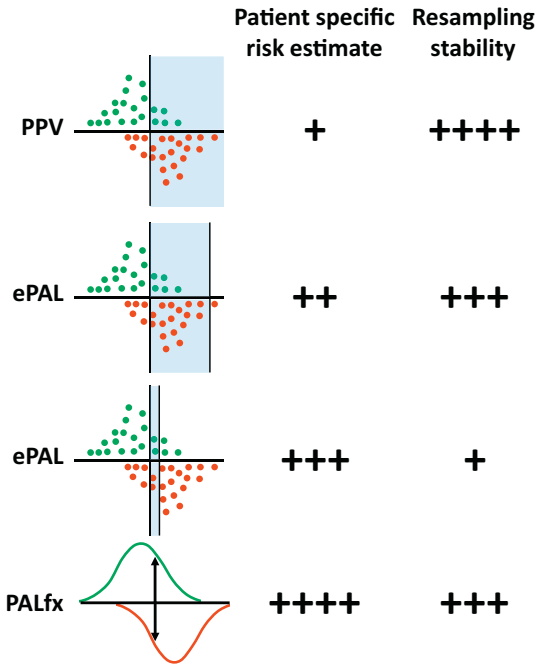

Fig. 1. Visual representation of three different ways to calculate the risk of myocardial infarction (MI). Simulated cTnT concentrations along an x axis from patients without (green) or with (red) MI. The Positive Predictive Value (PPV) is the fraction of patients with MI above a given decision limit (black vertical line). The PPV is relatively stable against resampling, if new data were to be collected from the same emergency department, as the PPV includes large amounts of data. On the negative side, the PPV only gives the risk of MI above the decision limit and is therefore often unable to estimate the patient-specific risk of MI. The empiric Predictive value Among Lookalikes (ePAL) is the fraction of patients with MI between two adjacent cTnT limits. The narrower the cTnT interval, the more patient-specific the risk estimate. But because the ePAL only uses data between these limits the risk estimate will be less stable against resampling. The Parametric PAL (PALfx) is based on density functions for patients without (green curve) or with MI (red curve). As the risk of MI is derived from density functions, the expected fraction with MI at any cTnT concentration can be estimated. Provided that the density functions capture the data distribution correctly, the PALfx is stable against resampling as all data were used to derive the density functions. The PALfx therefore results in patient-specific risk estimates and a high resampling stability.
(For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

patient's cTnT concentration, potentially giving a more personalized risk of MI. However, if we were to use a narrow cTnT range to improve the patient-specific risk estimate, the number of patients in the ePAL calculation is reduced. The resulting use of very sparse data lowers the resampling stability, that is, the ePAL variation may become unacceptably large if new data were to be collected from the same emergency department.

In the original description of the ePAL [7], biomarker limits were derived from percentiles to make sure that a minimum number of patients were included in the ePAL calculation. The use of biomarker ranges based on percentiles improved the ePAL resampling stability, especially at low or high biomarker concentrations. However, resampling stability remained a problem if the biomarker concentrations had a right-skewed distribution, with few patients displaying very high biomarker concentrations, as is the case for cTnT concentrations in the emergency department. In addition, cTnT concentrations from our ED datasets could not be transformed to normality by log-transformation.

A way to overcome this problem is to use a flexible transformation algorithm, like Box-Cox transformation, to transform the data. In our case, we were able to find an optimized Box-Cox transformation that converted the cTnT concentration distribution to normality. We were then able to use conventional normality mathematics with mean values and standard deviations to calculate point estimates of the risk of MI. It is of course possible that Box-Cox transformation to normality would not be possible with other datasets, possibly limiting the use of our method.

Here, we describe our ability to overcome the poor resampling stability with the ePAL using Box-Cox transformation to transform cTnT concentrations from our ED to normality density functions that allow calculation of the parametric PAL, the PALfx. We find that the PALfx estimates the risk of MI at specific cTnT concentrations with acceptable resampling stability.

2. Materials and methods

2.1. The Kolmogorov-Smirnov goodness-of-fit test

All calculations were performed in MATLAB (MathWorks, Inc.). To determine subgroups based on age and gender with distinct distributions of cTnT concentrations, the set was divided into disjoint subsets and the subsets' cumulative distribution functions were compared with a two-sample Kolmogorov-Smirnov goodness-of-fit hypothesis test [8] with $\alpha = 0.01$.

The null hypothesis is that the data in the two groups are drawn from the same continuous distribution and the test statistic is $D = \max_x(|F_1(x) - F_2(x)|)$, where $F_1(x)$ and $F_2(x)$ are functions giving the proportion of values less than or equal to x in the respective set. We thus look at the maximum distance between the two Empirical Cumulative Distribution Functions (ECDF) and reject the null hypothesis at concentration α if $D > c(\alpha)\sqrt{\frac{n_1+n_2}{n_1 n_2}}$, where n_1 and n_2 are the sizes of the respective sets and $c(0.01) = 1.63$.

2.2. Box-Cox transformation

A powerful feature of the Box-Cox transformation is that it can be used to normalize data by removing skewness [9] (Supplementary Figs. 1 and 2). It has been used extensively to transform both left and right-skewed biological data to normality [10–16]. The transformation in question is:

$$x_i^{(\lambda)} = \begin{cases} \dfrac{x_i^{\lambda} - 1}{\lambda}, & \lambda \neq 0 \\ \ln(x_i), & \lambda = 0 \end{cases}$$

To deduce the transformation when $\lambda = 0$, simple rewriting using the Maclaurin series of the exponential function is used:

$$\lim_{\lambda\to 0}\frac{x_i^{\lambda}-1}{\lambda} = \lim_{\lambda\to 0}\frac{e^{\lambda\ln(x_i)}-1}{\lambda} = \lim_{\lambda\to 0}\frac{\left(1+\lambda\ln(x_i)+\frac{1}{2}\lambda^2\ln^2(x_i)+\ldots\right)-1}{\lambda}$$
$$= = \lim_{\lambda\to 0}\ln(x_i)+\frac{1}{2}\lambda\ln^2(x_i)+\ldots = \ln(x_i)$$

An example demonstrating the capabilities of the Box-Cox transformation is given in Supplementary Fig. 2, where both right and left-skewed data were randomly generated and later normalized using the transformation with a selected λ value. To determine which λ value to use, a search algorithm was used, which found the λ value that maximized the Log-Likelihood Function. This algorithm was also used with the received cTnT data. Note that the algorithm finds a specific optimized λ value for each dataset provided, so some statistical testing must be carried out to ensure that the calculated λ value can be used for new data as well.

2.3. The predictive value among lookalikes function

The predictive value among lookalikes (PAL) is a method to describe

the percentage with disease among patients with similar biomarker concentrations from a set of data (Fig. 1), i.e. it calculates the posterior probability of the event happening. However, as the dataset used in the PALfx calculations is restricted to the study of patients with biomarker concentrations close to those of the actual patient, the resampling stability becomes a problem if small datasets are used (Fig. 1). To calculate the probability of having a myocardial infarction, given a certain cTnT value c, Bayes' theorem is used:

$$P(MI \mid cTnT = c) = \frac{P(cTnT = c \mid MI)P(MI)}{P(cTnT = c)}$$

The right-hand side can be rewritten as:

$$P(MI \mid cTnT = c) = \frac{P(cTnT = c \mid MI)\,P(MI)}{P(cTnT = c \mid MI)P(MI) + P(cTnT = c \mid nMI)\,P(nMI)} \quad (1)$$

where $P(MI)$ (resp. $P(nMI)$) is the prior probability of having (resp. not having) myocardial infarction and $P(cTnT = c|\ MI)$ (resp. $P(cTnT = c|nMI)$) is the conditional probability of having a certain cTnT-level given that you have (resp. not have) myocardial infarction.

If normality is assumed, based on optimal Box-Cox-transformed cTnT values, each transformed with the optimal λ for that group in the datasets, the probability density functions of patients with or without MI can be described as:

$$P(cTnT = c \mid nMI) = \frac{1}{\sigma_{nMI}\cdot\sqrt{2\pi}} \exp\left(-\frac{(c-\mu_{nMI})^2}{\sigma_{nMI}^2}\right) := f_{nMI}(c)$$

$$P(cTnT = c \mid MI) = \frac{1}{\sigma_{MI}\cdot\sqrt{2\pi}} \exp\left(-\frac{(c-\mu_{MI})^2}{\sigma_{MI}^2}\right) := f_{MI}(c)$$

The prevalence-adjusted PALfx can thus be calculated at any disease prevalence:

$$PALfx(c) = \frac{f_{MI}(c)}{f_{MI}(c) + \beta f_{nMI}(c)}$$

where $\beta = \frac{P(MI)}{P(nMI)}$.

If a distribution fitting is made of the collected data, a continuous PALfx function can be found. This function will not only approximate the probability of MI for the available data points, but for all possible cTnT values.

2.4. The study groups

The study cohort comprised 15,425 ED admissions with a chief complaint of chest pain, at the EDs at Mölndal hospital and Östra hospital between 1 February 2012 and 31 December 2013, and at Sahlgrenska hospital between 1 February 2012 and 15 December 2015, all in Gothenburg, Sweden (Table 1). These EDs did not perform point-of-care troponin analyses during the study period and all cTnT evaluations were made using the high-sensitivity cTnT assay in the central lab.

The number of ED admissions with MI was 941. The number of ED admissions without MI was 14,484. The numbers of ED admissions without MI in the four subgroups were 3413 for males > 60 years, 4331 for males ≤60 years, 4106 for females > 60 years and 2634 for females ≤60 years of age.

Study data were retrieved from the hospital's administrative database with information on diagnoses, primary complaint, personal identification number and time of arrival at the ED. Data entered in the local hospital database are used to distribute funds within the hospital, thus adding an economic incentive for the departments to keep this record complete.

All laboratory data were retrieved from the central lab database, including the personal identification number, time of blood sampling and biomarker concentrations. Entry of ordering time and patient identity in the laboratory database is a part of the test ordering procedure and is automatic. It is not possible to obtain a test result from the central lab without a complete registration. A characterization of admitted patients with chest pain and adjudication of the MI diagnoses [17,18] at these EDs from another time period has been published before [19,20]. Patients with creatinine concentrations > 100 μmol/L were excluded, as low kidney function increases cTnT concentrations, especially when creatinine levels are above 100 μmol/L, as we have shown before [21,22]. The clinical evaluation of cTnT concentrations in relation to creatinine levels was not systematic although clinicians were alerted to the fact that cTnT levels might overestimate the risk of MI in patients with low kidney function. At the time of the study clinicians were not provided with calculations of eGFR from creatinine concentrations and were not part of the clinical evaluation. We therefore excluded patients with creatinine levels > 100 μmol/L, which have previously been shown to affect cTnT levels, possibly making the clinical evaluation of the patients uncertain. A separate PALfx equation possibly needs to be developed for patients with increased creatinine concentrations. All cTnT measurements were made with the high-sensitivity cTnT assay (Roche), and concentrations down to 5 ng/L were reported to the attending clinician. The Cobas analytical machinery (Roche) that was used during the study does not give values below 3 ng/L. Therefore, cTnT concentrations ≤ 3 ng/L, which, in principle, could have values from 0 to 3 ng/L, were excluded to allow an optimal distribution fitting to normality.

The high-sensitivity cTnT decision limit point for MI was the 99th percentile of 14 ng/L, as recommended by the European Society of Cardiology [23]. MI patients were diagnosed at the discretion of the attending clinician by reviewing available clinical and laboratory data collected during the hospital stay. No external adjudication of the MI diagnosis was carried out. The study was approved by the Ethics Committee at the University of Gothenburg, and the study protocol followed the ethical guidelines of the Declaration of Helsinki.

2.5. Laboratory measurements

Cardiac troponin T was measured using the high-sensitivity cardiac

Table 1
Characteristics of patients included in this study.

Characteristics of the study cohort		
	All ED visits	ED visits with cTnT > 3 ng/L*
Number of ED visits	19,648	15,425
Male sex (%)	50	54
Age (median. IQR)	58 (43–71)	62(49–74)
Sahlgrenska Hospital (%)	49	47
Mölndal Hospital (%)	13	14
Östra Hospital (%)	38	39
Myocardial infarction† (%)	4.8	6.1
cTnT (all) (ng/L) (median. IQR)	6.3 (3.5–12.9)	8.2 (5.3–16.0)
cTnT (No MI) (ng/L) (median. IQR)	6.1 (3.3–11.4)	7.8 (5.2–14.0)
cTnT (MI) (ng/L) (median. IQR)	49.4 (22.4–129.8)	49.5 (22.6–130.2)
Creatinine (all) (μmol/L) (median. IQR)	73 (63–83)	75 (64–85)
Creatinine (No MI) (μmol/L) (median. IQR)	73 (62.9–83)	74.8 (64–85)
Creatinine (MI) (μmol/L) (median. IQR)	78.4 (68.1–87)	78.4 (68.2–87)

ED = Emergency department. cTnT = Cardiac troponin T. IQR = Interquartile range.

* This cohort was used to derive the PALfx functions.

† Only Non-ST-elevation myocardial infarction (NSTEMI) were included.

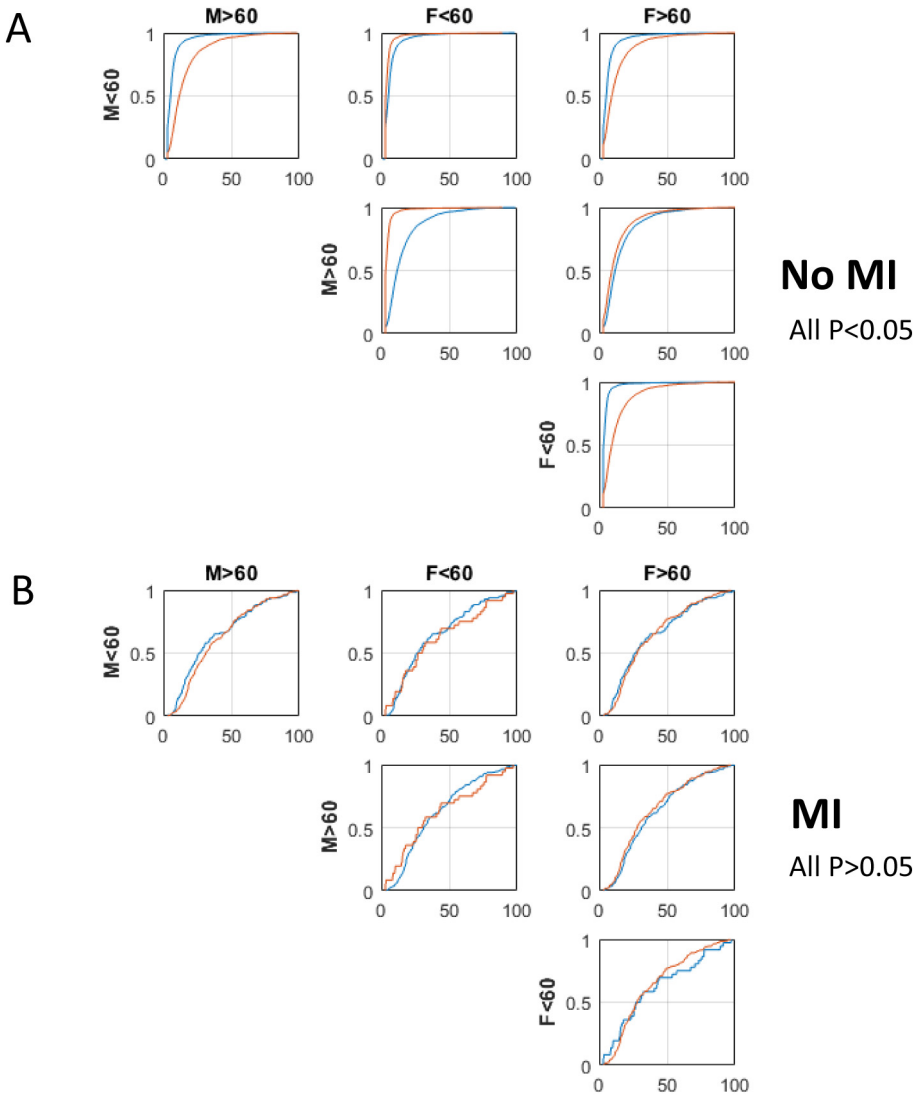

Fig. 2. Kolmogorov-Smirnov plots on subgroups. A. Kolmogorov–Smirnov plot of n males above 60 years of age and females below 60 years of age without myocardial infarction (MI). B. Kolmogorov-Smirnov plot of n males above 60 years of age and females below 60 years of age with myocardial infarction. M = Male, F = Female. The number of patients in each subgroup without *MI* was 3413 for $M > 60$, 4331 for $M \leq 60$, 4106 for $F > 60$ and 2634 for $F \leq 60$. The number of patients with MI was 415 for $M > 60$, 163 for $M \leq 60$, 316 for $F > 60$ and 47 for $F \leq 60$.

troponin T assay (cTnT) (Roche), analyzed by the accredited central lab (ISO 15189). The local performance of the cTnT assay has been reported previously [24]. Only the first cTnT analysis result recorded during each ED visit was used.

3. Results

3.1. Distribution of cTnT concentrations in the ED cohort

The Kolmogorov-Smirnov test suggested the existence of four subgroups with different cTnT distributions among ED patients without MI. In contrast, patients with MI had similar cTnT distributions in the entire dataset (Fig. 2). All subgroups had a highly right-skewed cTnT distribution (Supplementary Fig. 1) and Box-Cox transformation was therefore applied to transform non-normal datasets to normal distributions (Fig. 3). Similar to log transformation, Box-Cox transformation allows compression of data at the high end of the dataset, but has the added advantage of allowing graded compression of both right-skewed [13] and left-skewed [11] data (Supplementary Fig. 2) to normality with a choice of the λ factor used in the Box-Cox transformation. Hence, unique λs were used to transform each of the four different subgroups of patients without MI, whereas a single λ was used to transform data from all patients with MI (Table 2).

3.2. Predictive value among lookalikes (PAL) from density functions

Box-Cox-transformed data from the four subgroups without MI and all data from patients with MI followed the normality density functions with unique means and standard deviations. These density functions allowed parametric calculation of the PAL as a function of the cTnT concentrations, the PALfx (Fig. 4, Table 3). We compared the resampling stability of the PALfx and the empirical PAL (ePAL) [7] using 5000 random resamples with replacement; bootstrapping. As expected, since the PALfx was based on means and standard deviations from the datasets, the variation in the PALfx was relatively small when we bootstrapped from the entire dataset (Fig. 4, Supplementary Fig. 3). In contrast, the ePAL generated risk "steps" at different cTnT concentrations with wider margins of variation (Fig. 4). We further tested the stability of our PALfx method by bootstrapping smaller and smaller datasets. Each new dataset was Box-Cox-transformed and used to derive new density functions and recalculation of the PALfx at relevant cTnT concentrations (Fig. 5). The PALfx resampling stability was similar at

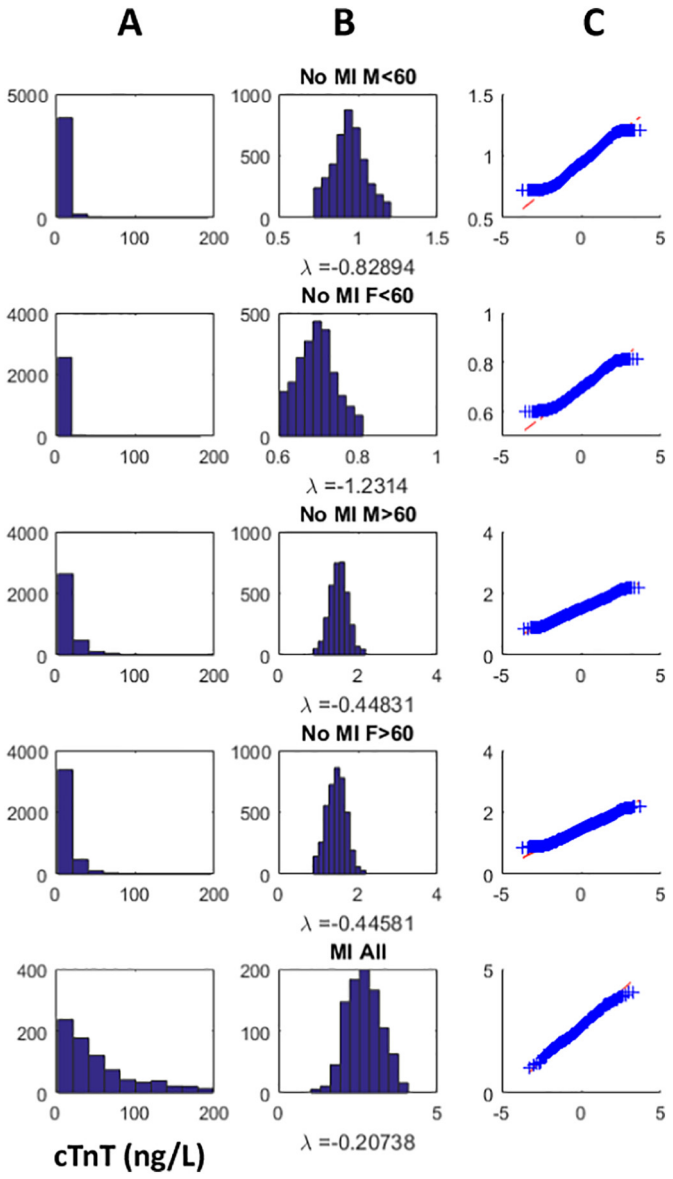

Fig. 3. Box-Cox transformation of cTnT concentrations. Distribution of cTnT concentrations without (A) or with (B) optimal Box-Cox transformation. Q-Q plots (C), a way to visualize a direct comparison of data to perfect normality, of Box-Cox-transformed cTnT concentrations in the four subgroups without MI and among patients with MI. The optimal lambda value used in the Box-Cox transformation is also included.

Table 2
Optimal λ for each subgroup from the entire dataset from three hospitals. M = Male, F = Female, MI = myocardial infarction. The mean optimal λ and its standard deviations were calculated from 5000 bootstraps.

Group	Optimal λ	Mean	Standard deviation
$M \leq 60$	−0.8289	−0.8288	0.0106
$M > 60$	−0.4483	−0.4482	0.0136
$F \leq 60$	−1.2314	−1.2312	0.0210
$F > 60$	−0.4458	−0.4452	0.0137
MI	−0.2074	−0.2076	0.0198

sample sizes above 1000. The sample size that resulted in a doubling of the sample error was around 500 and was similar in the subgroups (Supplemental Table 1). Finally, when data from the three hospitals were used separately, the PALfx remained similar (Fig. 6).

4. Discussion

Biomarker concentrations are often evaluated using decision limits aimed at separating patients with or without disease. Sometimes, small differences in biomarker concentrations around a decision limit result in markedly different workups and treatments [25]. This is especially problematic when the cardiac damage biomarker cTnT is used to evaluate the risk of MI in patients with chest pain. The internationally accepted decision limit of cTnT is 14 ng/L, a concentration based on cTnT concentrations in healthy individuals [26]. The cTnT concentrations found among patients without MI in the ED are very different and highly age-dependent [3,27], making the use of a single decision limit problematic. For instance, male cTnT concentrations are roughly twice as high as female concentrations [3,28], fostering a rich debate concerning sex-specific decision limits [29–31].

Among patients without MI in the ED, cTnT concentrations vary within a 20-fold range [4] and often rival the concentrations seen in patients with MI. This is especially true among patients of old age, in patients with reduced kidney function and in patients with heart failure. The risk of MI increases linearly between cTnT concentrations of 5–52 ng/L. At higher concentrations, the MI frequency levels out, and remains at roughly the same frequency even at cTnT concentrations over 100 ng/L as most patients with cTnT concentrations above 52 ng/L have MI [32,33]. Therefore, the risk estimation needs to be patient-specific for those patients whose cTnT concentrations are between 5 and 52 ng/L.

If biomarker concentrations could be converted to risk of disease, the patient-specific risk of disease could be used to complement the decision limits. This way of thinking is currently in use when patients are evaluated for cholesterol-lowering therapy [34] and the risk of deep venous thrombosis and pulmonary embolism with the D-dimer biomarker [35].

No conventional parameter answers the clinicians question, “How likely is it that my patient presenting with this specific cTnT concentration has MI?”

The project presented here started as predictive values, sensitivity and specificity, ROC plots or Baysean reasoning, and likelihood ratios do not result in a patient-specific risk of disease. All these methods use study individuals with biomarker concentrations above or below a given biomarker limit and therefore fail to estimate the risk of disease at a specific biomarker concentration. We have therefore developed the Predictive value Among Lookalikes, PAL, parameter, which calculates the percentage with disease within a range around the actual patient biomarker concentration (Fig. 1) [7].

In this report, we describe a parametric way to calculate the Predictive value Among Lookalikes (PAL) by converting cTnT concentrations from our emergency ward to normality by Box-Cox transformation. Biomarkers are often right-skewed with a small proportion of individuals with high concentrations. For this reason, it is often necessary to transform biomarker data before they can be fitted to normality functions. Several reports indicate that the more adjustable Box-Cox transformation is superior to log transformation for most biomarkers [36], and it is now the recommended transformation method when reference limits are calculated [10,37]. In agreement with this, we observed that it was not possible to log transform the cTnT datasets to normality.

There are several advantages of the PALfx, compared with positive Predictive Values (PPV) and other versions of binary classification including binary logistic regression.

Firstly, the PALfx gives the risk of disease at the patient-specific biomarker concentration. This is in contrast to the PPV and conventional binary logistic regression, which only gives the risk above a given limit. The PPV often overestimates the risk of disease of the actual patients, as the PPV is calculated using all patients with cTnT concentrations above the decision limit, often including many patients with higher concentrations than the actual patient.

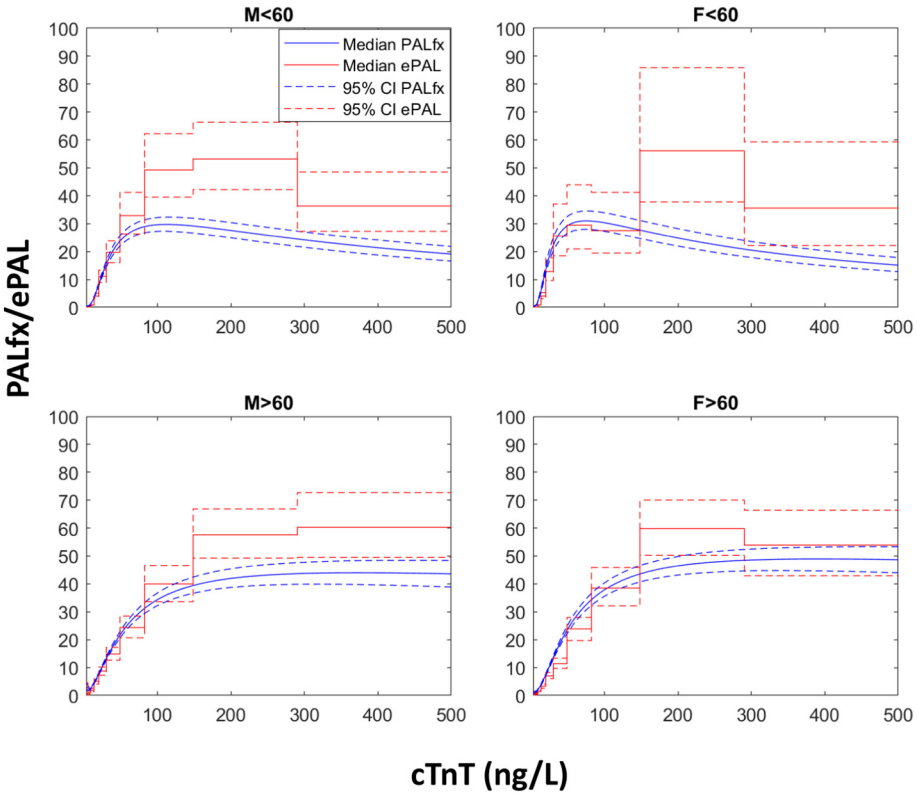

Fig. 4. PALfx and ePAL plots. PALfx (parametric PAL) (blue) and ePAL (empirical PAL) (red) calculated for the four different subgroups as a function of cTnT concentrations. The non-parametric 95% confidence interval based on 5000 bootstraps is shown as a dotted line. The number of ED admissions with *MI*, 941, was the same in each plot. For ED admissions without *MI*, the number of ED admissions in each plot was 3413 for $M > 60$, 4331 for $M \leq 60$, 4106 for $F > 60$, and 2634 for $F \leq 60$. Area scaling were used as described in materials and methods to compensate for pre-test prevalence and generate actual PALfx and ePAL values. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3
An example of the PALfx-calculated risk of myocardial infarction at a given baseline cTnT concentration, age and sex in the study cohort of patients with a main complaint of chest pain in our emergency department.

	cTnT (ng/L)[†]		
Sub group[*]	7 ng/L	14 ng/L	25 ng/L
M < 60	0.5 (0.4–0.6)	3.6 (3.3–3.9)	11.8 (11.0–12.7)
F < 60	0.5 (0.4–0.6)	6.3 (5.6–7.0)	18.4 (16.5–20.5)
M > 60	1.9 (1.5–2.2)	4.5 (4.1–4.9)	10.3 (9.8–10.8)
F > 60	1.2 (1.0–1.4)	3.9 (3.6–4.3)	10.3 (9.8–10.9)

[†] Median and 95% confidence intervals of risk of having MI (in percent) at cTnT concentrations of 7, 14 and 25 ng/L. 95% confidence intervals confidence intervals were from 5000 bootstraps.

[*] Sub groups were as follows: M < 60; Male with age < 60 years, F < 60; Female with age < 60 years, M > 60; Male with age > 60 years, F > 60; Female with age > 60 years.

Secondly, the PALfx is derived from continuous mathematical functions describing the distribution of the biomarker concentrations in patients with or without MI. Therefore, the PALfx gives a reasonable resampling stability despite being able to estimate the risk of disease at a specific biomarker concentration. This is due to the fact that all study data have been included when determining the density functions used in the PALfx. Therefore, in principle, data at the low range influence the PALfx at high concentrations and vice versa, making the risk estimates stable against resampling. The PALfx resampling stability was not very different when using datasets of 2000 (1900 without MI and 100 with MI) or 1000 (950 without MI and 50 with MI) ED admissions. The bootstrap sample-to-sample variation increased twofold when datasets of around 500 (475 without MI and 25 with MI) were used. This means that if the PALfx were to be calculated from a new, similar dataset where 5% had MI, our bootstrap estimates indicate acceptable variation when datasets of 500 ED patients or more are used. A high resampling stability from small datasets is important as we also see that separate

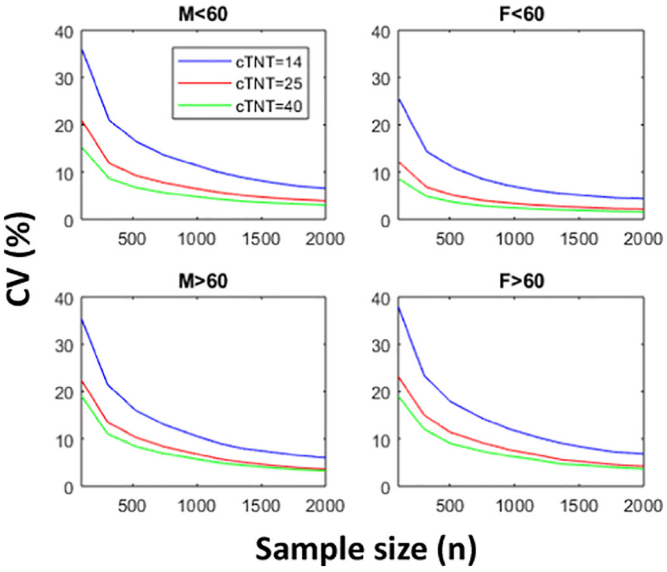

Fig. 5. The resampling stability of the PALfx at different sample sizes. The coefficient of variation (SD as % of the mean value) of the PALfx at clinically relevant cTnT concentrations of 14, 25 and 40 ng/L was calculated using 5000 bootstraps of different sample sizes (n). In each bootstrap, the relationship between patients with or without MI was preserved as in the full dataset. The full dataset consisted of 941 ED admissions with *MI*, used in all four plots, and for ED admissions without *MI*, the number of ED admissions was 3413 for $M > 60$, 4331 for $M \leq 60$, 4106 for $F > 60$, and 2634 for $F \leq 60$.

PALfx equations are required for different patient groups, limiting the number of patients in each group.

A promising possibility is to use local hospital statistics to identify stable admission parameters, including chief complaint, age and gender, which distinguish between patients with different biomarker distributions. Local hospital statistics and laboratory data can be used,

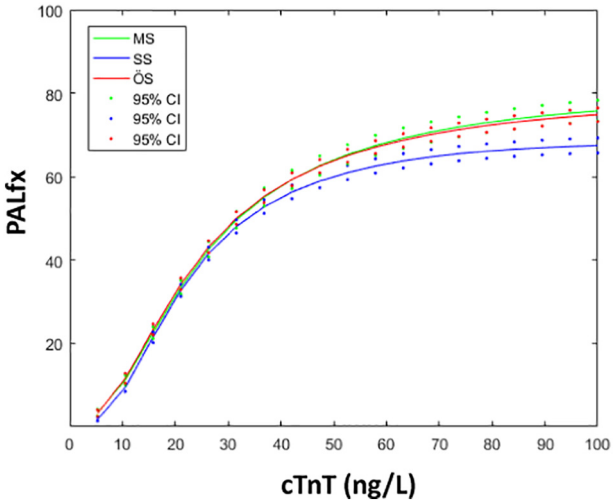

Fig. 6. PALfx calculations using datasets from three different hospitals (Mölndal Hospital (MS), Sahlgrenska Hospital (SS) and Östra Hospital (ÖS)) without area scaling and thus assuming identical pre-test prevalences of MI ($\beta = 1$) to allow comparison between hospitals as the pre-test prevalence of MI differed between hospitals. The 95% confidence interval based on 5000 bootstraps is shown as dots, as shown in the figure legend.

after appropriate Box-Cox transformation, to generate the PALfx for a biomarker, as we have done here for cTnT and MI at our ED. The PALfx-calculated risk of disease can then be used in combination with local procedures to triage a patient similar to how the HEART score and TIMI score are used at some EDs [38–40] and advocated by European guidelines when patients with suspected MI are triaged [41]. Thus, the final risk for MI in a patient will depend on several laboratory and clinical parameters that in principle could be transformed using our PALfx method and therefore further improve the risk stratification beyond what is provided by cTnT levels, age and sex.

Some diagnoses, like MI, are defined, in part, on the basis of biomarker concentrations. It is therefore not surprising that the distribution of cTnT concentrations is similar among patients with MI, although the distribution of cTnT concentrations among patients without MI differs depending on age and sex. This situation is fortunate, as the patients without disease always outnumber the patients with disease, more than by a factor of ten in most instances, and this is always the limiting factor when small datasets are used.

It is, however, important to note that these PALfx calculations were made using limited numbers of patients from three hospitals that use similar triage systems and the same decision limits for cTnT. Our PALfx algorithms must be validated before our PALfx equations for cTnT concentrations can be used as a general tool to estimate the risk of MI in the ED. Secondly, patients with elevated creatinine concentrations were excluded in this study because we have shown that poor kidney function results in increased cTnT concentrations and could make the clinical evaluation of patients with chest pain uncertain. Likely, a separate study needs to be done to generate a PALfx for patients with impaired kidney function. Third, other datasets may not be able to be transformed to normality by Box-Cox transformation and limit the general usability of our PALfx method. Lastly, cTnT concentrations are often evaluated at several time points during the ED visit to evaluate cTnT change. In this report we only use the baseline cTnT level to calculate the risk of MI, as it is still being debated which change in the cTnT level signifies MI [3,42,43]. Indeed, over 25% of patients with non-ST elevation MI (NSTEMI) have less than a 20% change in the cTnT concentration over six hours, likely because they present late in the infarction process [18]. However, once a cTnT change cutoff has been established, this could be included in the PALfx calculations to streamline the risk estimates further.

In principle, our data indicate that the PALfx can complement decision limits and reference intervals in clinical decisions.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clinbiochem.2019.02.003.

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