Opinion Paper

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Reference limits of high-sensitive cardiac troponin T indirectly estimated by a new approach applying data mining. A special example for measurands with a relatively high percentage of values at or below the detection limit

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Abstract: A new model for the indirect estimation of reference limits (RLs) has been proposed recently and was coined TMC approach (truncated minimum chi-square estimation) which can be performed with R statistic. A spline function is applied to the RLs to get a continuous function if age is graphically presented vs. the RLs avoiding artificial “jumps” between different age groups. Most indirect models assume a power normal distribution and fail if this assumption is not fulfilled as e.g. if a relatively high percentage of measured values is below the detection limit and the data are distributed extremely skewed. This problem is handled by the TMC model. High-sensitive cardiac troponin T (hs cTnT) was chosen as an example. The hs cTnT concentration in serum or plasma is well accepted as a valuable marker in the diagnosis of acute myocardial infarction. Currently, the 99th percentile derived from a “healthy” subpopulation is the decision limit recommended by consensus groups. However, this decision limit is questioned by several authors for many reasons. In the present report, the 97.5th and the 99th percentile limits were reinvestigated by the TMC model with different subpopulations stratified according to age and sex and were finally compared to presently recommended decision limits. In summary, the generally recommended 99th

percentile as a fixed decision limit should be reconsidered. It is suggested to apply more specific reference limits stratified for age and sex instead of a fixed decision limit.

Keywords: age partitioning; data mining; diurnal variation; indirect reference limits; sex partitioning; TMC approach; troponin T.

Introduction

The truncated minimum chi-square estimation (TMC) method was developed as an indirect approach to estimate reference intervals for the majority of measurands in laboratory medicine which provide values more or less far above the detection limit and which follow a power-normal distribution [1]. Values of non-diseased (“healthy”) subjects are distributed around a central value, while in case of a mixed population, the values of patients are either at the lower and/or at the higher end of the distribution.

For a few measurands, a relatively high percentage of values from non-diseased subjects (e.g. more than 10%) is located around or below the detection limit and the values of patients are located at the higher end of the distribution (e.g. cardiac troponin and C-reactive protein). Reference interval (RIs) of these measurands can be estimated by the TMC procedure, but not be correctly handled by other procedures for the indirect estimation of RIs reported in the literature [1].

Measurement of high-sensitive troponin T (hs cTnT) concentrations is performed in patients with chest pain admitted to emergency departments in order to diagnose (confirm) or rule out acute myocardial infarction (AMI). Furthermore, troponin levels (even below the recommended decision limit) have been found to be of prognostic value [2, 3]. Usually, only a single decision limit is given based on the 99th percentile of a “healthy” reference

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population, where “healthy” means individuals without a known history of heart disease [4]. However, this decision limit is questioned by several authors for many reasons.

Multiple studies have demonstrated that deviation of the 99th percentile can be problematic with the selected population, statistical data handling (e.g. outlier treatment), stratification according to age and sex, in the presence of renal insufficiency and high physical activity. Furthermore, the decision limits differ with the analytical systems applied. These factors can cause large changes in the derived upper limit [3]. Consensus documents have not sufficiently defined the reference population [5]. Differences in the cohort of healthy individuals lead to major differences in the resulting 99th percentile as decision limit. The younger the reference population is and the more stringent the criteria to define cardiac health are, the lower the resulting 99th percentile is [6].

In summary, there still is a controversial discussion going on what is the best (most efficient) diagnostic limit concerning diagnosis of AMI and prognosis of death. Therefore, the 97.5th and the 99th percentile limits were reinvestigated by the TMC approach with different sub-populations stratified according to age and sex to be finally compared with presently recommended decision limits.

Methods

The measured values were obtained from out-patients (out-clinic patients, ambulant patients) and in-patients (hospitalized patients) of the university hospital of Kiel. The data were collected within three years (2015–2018). Plasma was gained within approximately 2 h after blood collection and analysed as soon as possible. The stability of the analytical procedure was verified by plotting the monthly medians of the data collecting time with confidence limits as described elsewhere [7]. The analysis of hs-cTnT, creatinine concentration and the catalytic activity concentration of creatine kinase (CK) were performed on a Cobas 8000/e602 analyser (Roche Diagnostics, Basel). The Institute for Clinical Chemistry of the university hospital of Kiel was accredited according to ISO 15189) and followed the Guidelines of the German Medical Association on Quality Assurance in Medical Laboratory Examinations (Rili-BAEK) [8]. The detection limit for hs cTnT was 3 ng/L. The coefficient of variation (inter-assay imprecision) at 11 ng/L was 5.2%. Reference documents claim that the coefficient of variation at the cut-off limit should be $\leq 10\%$ [9, 10].

The assay applied for the determination of cardiac troponin T can be considered as a high-sensitive assay because its analytical performance (coefficient of variation less than

10% at the 99th decision limit) fulfils international guidelines and more than 50% of healthy subjects are above the detection limit [9]. The present modification of the TMC approach is the first report on uRLs for hs-cTnT estimated indirectly. So far, all other indirect methods cannot be used for data distributions if most values below the detection limit are from non-diseased subjects. Examples of such distributions are cardiac troponin, C-reactive protein and prostatic-specific antigen.

The laboratory results of patients were selected as described earlier [6]: only first values were used if several results were obtained during a hospital stay and patients from special wards (e.g. intensive care units, gynaecological units) were excluded.

The TMC method recently was described in detail [1] and slightly modified later [11]: the lower truncation starts at zero. In case that the RLs are obtained from several age intervals, a spline function is applied to them. This leads to a continuous line if the patients age is graphically presented versus the RLs avoiding artificial “jumps” between different age groups. A skript based on the R programming language with automatic partitioning for age and sex, continuous determination of RLs depending on age for children and adults, and calculation of confidence and tolerance limits can be requested from the authors. Within this programme, limiting percentiles of lower and upper decision limits can be varied by the user.

Results

Dependence of hs-cTnT concentration on age and sex

The RLs of hs-TnT values were higher in men than in women (Figure 1) and increased with age in both sexes (Figure 1 and Table 1). Between 18 and 40 years of patients age, hs-TnT values were almost constant. The mean upper 99th percentile limit (Table 1) was about 29% higher than the mean 97.5th percentile limit (upper reference limit [uRL]). The RLs of ambulant and hospitalised patients are similar up to an age of 49 years. Above 50 years, hospitalised patients have higher uRLs than ambulant patients. The reason for this difference may be due to a decreased glomerular filtration with hospitalised patients (not shown).

Kimienai et al. [12] reviewed 18 reports on hs-cTnT and found that the 99th decision limit of hs-cTnT varied from 8 to 25 ng/L. Reports in a limited literature survey vary considerably (Table 2), in women between 7 and 87 ng/L and in men between 11 and 77 ng/L (Table 2) at the 99th percentile of subjects considered to be “healthy” with regard to AMI. In the present study, the upper RLs of the age group 18–30 years were 9.8 ng/L for men (97.5th percentile;

Figure 1: Dependency of age (years) on reference limits (RLs) (ng/L) of high-sensitive cardiac troponin T (red, female patients $n=32,411$; blue, male patients $n=44,347$) in a mixed subpopulation (out-patients+in-patients).

The upper curves are upper reference limits (uRLs) (97.5th percentiles), and the lower curves are lower RLs (2.5th percentiles) with vertical confidence limits.

11.4 ng/L 99th percentile) and 5.5 ng/L for women (97.5th percentile; 6.1 ng/L 99th percentile). These values agreed with the upper RLs reported by several authors (Table 2). The best agreement occurred with the data of Masuch et al. [13] and of Giannitis et al. [21]. The latter authors used the same analytical system than that applied in the present study. Giannitis et al. [21] reported an uRL (99th percentile) =12.7 ng/L for men aged ≤ 55 years and we found 12.6 ng/L for men aged 18–50 years (97.5th percentile).

In comparison with data reported in the literature, it must be kept in mind that values in plasma are known to be 9–15% lower than those in serum [21, 22]. Therefore, the upper 99th limits of Hammersten et al. in serum [14] were higher than the corresponding TMC values in plasma. The upper 99th percentile limits of Monneret et al. [16, 18] were slightly higher than TMC values with the exception in older men (above 50 years). The differences may result from variations in the selection of reference subpopulations [18].

The 2.5th percentile limits reported are delivered automatically by the script used, but are clinically irrelevant.

Relation between hs-cTnT and the creatinine concentration

Several authors [20, 23–26] reported an inverse relationship between hs-cTnT concentration and glomerular filtration rate

Table 1: Reference limits (RLs) of hs-cardiac troponin T (ng/L) from patients of a university hospital estimated by the TMC method.

2.5 and 97.5 percentiles			1.0 and 99 percentiles		Number of values
Age, years	Lower RL	Upper RL	Lower RL	Upper RL	
Mixed population of ambulant and hospitalised patients					
Men					
18–29	2.03	9.84	1.75	11.41	1933
30–49	2.00	13.73	1.66	16.44	5544
50–90	3.67	63.74	2.81	83.22	35225
Women					
18–29	0.13	5.46	0.05	6.14	1605
30–49	0.21	6.78	0.08	7.56	4138
50–90	2.51	55.46	1.88	74.06	25874
Ambulant patients					
Men					
18–29	1.46	11.18	1.21	13.52	762
30–49	1.91	12.48	1.61	14.87	1646
50–90	3.27	48.97	2.54	63.07	5375
Women					
18–29	NA	NA	NA	NA	699
30–49	0.21	6.69	0.09	7.46	1488
50–90	1.90	38.66	1.44	51.24	3784
Hospitalised patients					
Men					
18–29	0.97	8.97	0.61	10.07	1231
30–49	1.04	9.16	0.60	10.10	3898
50–90	3.8	72.62	2.88	95.69	29850
Women					
18–29	NA	NA	NA	NA	906
30–49	0.75		0.58	87.39	2650
50–90	2.59		1.92		22090

NA means not available due to low number of data.

(eGFR). Higher decision limits were claimed for patients with renal failure [20]. If creatinine concentrations and hs-cTnT values measured simultaneously are graphically presented, a slight positive correlation was observed (Figure 2). The correlation for men was $r=0.52$ (intercept 4.12, slope 0.198), and for women $r=0.48$ (intercept 3.985, slope 0.167). Recently, we have reported that the plasma creatinine concentration increased with age in both sexes [11]. Therefore, the inverse relationship could also be described by the age dependency of hs-cTnT. It appears sufficient to consider the age dependency of hs-cTnT for the diagnosis of AMI independent of the creatinine concentration.

Relation between hs-cTnT and the catalytic activity concentration of creatine kinase

Masuch et al. [13] reported that the CK activity is non-linearly associated with the hs-cTnT concentration in the

Table 2: Upper reference limits of hs-cardiac troponin T(ng/L) reported by several authors (estimated by direct methods).

Authors	URL	Sex	Age, years	Percentile, %	Number of values	Remark
Hammersten et al. [14]	12		<65	99		
	82		>65	99		
	24.3	Women	52 (median)	97.5	342	
	44.1	Men	53 (median)	97.5	309	
	32.5	Women	52 (median)	99	342	
	76.5	Men	53 (median)	99	309	
Wildi et al. [6]	14	Men		99		
	7.1	Women		99		
Masuch et al. [13]	10.5	Women, men		99		CK adjusted
Saenger et al. [15]	8.9	Women	36 (median)	99		
	15.5	Men	38 (median)	99		
	10.7	Men	20-29	99		
	19.9	Men	50-59	99		
Monneret et al. [16]	9.9			99	394	eGFR 120(111–144)
	124			99	451	eGFR 45 (13–59)
Apple et al. [17]	13	Women		99		
	2D	Men		99		
Kimenai et al. [12]	9	Women	40–75	99		
	16	Men	40–75	99		
Monneret et al. [18]	8	Women	18–50	95		
	19	Women	51–70	95		
	45	Women	71–98	95		
	16	Women	18–50	99		
	3D	Women	51–70	99		
	87	Women	71–98	99		
	13	Men	18–50	95		
	23	Men	51–70	95		
	41	Men	71–98	95		
	19	Men	18–50	99		
Mueller et al. [19]	33	Men	51–70	99		
	66	Men	71–98	99		
	11	Women		99		Blood donors
	14	Men		99		Blood donors
Chuang et al. [20]	2D			99		eGFR≥90
	144			99		eGFR<90
Giannitsis et al. [21]	13.1	Women		99		cohort C ^a
	16.8	Men		99		cohort C
	12.7	Men	≤55	99		cohort C
	16.8	Men	>55	99		cohort C

^aMost specific criteria on cardiac imaging; number of values=626.

plasma of a well characterized DONOR SHIP cohort (age median 26 years). The CK activity was used to characterize the physical activity and the authors found that the hs-cTnT concentration was higher in subjects with higher CK activity than in those with lower CK activity. They suggested that the upper RIL should be reduced in individuals with relatively low physical activity from 14 ng/L to 9.4 (8.4–10.3). However, the association was only observed in men and not in women. With hs-cTnI, the effect of physical activity depended on the analytical system applied [27]. With mixed populations, it does not make sense to consider a possible association between the CK activity and hs-cTnT, because a CK activity increase may have multiple

causes (e.g. myocardial and skeletal muscle diseases, hypothyroidism, drug and alcohol, malignant tumours, high muscle activity). We did not find any correlation (Figure 3) if hs cTnT and CK activity was measured in the same samples from the same cohort used in Figure 1 ($r=0.033$, intercept 4.83, slope -0.027).

Diurnal variation

The diagnosis of AMI relies strongly on serial testing [3]. Therefore, it is essential to regard diurnal variation if it occurs. Klinkenberg et al. [28] observed a peak of 17.1 ng/L

Figure 2: Creatinine concentrations and high sensitive cardiac troponin T (hs cardiac troponin) concentrations presented on ln scales. The data were from male ambulant patients of a university hospital. Analysis for both hs-cTnT and creatinine were performed on the same sample. The red line is the regression function.

Figure 3: Creatine kinase activity and high sensitive cardiac troponin T (hs cardiac troponin) presented on a ln scale. The data were from male ambulant patients of a university hospital. Analysis for both hs-cTnT and creatinine were performed on the same sample. The red line is the regression function.

during the morning at 8:30 and a minimum at 20:30 of 11.9. However, there is a large interindividual difference between the peak and the nadir of cTnT concentration ranging from 3.4 to 11.8 ng/L depending on the start

Figure 4: Diurnal variation of the high sensitive cardiac troponin T (hs cardiac troponin) concentration in heparinised plasma (ambulant patients).

Medians of observations were calculated per hour of daytime from Monday to Friday (Mo–Fr). The yellow areas represent the approximate 95% confidence limits based on the median absolute deviation.

concentration. A relatively low concentration of about 10 ng/L led to the lowest difference, and concentrations between 20 and 30 ng/L led to the highest variations in the absence of AMI. The concentrations between 20 and 30 ng/L were observed in patients with type 2 diabetes and overweight persons. In the present study, a peak (Figure 4) was observed at 10:30 (sample arrival time in the laboratory) with ambulant patients. Assuming a delay time of about 2 h for sample transport times, the peak time observed by Klingenberg et al. and ours appears to agree. A similar diurnal variation with a peak at the late morning was also observed with hospitalised patients (data not shown).

Discussion

It has been stressed by consensus documents [3, 28, 29] that the sole elevation of the cTn is not indicative for AMI. Several other diseases may increase the blood concentration of cTn in the absence of AMI. Examples of these situations are type 2 diabetes [17, 30] and chronic renal impairment [28]. It is well known the creatinine concentration also increases with age. Chung and Jones hypothesised that a change in renal clearance may mediate cTnT concentrations rather than changes in cardiac damage. The underlying mechanism remains unclear [20]. Thus, higher cTnT concentrations may be related to an increasing renal

insufficiency. In older subjects, Schneider concluded that in the population with renal failure, there is a substantial fraction of patients that will require repeated testing and further investigations before the final diagnosis of AMI can be made [25]. However, regardless of the mechanism of elevated cTnT, acute myocardial injury, when associated with rising and/or falling patterns of cTnT values and with at least one value above the 99th percentile limit, is designated as AMI [28]. Elevated cTnT levels may be indicative of AMI if the pattern of values is rising and/or falling even in the absence of clinical symptoms.

The present study confirms the increase of cardiac troponin values with age [30]. The age-dependent increase of cTn level has been related to a progressive increase of heart failure due to the ageing of myocardial tissue [28] and to increasing renal insufficiency. The difference between men and women may be due to a positive relation of the circulating cTn level to left ventricular mass which is lower in women than in men [31]. Sex and age dependency of hs cTnT, although already known has not been considered in the 2015 guidelines of the European Society of Cardiology for management of non-ST elevation acute coronary syndrome [9, 29]. In the future, age and sex dependency should be integrated in such guidelines to increase sensitivity and specificity of hs cTnT concentrations in diagnosing cardiac infarction.

Furthermore, differences in RLs may be due to different analytical procedures, type of sample (serum or plasma), timing of sampling (diurnal rhythm), in subpopulations selected, and mathematical approaches used to identify and exclude outliers [31]. Specific decision limits for each analytical system are required as also requested for other measurands [32]. Indirect approaches have several advantages over direct methods as summarized by Jones et al. [33]. One important issue is the number of selected reference individuals. Each group should contain at least 300 subjects [31]. This number usually causes a lot of expenses with direct methods, but can be obtained relatively easy with indirect approaches.

Lippi and Cervellin [34] reviewed many factors which influence diagnostic decision limits of cardiac troponin and concluded that there are many doubts as to whether the use of the 99th percentile uRL may be really suitable to be used as diagnostic threshold. The assumption that an uRL calculated on healthy subjects could be efficiently used in patients with suspected myocardial ischaemia (usually older than 50 years with comorbidities which impact the actual cTn concentration) can be seriously questioned. No global consensus exists on how to define the population used to determine the 99th percentile upper RIL [35]. A perfect healthy population without hs-

cTnT influencing co-morbidities and medication will not be a representative population of patients presenting with suspected AMI to the emergency departments [34]. The younger the reference population is and the more stringent the criteria to define cardiac health, the lower the resulting 99th percentile [6]. Therefore, it is not surprising that variable AMI cut-off values are reported in the literature.

Wildi et al. [6] also claimed to revise the present recommended strategy and to lower the official decision limit. Therefore, the usual 97.5th uRL, which is the consensus reference limit for most clinical chemistry measurands, may be more appropriate. However, further clinical studies on sensitivity and specificity in comparison with the established 99th percentile are required.

RLs covering the 95% interval are defined by the lower RL at the 2.5th percentile and the URL at the 97.5th percentile. Consensus recommendations often suggest the 99th percentile as uRL for hs-cTnT [36, 37]. In a recent editorial [38], it was pointed out that the term reference interval should be restricted to the 95% inner interval and that, in the case of troponin, the 99% interval may be used as a diagnostic decision point. Diagnostic or therapeutic “cut-off values” should be termed clinical decision limits [38, 39]. If the 99% limit is chosen as clinical decision limit, it has a high transparency in comparison with less transparent limits as e.g. the 200 mg/dL plasma cholesterol concentration or the 7 mmol/L blood glucose concentration. However, regarding the 99% limit as a RL or decision limit may be a pure semantic problem. In the light of the controversial discussion on the most efficient decision value for the diagnosis of AMI, the delta 1 h/3 h rule appears more relevant than an uncertain decision limit.

Another advantage of the TMC method is that it does not require using outlier tests. Most other approaches start with the application of various so-called outlier tests. Their use is controversially discussed in the literature, because no generally accepted cut-points exist so far. The present study is limited by the assumption that the reference interval does not contain diseased individuals in a percentage which relevantly affects the RLs. However, this assumption is also made by all other models, even by the direct approaches which are presently considered as the “gold standard”. Furthermore, data pools of patients with a low prevalence of cardiac diseases (e.g. from private laboratories serving primarily practitioners) are not suited for indirect procedures.

Although a diurnal variation has been observed (Figure 4), it was neglected by the present estimations of the uRLs as all other studies previously made have also not considered this variable.

Conclusions

The RLs presented are only valid for the analytical platform applied [4]. Even with the same analytical system, a single decision limit (as e.g. 14 ng/L) cannot be recommended, because stratification according to race, age, sex and probably other biological variables is required [40]. It should be differentiated between RLs and clinical decision limits. Consensus recommendations claim that any decision limit should be determined in each local laboratory [7]. Because most laboratories are overburdened to apply direct approaches, the present indirect approach may be a useful tool for many laboratories.

It is suggested to reconsider the fixed 99th percentile decision limit for AMI derived of a “healthy” subpopulation and it is suggested to use uRLs stratified for age and sex instead of decision limits adjusted for renal dysfunction and physical activity by considering GFR and/or the CK activity. The age dependent 97.5th percentile stratified by sex may be an empirical alternative for the well-known 99th clinical decision limit which can be easily estimated by each laboratory which generates sufficient data.

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