

Contents lists available at [ScienceDirect](http://ScienceDirect.com)

# Clinical Biochemistry

journal homepage: [www.elsevier.com/locate/clinbiochem](http://www.elsevier.com/locate/clinbiochem)

## Risk of myocardial infarction at specific troponin T levels using the parameter *predictive value among lookalikes* (PAL)

Ola Hammarsten <sup>a,\*</sup>, Elvar Theodorsson <sup>b</sup>, Christian Bjurman <sup>a</sup>, Max Petzold <sup>c</sup><sup>a</sup> Department of Clinical Chemistry and Transfusion Medicine, Institute of Biomedicine, The Sahlgrenska Academy at the University of Gothenburg, SE-41345 Gothenburg, Sweden<sup>b</sup> Department of Clinical Chemistry and Department of Experimental Medicine, Linköping University, Linköping, Sweden<sup>c</sup> Centre for Applied Biostatistics, The Sahlgrenska Academy at the University of Gothenburg, SE-41345 Gothenburg, Sweden

### ARTICLE INFO

#### Article history:

Received 14 July 2016

Received in revised form 7 September 2016

Accepted 8 September 2016

Available online xxxxx

### ABSTRACT

**Background:** Myocardial infarction is more likely if the heart damage biomarker cardiac troponin T (cTnT) is elevated in a blood sample, indicating that cardiac damage has occurred. No method allows the clinician to estimate the risk of myocardial infarction at a specific cTnT level in a given patient.

**Methods:** *Predictive value among lookalikes* (PAL) uses pre-test prevalence, sensitivity and specificity at adjacent cTnT limits based on percentiles. PAL is the pre-test prevalence-adjusted probability of disease between two adjacent cTnT limits. If a chest pain patient's cTnT level is between these limits, the risk of myocardial infarction can be estimated.

**Results:** The PAL based on percentiles had an acceptable sampling error when using 100 bootstrapped data of 18 different biomarkers from 38,945 authentic lab measurements. A PAL analysis of an emergency room cohort (n = 11,020) revealed that the diagnostic precision of a high-sensitive cTnT assay was similar among chest pain patients at different ages. The higher incidence of false positive results due to non-specific increases in cTnT in the high-age group was counterbalanced by a higher pre-test prevalence of myocardial infarction among older patients, a finding that was missed when using a conventional ROC plot analysis.

**Conclusions:** The PAL was able to calculate the risk of myocardial infarction at specific cTnT levels and could complement decision limits.

© 2016 The Authors. Published by Elsevier Inc. on behalf of The Canadian Society of Clinical Chemists. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

### 1. Introduction

The clinical utility of a biomarker is often evaluated using binary classification that results in a fraction of patients with or without the disease below or above a given biomarker level [1–6]. Some examples are sensitivity (Sn) and specificity (Sp), where patients with or without disease are analyzed separately (Fig. 1A), and negative and positive predictive values (NPV and PPV), where patients with and without disease are analyzed together (Fig. 1A and Supplemental Tables 1 and 2).

An emergency room physician assessing the result of the heart damage biomarker cardiac troponin T (cTnT) in a patient with chest pain is often occupied with the following two questions:

1. If I exclude using the cTnT level in my patient, what fraction of patients with myocardial infarction (MI) will I miss?
2. At the patient level of cTnT, what is the probability of MI?

The answer to the first question is simply  $1 - S_n$  at the patient's cTnT level; that is, the fraction of patients with MI presenting with cTnT levels below the actual patient's level.

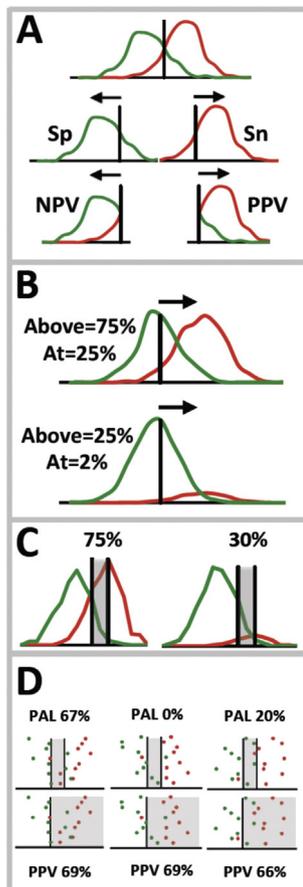
The probability of disease at a given cTnT level is more complicated. The MI prevalence at a specific cTnT level increases along with the pre-test MI prevalence, and is increased by age, smoking habits, male sex and so forth [7]. Therefore, to answer how likely it is that this particular patient has an MI, the calculations must also involve an estimate of the patient's pre-test prevalence of MI.

Unfortunately, most binary classifications do not adjust for the pre-test prevalence of disease. In addition, binary classification results in a fraction with disease above or below a given biomarker level, not the probability of disease at the patient's biomarker level (Fig. 1B).

There are ways to use binary classification in a pre-test prevalence-sensitive manner, such as the likelihood ratio combined with Bayesian reasoning [8] and prevalence-adjusted predictive values [9,10].

However, like all binary classification, these calculations use the fraction of patients above or below a given biomarker level and hence fail to estimate the prevalence of disease at a specific biomarker level (Fig. 1B). Slopes calculated from an ROC plot is one way to do this [11,12]. However, this method has not been extensively used.

\* Corresponding author at: Newcastle University, Australia, Institution of Biochemistry, Department of Clinical Chemistry and Transfusion Medicine, Bruna Stråket 16, Sahlgrenska Academy at the University of Gothenburg, SE-41345 Gothenburg, Sweden.  
E-mail address: [ola.hammarsten@clinchem.gu.se](mailto:ola.hammarsten@clinchem.gu.se) (O. Hammarsten).



**Fig. 1.** A. Visual demonstration of calculation of the specificity (Sp), the sensitivity (Sn), the negative predictive value (NPV), and the positive predictive value (PPV). Frequency plots of simulated data from individuals without (green line) and with (red line) disease. The black vertical line indicates the biomarker action limit (see also Supplemental Tables 1 and 2). B. Frequency plots of simulated patient populations without (green line) or with (red line) disease at a 50% and 10% disease prevalence. Numbers indicate the disease prevalence above or at the biomarker level indicated by black vertical lines. C. Visual representation of calculation of the *predictive value among lookalikes* (PAL). Frequency plots of simulated patient populations without (green line) or with (red line) disease at a 50% or 10% disease prevalence. The pairs of vertical lines represent the low and high biomarker level used to calculate the PAL (see text and Supplemental Fig. 1 for details). The PAL is the disease prevalence (numbers) in the shaded area between the two biomarker levels. D. Visual representation of the sampling error of the PAL in relation to the PPV from 20 normally distributed simulated patient data without (green dots) or with (red dots) disease at a 50% disease prevalence. The numbers are the PAL calculated from data between the two biomarker levels and the PPV calculated from data above the action limit, indicated by black vertical lines. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

At present, common methods using binary classification are unable to calculate the answer to the clinician's second question: "What is the risk of MI at my patient's level of cTnT?"

Here, we describe a parameter that makes it possible to calculate the probability of disease at a specific biomarker level, *predictive value among lookalikes* (PAL). The PAL is calculated using the pre-test prevalence in combination with Sn and Sp between two adjacent biomarker levels. The PAL can be used to generate pre-test prevalence-sensitive PAL plots that visualize the interplay between the probability of disease and the risk of missing a patient with the disease at specific biomarker levels.

## 2. Materials and methods

### 2.1. Simulations

Simulations were done in Excel 2011 for Mac OS 2011. Biomarker data from healthy individuals were retrieved from the Nordic Reference

Interval Project 2000 (NORIP) database [13] (n ranging from 952 to 2740 for the 18 biomarkers analyzed). Diseased populations were simulated by scaling, where a fixed factor was added to each lab data from the NORIP database. These simulated disease data were used to assess the sampling error of PAL.

The sampling error is the variation in PAL when PAL is repeatedly calculated, typically 1000 times, from randomly selected subgroups of data. If the data in the subgroups are randomly selected with replacements called bootstrapping, each randomly selected subgroup of data will essentially be unique.

In these calculations, data with or without the added factor were randomly sampled with replacements. In each bootstrap, 100 authentic lab data from the NORIP database from individuals without disease and 100 lab data with the added factor (i.e., the simulated diseased population) were randomly selected.

From the bootstrapped data the Sn and the Sp were calculated at 21 biomarker limits derived from percentiles from the bootstrapped data (Supplemental Fig. 1).

The Sn and the Sp were either the fraction of data above or below the 21 percentiles (non-parametric PAL calculation) or the integrated area under the normal distribution at the 21 percentiles using the mean and standard deviation (SD) from log-transformed data (parametric PAL calculation, Supplemental Fig. 1).

The Sn and the Sp were used to calculate the PAL at 20 regions; consequently, each PAL calculation included 5% of the combined bootstrapped data from healthy and simulated diseased populations. The mean and the 2.5th and the 97.5th percentiles of the Sn, Sp and PAL from 1000 bootstraps were plotted to examine the general trend and the sampling error of the different methods to calculate the Sn, Sp and PAL.

### 2.2. Analysis of PAL of high-sensitive cardiac troponin T

The study cohort comprised 11,020 visits to the emergency department (ED) at Mölndal or Östra Hospital at the Sahlgrenska University Hospital in Göteborg, Sweden, with a chief complaint of chest pain between January 2010 and December 2013. A characterization of admitted patients with chest pain at these EDs from another time period has been published before [14,15]. Only males with creatinine levels below 100  $\mu\text{mol/L}$  were included, as kidney function strongly affects cardiac troponin levels [16]. This resulted in the exclusion of 601 patients who lacked a creatinine measurement in the laboratory database. Until the end of January 2012, the hs-cTnT cutoff point for myocardial infarction (MI) was 40 ng/L. In February 2012, the hs-cTnT cutoff point for MI was changed to 14 ng/L. No point-of-care troponin assay was in use during the study and all troponin evaluations were made using the high-sensitive cardiac troponin T assay (hs-cTnT) (Roche), analyzed by the central lab. The local performance of the hs-cTnT assay has been reported [17]. Only the first hs-cTnT analysis result recorded during each ED visit was used in Figs. 4 and 5. The final diagnosis of each ED visit was retrieved from the hospital registry. Laboratory data were retrieved from the local laboratory database. The study was approved by the Ethics Committee at the University of Gothenburg. The calculations of parametric and non-parametric Sn, Sp and PAL were made using Excel 2011 for Mac OS 2011, as described in the text on log-transformed hs-cTnT data. hs-cTnT limits were derived from 16 percentiles across the MI cohorts.

## 3. Results and discussion

If the pre-test prevalence of disease is 50%, then the PPV, the probability of disease above a given biomarker level, can be calculated from the Sn and Sp.

$$\text{PPV} = \frac{\text{Sn}}{\text{Sn} + (1 - \text{Sp})} \quad (1)$$

If the odds of not having the disease are included in this equation, a prevalence-adjusted PPV (PAP(+)) can be calculated at any disease prevalence [9,10]:

$$PAP(+) = \frac{Sn}{Sn + \beta(1-Sp)} \quad (2)$$

where  $\beta$  is the ratio of patients with the disease to those without the disease.

$$\beta = \frac{n_{no\ disease}}{n_{disease}} \quad (3)$$

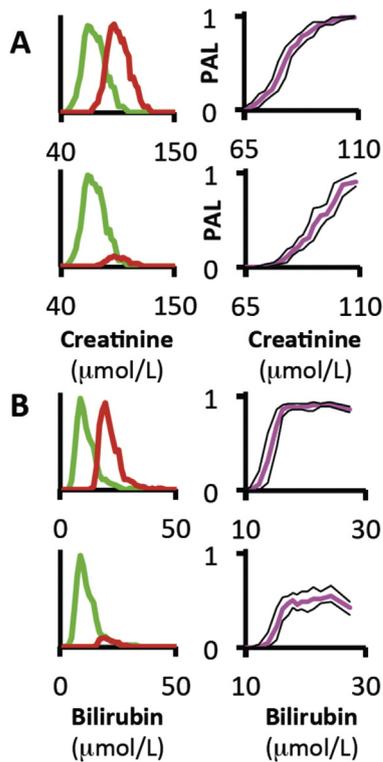
The same principle can be used to calculate the disease prevalence between two biomarker levels, the *predictive value among lookalikes* (PAL) (Fig. 1C, Supplemental Fig. 1):

$$PAL = \frac{Sn_{Low} - Sn_{High}}{(Sn_{Low} - Sn_{High}) + \beta(Sp_{High} - Sp_{Low})} \quad (4)$$

where  $Sn_{Low}$  and  $Sn_{High}$  are the sensitivity and,  $Sp_{Low}$  and  $Sp_{High}$  are the specificity at the adjacent lower and upper biomarker limits, respectively.

If the range between the upper and the lower biomarker level includes the biomarker level found in a patient, the probability of disease can be estimated in a pre-test-sensitive way.

However, calculation of the PAL only involves study data between the upper and lower biomarker limits. For that reason, the PAL sampling error (see Materials and methods) may become unacceptably large if the number of study data or the distance between the two adjacent biomarker levels is small (Fig. 1D).



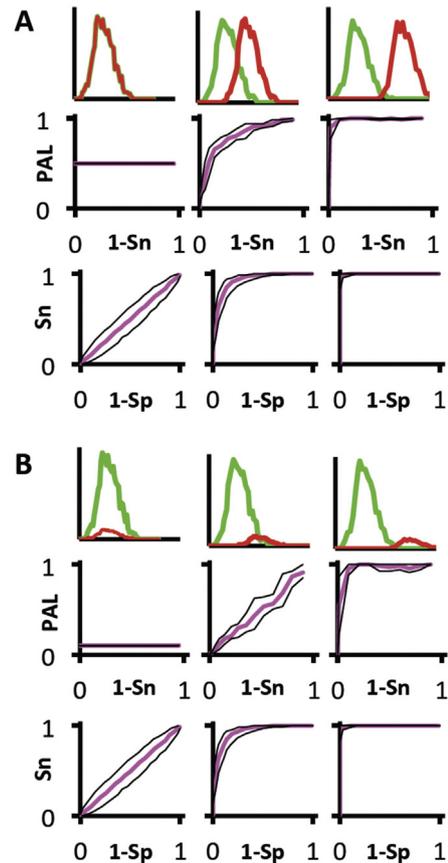
**Fig. 2.** Examples of PAL analysis of biomarker data. Frequency plots of creatinine (A,  $n = 2636$ ) or bilirubin (B,  $n = 2740$ ) levels in healthy individuals (green line) and simulated data on diseased individuals (red line) at a 50% or 10% disease prevalence. The mean PAL from 1000 bootstraps of 100 lab data from the green and red population is plotted (magenta line). The non-parametric 95% confidence limits from 1000 bootstraps are shown with thin black lines. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

We find, however, that the overall trend of how the PAL changes with biomarker levels is stable with an acceptable sampling error if the biomarker limits for the PAL are derived from percentiles. We have tested this using 21 limits across bootstrapped data sets of 100 lab data without disease and 100 simulated lab data with disease, so that the 20 PAL calculations involve at least 5% of the data (Fig. 2). This was done in order to avoid erratic sampling errors [18], if the  $Sp$  or the  $Sn$  is close to zero at the low or high end of the data sets.

The 95% confidence limits of the PAL grow wider as the data from individuals without disease are amplified with the  $\beta$  factor in the PAL equation (Fig. 2). This is particularly evident if the data have a log-normal distribution like bilirubin (Fig. 2B).

It is possible to plot the  $1-Sn$  against the PAL (Fig. 3). The resulting PAL plot behaves like a ROC plot. The advantage, compared with the ROC plot, is that the data on the longitudinal axis are affected by pre-test prevalence and that the data on the horizontal axis describe the risk of missing the disease ( $1-Sn$ ). Therefore, the area under the PAL plot (AUC) is dependent both on the pre-test prevalence and the biomarkers' ability to separate patients with and without disease (Fig. 3)

In this setting, a biomarker that fails to separate individuals with or without disease generates a PAL plot with a horizontal line at the disease prevalence. The horizontal line gives an AUC of 0.5 at a 50% disease prevalence (Fig. 3A) and an AUC of 0.1 at a 10% disease prevalence (Fig. 3B). A perfect separation of individuals with or without disease generates an AUC close to 1 at any disease prevalence. Unlike ROC



**Fig. 3.** Comparison of a PAL plot and ROC plot analysis. Frequency plots of creatinine ( $n = 2636$ ) levels from a healthy population (green lines) and a simulated diseased population (red lines) at a 50% (A) and a 10% (B) disease prevalence. The separation between the green and red population was made to generate an area under the ROC curve (AUC) of 0.5, 0.95 and 0.99. The magenta PAL (upper) and ROC plots (lower) are means from 1000 bootstraps of 100 lab data from the green and the red populations. The non-parametric 95% confidence limits from 1000 bootstraps are shown with thin black lines. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

plots, PAL plots visualize the interplay between the pre-test-dependent disease prevalence and the risk of missing the disease at specific biomarker levels.

To be able to calculate the PAL at the patient's specific biomarker level, we propose to use parametric Sp and Sn. The Sp and the Sn are proportions that can be estimated from the cumulative area under the normal distribution function.

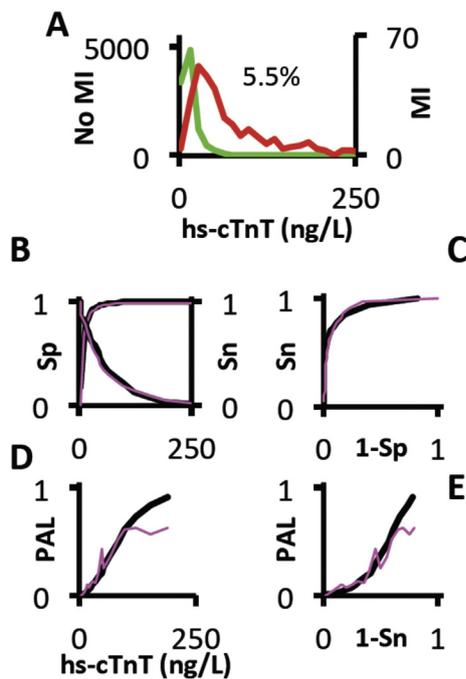
$$Sp = \left( \frac{1}{SD_h \sqrt{2\pi}} \right) \int_0^x e^{-\frac{(x-M_{no\ disease})^2}{2SD_{no\ disease}^2}} dx \quad (5)$$

$$Sn = 1 - \left( \frac{1}{SD_d \sqrt{2\pi}} \right) \int_0^x e^{-\frac{(x-M_{disease})^2}{2SD_{disease}^2}} dx \quad (6)$$

where x is the biomarker level,  $SD_{no\ disease}$  and  $SD_{disease}$  is the standard deviation, and  $M_{no\ disease}$  and  $M_{disease}$  is the mean from the study data for individuals with or without the disease (Supplemental Fig. 1).

As biomarkers often display non-Gaussian distribution, the parametric estimates of the Sp and the Sn are often better when using log-transformed data (Fig. 4). With the parametric approach using log-transformed data, the goodness of fit with the non-parametric PAL and sampling error (see Materials and methods) are often acceptable when the parametric PAL is calculated from 100 authentic lab data without disease and 100 simulated lab data with disease (Supplemental Figs. 2–4). However, for some biomarkers, like creatine kinase, AST and bilirubin, the parametric PAL is unable to generate an acceptable fit (Supplemental Figs. 2–4), as these biomarkers do not follow a normal or log-normal distribution when analyzed with Q-Q plot and P-P plot (data not shown).

In addition, the sampling error of a parametric PAL increases to a larger extent compared with a non-parametric PAL when the data from individuals without disease are amplified by the  $\beta$  factor



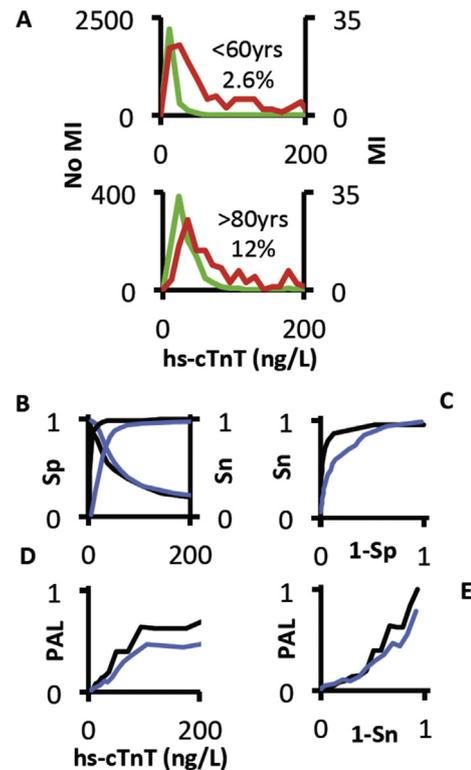
**Fig. 4.** Comparison of parametric and non-parametric PALs. Male patients with chest pain (n = 11,020) in the emergency department were analyzed with a high-sensitive troponin T analysis (hs-cTnT) at presentation. (A) Frequency plots of hs-cTnT levels in patients with (red lines) or without (green lines) myocardial infarction (MI). No MI = 10,369, MI = 608, MI frequency = 5.5%. Parametric (black lines) and non-parametric (magenta lines) calculations of specificity, sensitivity (B), ROC plot (C) and PAL plots (D, E) at 16 hs-cTnT percentiles across the MI cohort. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

(Supplemental Figs. 2–4). However, if the Sp and the Sn are defined in a mathematical equation, the PAL can be calculated at patient-specific biomarker levels.

The pre-test prevalence is a missing factor. Sometimes, the pre-test prevalence is known. For instance, the pre-test prevalence of MI is often 5%, if a patient presents with chest pain at an emergency department in Sweden [14]. It is, however, important to note that the pre-test prevalence depends on the clinical setting. It is likely that the chance of MI being present given a set of symptoms will be different in an emergency department with an efficient triage process than in a primary care setting.

The pre-test prevalence is often estimated using different score systems, like Well's score for deep venous thrombosis [19]. However, score systems use clinical findings and binary classification to calculate the probability of disease. Score systems are therefore also affected by the pre-test prevalence. Hence, at a given Well's score, the risk of deep venous thrombosis will be different, if Wells score is applied in an emergency department with an efficient triage or in a primary care setting. This possibly contributes to the variation in deep venous thrombosis prevalence of 0–38% at a moderate Well's score in validation studies [20].

A promising possibility is to use local hospital statistics to identify stable baseline parameters, like chief complaint, age and gender, which distinguish between patients with different pre-test disease prevalences. Local hospital statistics and laboratory data can be used to calculate the PAL for a biomarker in these risk categories, as we have done for myocardial infarction (MI) and the myocardial infarction biomarker, high-sensitive cardiac troponin T (hs-cTnT) (Figs. 4 and 5).



**Fig. 5.** PAL analysis of the cardiac damage biomarker high-sensitive cardiac troponin T (hs-cTnT) in different age groups. Male patients with chest pain in the emergency department were analyzed with a high-sensitive troponin T analysis (hs-cTnT) at presentation. Frequency plots of hs-cTnT levels in patients with (red lines) or without (green lines) MI in the age range <60 years (No MI = 5723, MI = 153, MI frequency = 2.6%) and >80 years (No MI = 1070, MI = 146, MI frequency = 12%). Non-parametric calculations of specificity, sensitivity (B), ROC plot (C) and PAL plots (D, E) at different levels of hs-cTnT on patients <60 years (black line) or >80 years old (blue line). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

PAL plot analysis on this chest pain population shows that at a given chance of missing MI ( $1-S_n$ ), the probability of MI is not very different among older and younger chest pain patients (Fig. 5). Apparently, the lower  $S_p$  of the hs-cTnT test due to non-specific minor hs-cTnT elevations among older patients (Fig. 5B) [21] is balanced by a higher pre-test prevalence of MI in older patients (Fig. 5E). According to the PAL plot, the hs-cTnT test ability to distinguish chest pain patients with or without MI was similar at different ages, a fact not visualized by the ROC plot analysis (Fig. 5D).

It is, however, important to note that these PAL calculations were made using non-parametric  $S_n$  and  $S_p$ , and can therefore not be applied to individual patients. In addition, the PAL for hs-cTnT must be further validated in a separate cohort prior to clinical use.

In summary, the PAL and PAL plots can visualize the interplay between “what fraction of patients with the disease will I miss if I exclude using my patient’s biomarker value?” and “what is the probability of disease in my patient?” and thereby complement decision limits.

### Acknowledgements

We wish to thank Catrin Wessman and Marija Cvijovic for their help with the mathematics and the Nordic Society of Clinical Chemistry (NFKK) for making samples and data from the Nordic Reference Interval Project Bio-bank and Database (NOBIDA) available to this project. We also wish to thank the Swedish Cancer Society, the Swedish Research Council, the Swedish Pain Foundation (SSF), the Assar Gabrielsson Cancer Research Foundation, LUA/ALF funding at the Sahlgrenska University Hospital, and the County Council of Östergötland for financial support.

### Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.clinbiochem.2016.09.012>.

### References

- [1] R.S. Galen, Predictive value and efficiency of laboratory testing, *Pediatr. Clin. N. Am.* 27 (4) (1980) 861–869.
- [2] R.S. Galen, S.R. Gambino, *Beyond Normality: Predictive Value and Efficiency of Medical Diagnoses*, John Wiley and Sons, Wiley Biomedical-Health Publication, 1975.
- [3] J.A. Knottnerus, F. Buntinx, *The Evidence Base of Clinical Diagnosis: Theory and Methods of Diagnostic Research*, 2nd ed. Wiley-Blackwell, Oxford, 2009.
- [4] X.-h. Zhou, N.A. Obuchowski, D.K. McClish, *Statistical Methods in Diagnostic Medicine*, Wiley-Interscience, New York, 2002.
- [5] M.S. Pepe, *The Statistical Evaluation of Medical Tests for Classification and Prediction*, Oxford University Press, Oxford, 2003.
- [6] W. Gerhardt, H. Keller, Evaluation of test data from clinical studies. I. Terminology, graphic interpretation, diagnostic strategies, and selection of sample groups. II. Critical review of the concepts of efficiency, receiver operating characteristics (ROC), and likelihood ratios, *Scand. J. Clin. Lab. Invest. Suppl.* 181 (1986) 1–74.
- [7] R. Body, S. Carley, C. Wibberley, G. McDowell, J. Ferguson, K. Mackway-Jones, The value of symptoms and signs in the emergent diagnosis of acute coronary syndromes, *Resuscitation* 81 (3) (2010) 281–286.
- [8] L.D. Broemeling, *Bayesian Biostatistics and Diagnostic Medicine*, Chapman & Hall/CRC, Boca Raton, 2007.
- [9] D.G. Altman, *Practical Statistics for Medical Research*, Chapman and Hall/CRC, 1991.
- [10] N.D. Mercaldo, X.-H. Zhou, K.F. Lau, Confidence intervals for predictive values using data from a case control study, *UW Biostatistics Working Paper Series*, December (Working Paper 271), 2005.
- [11] B.C. Choi, Slopes of a receiver operating characteristic curve and likelihood ratios for a diagnostic test, *Am. J. Epidemiol.* 148 (11) (1998) 1127–1132.
- [12] N.P. Johnson, Advantages to transforming the receiver operating characteristic (ROC) curve into likelihood ratio co-ordinates, *Stat. Med.* 23 (14) (2004) 2257–2266.
- [13] P. Rustad, P. Simonsson, P. Felding, M. Pedersen, Nordic Reference Interval Project Bio-bank and Database (NOBIDA): a source for future estimation and retrospective evaluation of reference intervals, *Scand. J. Clin. Lab. Invest.* 64 (4) (2004) 431–438.
- [14] A. Ravn-Fischer, T. Karlsson, M. Santos, B. Bergman, P. Johanson, J. Herlitz, Chain of care in chest pain—differences between three hospitals in an urban area, *Int. J. Cardiol.* 166 (2) (2013) 440–447.
- [15] C. Bjurman, M. Larsson, P. Johanson, M. Petzold, B. Lindahl, M.L. Fu, O. Hammarsten, Small changes in troponin T levels are common in patients with non-ST-segment elevation myocardial infarction and are linked to higher mortality, *J. Am. Coll. Cardiol.* 62 (14) (2013) 1231–1238.
- [16] C. Bjurman, M. Petzold, P. Venge, J. Farbemo, M.L. Fu, O. Hammarsten, High-sensitive cardiac troponin, NT-proBNP, hFABP and copeptin levels in relation to glomerular filtration rates and a medical record of cardiovascular disease, *Clin. Biochem.* 48 (4–5) (2015) 302–307.
- [17] O. Hammarsten, C.E. Jacobsson, M. Widegren, T. Danylchenko, A.S. Jaffe, Long-time quality assessment of the Elecsys troponin T hs assay, *Clin. Biochem.* 46 (12) (2013) 1055–1057.
- [18] H.W. Franz, Ratios: A Short Guide to Confidence Limits and Proper Use, Cornell University Library arXiv:0710.2024 [stat.AP], October 2007 1–60.
- [19] P.S. Wells, D.R. Anderson, M. Rodger, M. Forgie, C. Kearon, J. Dreyer, G. Kovacs, M. Mitchell, B. Lewandowski, M.J. Kovacs, Evaluation of d-dimer in the diagnosis of suspected deep-vein thrombosis, *N. Engl. J. Med.* 349 (13) (2003) 1227–1235.
- [20] L.J. Tamariz, J. Eng, J.B. Segal, J.A. Krishnan, D.T. Bolger, M.B. Streiff, M.W. Jenckes, E.B. Bass, Usefulness of clinical prediction rules for the diagnosis of venous thromboembolism: a systematic review, *Am. J. Med.* 117 (9) (2004) 676–684.
- [21] O. Hammarsten, M.L. Fu, R. Sigurjonsdottir, M. Petzold, L. Said, K. Landin-Wilhelmsen, B. Widgren, M. Larsson, P. Johanson, Troponin T percentiles from a random population sample, emergency room patients and patients with myocardial infarction, *Clin. Chem.* 58 (3) (2012) 628–637.