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Determination of hemolysis cut-offs for biochemical and immunochemical analytes according to their value

Abstract

Background: All general biochemistry instruments allow the measure of hemolysis index (HI), and suppliers provide an acceptable HI for each assay without consideration of the analyte value or its clinical application. Our first objective was to measure the impact of hemolysis degree on plasma biochemical and immunochemical analytes to determine the maximum allowable HI for each of them using four calculation methods as significant bias in comparison to manufacturer's data. The second objective was to assess whether the maximum allowable HI varied according to the analyte values.

Methods: Twenty analytes were measured in hemolyzate-treated plasma to determine the HI leading to a significant change compared to baseline value. Analytes were assessed at one (3 analytes), two (5 analytes) and three (12 analytes) values according to their sensitivity to hemolysis and their clinical impact. We used four calculation methods as significant limit from baseline value: the total change limit (TCL), the 10% change (10%Δ), the analytical change limit and the reference change value.

Results: Allowable HI was significantly different according to the threshold chosen for most analytes and was also dependent on the analyte value for alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, creatine kinase, iron, haptoglobin and high sensitivity troponin T. No hemolysis interference was observed for

albumin, creatinine, C-reactive protein, and procalcitonin even at an HI value of 11 g/L.

Conclusions: This study highlights that TCL is the most appropriate calculation method to determine allowable HI in practice for biochemical and immunochemical parameters using Cobas 8000© from Roche Diagnostics. In addition, different allowable HI were found according to analyte value leading to optimization of resampling to save time in patient care.

Keywords: analytes; hemolysis index; interference; total change limit; validity of results.

Introduction

High sample quality is essential to ensure the reliability of results for appropriate patient care. The laboratories must manage the entire analytical process from pre- to post-analytical stages. Among the many indicators of sample quality, hemolysis is a major issue in laboratory medicine which can lead to sample rejection, resampling and delay in the laboratory result [1]. Hemolysis is more frequently observed in emergency department (ED) samples than in other medical units [2, 3]. In our laboratory, based on the results of 13,351 consecutive samples, we determined that up to 4.0% vs. <1.0% of samples from ED vs all other medical units, respectively, had an HI > 3.0 g/L. A recent survey reported an overall hemolysis rate of 3.4% with a higher occurrence in samples from inpatients compared to outpatients [4]. The possible solutions to reduce hemolysis rates in the ED or ICU could be the implementation of low vacuum tubes in the use of IV catheter blood collection [5]. The management of hemolysis varies according to the institution and the methods of determining interference. Hemolyzed samples can have detrimental consequences on the quality of patient care.

For example, great hemolysis can lead to higher potassium or lower troponin T levels, which can delay diagnosis and patient care [4]. These consequences, along with the repetition of analysis, can also cause an increase in cost.

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Currently, only two manufacturers (Roche and Abbott) offer an automated measurement of the hemolysis index (HI) which can be converted into the corresponding hemoglobin value in g/L, along with icterus and lipemia indices. However, the HI associated to a significant interference for each assay and provided by manufacturers has been determined using a classical 10% change [6] from baseline value (10%Δ) within reference interval, while the impact of interference likely depends on the value of the analytes. For this reason, manufacturer's data on allowable HI are frequently over- or underestimated [7]. To overcome this, the Clinical and Laboratory Standards Institute (CLSI) recommends measuring the index at a minimum of two medical decision values to evaluate interference [8]. In addition to analytical and biological criteria, a clinical basis should be considered due to the clinical impact of hemolysis interference. The CLSI guideline states that an assay's instruction for use (IFU) should document the values of both hemoglobin and analyte that interfere as well as the amount of bias observed [9]. However, to date, IFUs are not fully compliant with CLSI guidelines. In consequence, laboratories cannot simply assume that manufacturers are performing testing at medically relevant decision levels, and the information contained within IFUs may be inadequate. So, it appears useful to assess hemolysis interference at several values of a given analyte in order to avoid erroneous results and to limit the collection of additional samples. Moreover, the maximum allowable interference resulting in the bias of a measured analyte could be dependent on the choice of calculation method which must be carefully considered [10]. Beyond the classical 10%Δ threshold, other calculation methods of interest are based on the analytical change limit (ACL), the total change limit (TCL) or the reference change value (RCV) [11]. The ACL, based on analytical variation and often used for stability assessment, enables to define whether the observed variation is statistically significant. However, it is more stringent than 10%Δ and too restrictive according to different authors [10, 12]. The TCL is widely used to assess interferences or stability of biochemical analytes in biological fluid, and it takes both analytical and biological variations into account [13]. RCV, which also accounts for both analytical and biological variations, is currently designed for the interpretation of serial measurements but is not restrictive enough for some analytes such as troponin [10]. Clearly, biological variation is important; however, regarding some analytes for which data on biological variation do not exist, the ACL calculation method may represent here an alternative solution.

The first objective of our study was to measure the impact of hemolysis degree on plasma biochemical and

immunochemical analytes to determine the maximum allowable HI for each of them using four calculation methods as significant bias from baseline value (TCL-based HI cut-off, 10%Δ-based HI cut-off, ACL-based HI cut-off and RCV-based HI cut-off). Then, our own HI cut-off limits were compared to manufacturer's data. The second objective was to assess whether the maximum allowable HI varied according to the analyte value. Three levels of values (upper limit, lower limit and within reference interval) were selected for 12 analytes known to be sensitive to hemolysis [1]. Two levels of values for five analytes and one for three analytes were selected guided by clinical application to confirm and/or complete literature's data [6, 8].

Materials and methods

Study design

Twenty routine biochemical and immunochemical analytes were measured on heparinized plasma pools free of hemoglobin (baseline value) and after overload cascade with hemoglobin-titrated hemolyzate. Measurements were performed on a Cobas® 8000 analyzer (Roche diagnostics, Meylan, France), which has the ability to assess HI which is then converted into hemoglobin value. We considered as allowable HI the highest HI measurement without significant change of analyte value from baseline. After calculating the bias (overloaded sample value – initial value) we compared this value with the different interference criteria such as 10%Δ, ACL, TCL or RCV. When the bias was higher than interference criterion, the change was considered as significant. This process was approved by the local Ethics Committee (Lapeyronie University Hospital, Montpellier, France).

Analytes investigated and HI measurement

We focused on analytes with low HI, with discordant data in the literature, with discordant results compared to Roche's HI, or not studied by other authors. Three values of baseline value were tested for the 12 following analytes: alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin (direct), creatine kinase (CK), γ-glutamyltransferase (GGT), haptoglobin (Hp), high sensitivity troponin T (hs-cTnT), iron, lactate dehydrogenase (LD), phosphorus and potassium. Five additional analytes were studied at two levels of values: albumin, creatinine, ferritin, lipase and protein. Three additional analytes known to be less influenced by hemolysis or widely studied were assessed at only one value: bicarbonates, C-reactive protein (CRP) and procalcitonin (PCT). Values were chosen to be either in the reference interval of lab or close to clinical decision values, or in pathological range. All measurements were performed in duplicate on a Kryptor Compact-Plus© (ThermoFisher, Villebon Sur Yvette, France) for PCT and on a Cobas® 8000 Analyzer (Roche Diagnostics, Meylan, France) for the 19 other analytes. The Cobas® 8000 is formed by an ion selective

Table 1: Principles of methods and main characteristics of the analytes investigated in our study.

Analyte	Manufacturer	Technology	Method	Wavelength, nm	Reference values	CV ^a , %	CV ^b , %	TCL, %	ACL, %	RCV, %
Albumin	Roche	Spectrophotometry	Bromocresol green	570–505	M, F = 35–52 g/L	2	3.2	5.7	5.5	14.2
ALP	Roche	Spectrophotometry	IFCC	450–480	M < 130 IU/L F < 105 IU//L	2.6	6.4	7.8	7.2	19.2
ALT	Roche	Spectrophotometry	IFCC without P-5'-P	340–700	M < 41 μmol/L F < 33 μmol/L	2.3	19.4	11.6	6.3	54.1
AST	Roche	Spectrophotometry	IFCC without P-5'-P	340–700	M < 40 IU/L F < 32 IU/L	2.2	12.3	8.6	6.0	34.6
Bicarbonates	Roche	Spectrophotometry	Enzymatic	415–505	M, F = 22–29 mmol/L	2.7	4.0	7.7	7.5	13.3
Bilirubin (direct)	Roche	Spectrophotometry	Colorimetric diazo method	552–659	< 3.4 μmol/L	2.3	36.8	19.5	6.5	102.1
CK	Roche	Spectrophotometry	IFCC	340–546	M < 190 IU/L F < 170 IU/L	2.3	22.8	13.0	6.3	63.4
Creatinine	Roche	Spectrophotometry	Enzymatic	512–583	M = 59–104 μmol/L F = 45–84 μmol/L	2.5	6.0	7.5	6.9	18.0
CRP	Roche	Turbidimetry	Specific antibodies	570–800	M, F < 5 g/L	2.4	42.2	22.1	6.6	117.0
Ferritin	Roche	ECL	Specific antibodies	NA	M < 400 μg/L F < 150 μg/L	2.6	14.2	10.1	7.2	39.9
GGT	Roche	Spectrophotometry	Enzymatic	415–700	M < 60 IU/L F < 40 IU/L	1.5	10	6.5	4.1	28.0
Hp	Roche	Turbidimetry	Specific antibodies	340–700	M, F = 0.3–2 g/L	1.3	20	10.6	3.6	55.5
Hs-cTnT	Roche	ECL	Specific antibodies	NA	M, F < 14 pg/mL	2.6	6.3	7.8	7.2	18.8
Iron	Roche	Spectrophotometry	Colorimetry	570–700	M, F = 8.4–28.8 μmol/L	1.3	26.5	13.7	3.4	73.4
LD	Roche	Spectrophotometry	Enzymatic	340–546	M < 225 IU/L F < 214 IU/L	1.8	8.6	6.5	4.8	24.3
Lipase	Roche	Spectrophotometry	Enzymatic	570–700	M, F < 60 IU/L	2	32.2	17.0	5.5	89.3
PCT	Thermo Fisher	TRACE™	Specific antibodies	340–665	M, F < 0.5 ng/mL	5.2	16	16.6	14.7	46.6
Phosphorus	Roche	Spectrophotometry	Phospho-molybdate/UV	340–700	M, F = 0.81–1.45 mmol/L	1.8	8.1	6.4	5	23.1
Potassium	Roche	Indirect potentiometry	Ion selective electrodes	NA	M, F = 3.4–4.5 mmol/L	2	4.6	6.0	5.5	13.8
Protein	Roche	Spectrophotometry	Colorimetry	546–700	M, F = 66–87 g/L	2	2.7	5.7	5.5	9.4

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CK, creatine kinase; CRP, C-reactive protein; GGT, gamma-glutamyltransferase; Hp, haptoglobin; hs-cTnT, high sensitivity troponin T; LD, lactate dehydrogenase; PCT, procalcitonin; ECL, electro chemiluminescence; TRACE, time resolved amplified cryptate emission; IFCC, International Federation of Clinical Chemistry and laboratory medicine; M, male; F, female; TCL, total change limit; ACL, analytical change limit; RCV, reference change value; ^aCV_a, analytical coefficient of variation. For each analyte, the CV_a was calculated from Roche and ThermoFisher internal quality control (IQC) used in our lab over a 12-month period. ^bCV_w, within-subject biological variation. The data are those given by the tables of Ricos et al. [14] and by the literature for PCT [15] and hs-cTnT [16].

electrode module, c701 and c502 modules (for photometric assays) and an e602 module (for immunoassays). HI was measured on the c701 module by a bichromatic wavelength-paired measurement at 570 and 600 nm. The final result of HI was expressed as an absolute number which corresponds approximately to the hemoglobin value in g/L (measuring range 0.05–11 g/L). The principles of methods and the main characteristics of the analytes investigated are reported in Table 1.

Preparation of samples

The preparation of samples followed three phases: phase 1, preparation of hemolyzate; phase 2, selection of hemoglobin-free samples to form a heparinized plasma pool with analytes of value of interest; and phase 3, overload of each pool of plasma with hemolyzate. Hemolyzate was prepared according to the protocol CLSI EP7-A2 using the osmotic shock procedure [8]. After removing plasma from heparinized whole blood pool of 10 samples, the red blood cells were washed three times in a 0.9% NaCl solution and then lysed in distilled water and frozen overnight at -20°C . After thawing, hemoglobin value in the hemolyzate was determined and adjusted with distilled water.

We selected from routine plasma free of hemoglobin (with HI < 0.05 g/L) near the test value desired, and we prepared plasma pools at different values for each analyte.

Each pool of plasma free of hemoglobin (<0.05 g/L) was split in equal volumes to be overloaded with increasing amounts of hemoglobin-titrated hemolyzate under a constant total volume of tube of 1 mL with compensation by addition of NaCl to obtain the theoretical final values of hemoglobin ranging from 0.1 to 11 g/L.

Allowable HI determination

The HI and the value of analytes were measured in duplicate on Cobas 8000 and Kryptor on each overloaded plasma.

The formula for calculating the hemolysis index on the Cobas8000 is as follows:

$$H = 1 / A \cdot (Abs_2 - B \cdot Abs_1)$$

with H = serum indices for hemolysis; A = factor for conversion of absorbance values ($\times 10^4$) to serum indices; Abs_2 = bichromatic absorbance readings at 505 and 480 nm for hemolysis; Abs_1 = bichromatic absorbance readings at 700 and 660 nm for lipemia; and B = corrects hemoglobin measurement Abs_2 for lipemia.

For each analyte, the bias was determined as the difference between average of baseline values determined in pools free of hemolysis (T_0) and average of values obtained from each overload of pools (T_x). The percentage deviation from T_0 was calculated using Eq. (1):

$$\left[\frac{T_x - T_0}{T_0} \right] \times 100$$

Each calculated bias was compared to the four cut-offs (10% Δ , ACL, TCL or RCV), for all analytes.

The TCL, as described by Oddo et al., was calculated using Eq. (2) [13]:

$$[(2.77 \times CV_a)^2]^{1/2} + [(0.5 + CV_w)^2]^{1/2},$$

where CV_a is analytical imprecision and CV_w is within-subject biological variation.

The ACL was calculated using Eq. (3) [17]:

$$2.77 \times CV_a$$

The RCV was calculated according to Eq. (4) [9]:

$$2.77 \times (CV_a^2 + CV_w^2)^{1/2}.$$

For each analyte, the CV_a (Table 1) was calculated from Roche and ThermoFisher internal quality control (IQC) used in our lab over a 12-month period. The lot number of IQC from Roche was PreciControl ClinChem Multi-160407 for chemistry analysis, PreciControl Varia-276843 for ferritin, PreciControl Cardiac-175878 for myoglobin and NT-proBNP and PreciControl Troponin-195257 for hs-cTnT. The lot number was 25032A for PCT on Kryptor.

The CV_w are the specifications (2014) provided by Ricos et al. [14] and by the literature for PCT [15] and hs-cTnT [16] (see Tables 1 and 2).

The allowable HI for each analyte was defined as the last HI value without bias higher than cut-off criteria, using either TCL, 10% Δ , ACL or RCV as significant limit of bias. Effectively, when the bias (in percentage) was higher than TCL, 10% Δ ACL or RCV values, the variation was considered as significant.

Results

Results of allowable HI determined for each analyte according to the four calculation methods (TCL, 10% Δ , ACL, RCV) as well as data provided by the manufacturers are reported in Table 2. Whatever the calculation methods used, there was no hemolysis interference on albumin, creatinine, CRP and PCT even when the HI values were as high as 11 g/L. Hemolysis led to a positive bias for ALT, AST, CK, iron, LD, lipase, phosphorus, potassium and protein measurement, while a negative bias was observed for ALP, bicarbonates, direct bilirubin, Hp and hs-cTnT.

For ALP, ALT, bilirubin, CK, GGT, iron and lipase, the allowable HI was found higher using TCL or RCV compared to the 10% Δ -based HI, and these resulted in higher allowable HI than with the ACL-based HI. The lowest HI were obtained for AST, direct bilirubin and LD. Nevertheless, for CK, iron, phosphorus and lipase the HI claimed by Roche were higher than our own measured HI using the 10% Δ -based HI. All other analytes were less affected by hemolysis than the manufacturer's claim. Ferritin, iron, Hp and GGT showed the highest difference between the allowable HI we determined and those provided by Roche (Table 2). To the best of our knowledge, such study including PCT and ferritin have not been previously investigated in the literature.

Allowable HI was found to be value-dependent for most analytes especially for ALP, ALT, AST, CK, ferritin,

Table 2: Hemolysis interference with significant limit based on TCL, 10% variation, ACL or RCV according to the values of analytes.

Analytes	Units	Allowable HI from manufacturers', g/L	Allowable HI determined in our study, g/L									
			Baseline concentration	TCL based HI cut-off	10%Δ based HI cut-off	ACL based HI cut-off	RCV based HI cut-off	Baseline concentration	TCL based HI cut-off	10%Δ based HI cut-off	ACL based HI cut-off	RCV based HI cut-off
Albumin	g/L	10	29 >11	>11	>11	>11	>11	36 >11	>11	>11	>11	>11
ALP	IU/L	2	69 2.5 (D)	1.8 (D)	1.8 (D)	1.8 (D)	2.5 (D)	136 2.5 (D)	2.5 (D)	2.5 (D)	1.8 (D)	5.1 (D)
ALT	IU/L	0.9	24 2.5 (D)	1.4 (D)	1.4 (D)	1.0 (D)	7.9 (D)	42 5.1 (D)	2.5 (D)	2.5 (D)	1.8 (D)	7.9 (D)
AST	IU/L	0.4	25 0.3 (D)	0.3 (D)	0.3 (D)	0.3 (D)	1.4 (D)	46 0.7 (D)	0.7 (D)	0.7 (D)	0.2 (D)	1.8 (D)
Bicarbonates	mmol/L	6	19 7.9 (D)	7.9 (D)	7.9 (D)	7.9 (D)	7.9 (D)	- -	-	-	-	-
Bilirubin (direct)	μmol/L	0.2	4 0.2 (D)	0.1 (D)	0.1 (D)	0.1 (D)	2.5 (D)	16 0.3 (D)	0.1 (D)	0.1 (D)	0.1 (D)	11
CK	IU/L	1	96 1.4 (D)	0.7 (D)	0.7 (D)	0.3 (D)	2.5 (D)	145 1.8 (D)	1.0 (D)	1.0 (D)	0.7 (D)	5.1 (D)
Creatinine	μmol/L	8	70 >11	>11	>11	>11	>11	100 >11	>11	>11	11	>11
CRP	mg/L	10	104 >11	>11	>11	>11	>11	- -	-	-	-	-
Ferritin	ng/mL	5	132 7.9 (D)	7.9 (D)	7.9 (D)	7.9 (D)	>11	1619 >11	>11	>11	>11	>11
GGT	IU/L	2	35 11	5.1 (D)	5.1 (D)	5.1 (D)	11	122 >11	>11	>11	5.1 (D)	>11
Hp	μmol/L	0.1	0.6 0.3 (D)	0.3 (D)	0.3 (D)	0.2 (D)	11	1.4 11	1.0 (D)	1.0 (D)	0.7 (D)	11
Hs-cTnT	pg/mL	1	16 1.4 (D)	1.4 (D)	1.4 (D)	0.7 (D)	1.8 (D)	21 1.8 (D)	1.8 (D)	1.8 (D)	1.8 (D)	2.5 (D)
Iron	μmol/L	2	12 2.5 (D)	1.8 (D)	1.8 (D)	0.7 (D)	11	16 2.5 (D)	2.5 (D)	2.5 (D)	0.7 (D)	11
LD	IU/L	0.1	181 0.1 (D)	0.1 (D)	0.1 (D)	0.1 (D)	0.2 (D)	215 0.1 (D)	0.1 (D)	0.1 (D)	0.1 (D)	0.3 (D)
Lipase	IU/L	10	27 >11	7.9 (D)	7.9 (D)	2.5 (D)	>11	50 2.5 (D)	2.5 (D)	2.5 (D)	2.5 (D)	11
PCT	ng/mL	5	0.5 >11	>11	>11	>11	>11	- -	-	-	-	-
Phosphorus	mmol/L	3	0.84 1.4 (D)	1.8 (D)	1.8 (D)	1.4 (D)	2.5 (D)	1.06 1.4 (D)	1.8 (D)	1.8 (D)	1.0 (D)	5.1 (D)
Potassium	mmol/L	0.9	3.5 0.7 (D)	1.0 (D)	1.0 (D)	0.7 (D)	1.4 (D)	4.3 0.7 (D)	1.4 (D)	1.4 (D)	0.7 (D)	1.8 (D)
Protein	g/L	5	57 2.5 (D)	7.9 (D)	7.9 (D)	2.5 (D)	5.1 (D)	77 2.5 (D)	7.9 (D)	7.9 (D)	2.5 (D)	5.1 (D)

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CK, creatine kinase; CRP, C-reactive protein; GGT, gamma-glutamyltransferase; Hp, haptoglobin; hs-cTnT, high sensitivity troponin T; LD, lactate dehydrogenase; PCT, procalcitonin; TCL, total change limit; ACL, analytical change limit; RCV, reference change value; D, decrease-negative % change of value from baseline pool; I, increase-positive % change of value from baseline pool. ^aManufacturer's data as included in package inserts from ThermoFisher for PCT and from Roche for all analytes. ^bCV_w, within-subject biological variation. The data are those given by the tables of Ricos et al. [14] and by the literature for PCT [15] and hs-cTnT [16].

Figure 1: Graphs demonstrating the interference due to hemolysis at different values of analytes tested.

Considering the TCL as maximum percentage acceptable limit, graphs represent the percentage deviation from baseline value of direct bilirubin (A), CK (B), hs-cTnT (C) and potassium (D) according to the hemolysis index. Horizontal dotted line indicates TCL value for each analyte in %, established from our data. CK, creatine kinase; Hp, haptoglobin; hs-cTnT, high sensitivity troponin T; HI, hemolysis index; TCL, total change limit.

Hp, Hs-cTnT, lipase and potassium. Two schemes were observed: either a linear relationship between the increases in analyte value and HI (e.g. CK, Hp, hs-cTnT and potassium) or an inverse relationship (e.g. lipase). As seen in Figure 1, the effect of hemolysis for direct bilirubin and hs-cTnT led to a negative bias with increasing amount of hemolyzate and at all analyte values. For potassium, the bias was positive with increasing hemolysis. Interference from hemolysis was significant for the lowest CK value but negligible for the highest. Interferographs of other analytes with percentage changes of analyte values according to the degree of hemolysis are represented in Figure A (Supplementary material).

Discussion

Our data indicate that some hemolysis index values are not fully concordant with those provided by the manufacturers or published in the literature. In addition, this study clearly demonstrates that the impact of hemolysis depends on analyte values for most analytes tested.

The effect of hemolysis on the analytes studied is in agreement with previous studies [10, 18] such as negative biases observed for hs-cTnT and positive biases for others such as potassium. As expected, the more sensitive analytes to hemolysis were AST, direct bilirubin, Hp and LD in agreement with published data [19]. No hemolysis interference was observed for albumin, creatinine and CRP even at an HI value of 11 g/L, which is in line with previous reports [7, 18]. Our data extend the lack of interference to hemolysis (HI > 11 g/L) for high values of ferritin and decisional values of PCT whatever the thresholds used. This could contribute to an appropriate interpretation of data in the event of such a level of hemolysis and could thus avoid resampling.

Hemolysis may interfere with chemical and immunochemical analytes through several mechanisms [9]. Due to the rich cell content in ALT, AST, LD, phosphate and potassium, lysis results in a release of these analytes in plasma, leading to an increase in their plasma value, even at a microscopic level of hemolysis. This is particularly difficult for potassium interpretation, especially in the case of a patient with hypokalemia for whom the interpretation may be erroneous by the presence of hemolysis.

The additive effect of hemoglobin on total protein value is small but still significant. Dilution is another possible effect especially for gross hemolysis and may result in decreased values. This is typically the case for albumin. At ~417, 540 and 575 nm, wavelengths at which hemoglobin strongly absorbs light, hemolysis results in an increase in absorption and a subsequent apparent increase in analyte values measured at these wavelengths. Spectral overlap and chemical reaction between hemolyzate and reaction components represent the cause of hemolysis interference for iron, creatinine and GGT. Regarding ALP, the interference is due to a denaturation of hemoglobin during reaction leading a decrease in ALP values. Increased values of bilirubin even with a slight hemolysis are explained by the pseudo-peroxidase activity of free hemoglobin in inhibiting diazonium color formation. CK is not a constituent of erythrocyte, but its increase during hemolysis is due to the release of active intracellular adenyl cyclase.

In our lab, the measurement of HI is performed on all plasma/serum tubes on Cobas 8000 through a programming of the data manager in parallel with analyte measurement and is reported for all patient records in the laboratory information system. In view of our experience, it is clear that the automated hemolysis index is of great interest for the monitoring and management of patients (in particular, those from ICU and ED). Several approaches within our lab have been implemented including the use of partial draw tubes with reduced vacuum (BD Vacutainer® Barricor tube) for ED to reduce hemolyzed samples. In addition, HI is recorded in the laboratory information system. An algorithm has been established taking into account the analyte and its value and the value of HI to either trigger an alert on the presence of hemolysis and add a specific comment under the result by specifying the direction of variation of the result or to remove the result.

If most clinicians are aware of the potential for overestimation due to hemolysis, they are less sensitive about negative interference, for example, on troponin T, underlining the need for laboratories to implement actions for hemolyzed samples. For this, there are two main challenges which are the speed of hemolysis detection and the definition of acceptability criteria to assess the interference of hemolysis in order to cancel the results or report a comment. The establishment of thresholds should be discussed with clinicians, to appreciate under what circumstances the results should be suppressed or not depending on the clinical context. For example, low potassium values in the presence of hemolysis can be a concealed severe hypokalemia. In this context, maintaining the result on the report should be mandatory to alert clinicians.

Today, there is a lack of harmonization for automated HI measurements and for the actions to be taken following the report of results [20]. Allowable HI can be used as supplied by the manufacturers or after own determination. The reasons for the difference between Roche's data and ours could be the method of obtaining the interferent (hemolyzate/Hb overload), the value of analyte studied (no information in the technical sheet on the value of analyte tested) and the cut-off used different from ours. As previously reported [10] and illustrated in the present study, the influence of hemolysis strongly depends on the calculation method used to define the significant bias. Allowable HI was systematically lower when using ACL-based HI cut-off – which considers analytical variation only, compared to the TCL- or RCV-based HI cut-offs [10]. Thus, the clinical impact of the analyte measurement should be taken into account to define a significant bias. For troponin, determining a TCL-based HI cut-off (9.4%) or a 10% Δ -based HI cut-off seems more appropriate than using a RCV-based HI cut-off (which was close to 20%) in line with the European Society of Cardiology (ESC) recommendations for troponin kinetic interpretation [21].

As illustrated in the present study, using own determination of TCL-based HI cut-off would be more appropriate for clinical application than 10% Δ as a significant limit of bias. This process could reduce the number of invalid results and the useless need for resampling as well as faster patient management, especially in the ED. This is of particular interest for PCT with an allowable HI value at 5 g/L provided by the manufacturers, while our results did not highlight any significant variation up to 11 g/L around the cut-off value of 0.5 ng/mL.

Moreover, the HI provided by manufacturers was mainly established using one analyte value within the physiological range. However, the CLSI guidelines [8] recommend that hemolysis interference evaluation should be carried out at a minimum of two values of the analyte. Based on these data and the clinical application of the analytes, we assessed two or three values for 17 analytes. For all of them, except proteins and LD, hemolysis interference was dependent on their value. Surprisingly, for lipase the allowable HI value decreased with a slight increase in the baseline value. For other analytes, we observed that the allowable HI value was higher when the baseline analyte value was high even for an analyte known to be very sensitive to hemolysis like Hp. Indeed, using the TCL-based HI cut-off, the allowable HI value was 0.8 g/L for an Hp value of 0.6 g/L compared to a HI value of 11 g/L for a value in Hp higher than 1.4 g/L. Likewise, the allowable HI value for baseline hs-cTnT around the upper limit of 99th percentile value of 14 ng/L was

1.4 g/L compared to the HI value at 2.5 g/L if the troponin value was higher than 50 ng/L. High caution should be taken when troponin results are reported in the presence of hemolysis as a significant negative bias could compromise the diagnosis of myocardial infarction based on serial change of troponin value >20% according to the ESC [21]. Our results are consistent with previous data which reported an effect of hemolysis on value-dependent troponin T [7, 22]. Both of these studies reported a negative interference with increasing degrees of hemolysis for the hs-cTnT assay. However, Florkowski et al. [22] observed no interference regardless of the degree of hemolysis on the pool at <3 ng/L and a bias >10% for the other three pools (pools at 19, 87 and 273 ng/L) when the HI was close to 1.32 g/L. To illustrate the complexity for clinicians, this previous study reported an opposite effect of hemolysis on troponin I value for some assays but not all [22]. The Abbott cTnI assay showed no interference with increasing degrees of hemolysis in a low pool, whereas with the Vitros cTnI assay, the authors reported a positive interference (up to 576% in low pool). The mechanisms by which hemolysis interferes with the immunoassays appear to be multifactorial and specific to the assay [23]. Indeed, hemolysis would release proteases that cleave the antigenic region of the hs-cTnT recognized in the Roche hs-cTnT immunoassay. On the other hand, the increase in analyte value with hemolysis for Vitros cTnI could be due to the fact that hemolysis at low analyte values causes a weak affinity binding of an erythrocyte component to unoccupied capture antibodies [23].

Limitations of the study

We determined the impact of hemolysis in our study on an experimental hemolysis, which is different from natural hemolysis. The study of Dimeski et al. [24] pointed out the importance of the strategies to evaluate the impact of hemolysis on the Roche ammonia. Adding hemolyzate to the samples had a dramatic effect on the ammonia results. In the hemolyzate the deamidation of cellular proteins causes an increase in ammonia. However, until now the effect of hemolysis was often studied by adding hemolyzate to plasma/serum based on CLSI. In a recent study [25], no difference was observed on biochemistry results regardless of the method (addition of hemolyzate or naturally hemolyzed patient specimens).

It should be noted that among suppliers there is no consensus on the method of obtaining the interferent (hemolyzate/Hb overload), on the choice of wavelengths (monochromatism, bichromatism or multichromatism),

on the algorithm of calculation of the index and on the mode of expression of the importance of hemolysis (quantitative or semi-quantitative). As a result, the allowable HI cut-offs determined in our study on Cobas8000 are not transferable to another. The HI measurements are homogeneous for a couple of identical analyzers but vary from one type of analyzer to another.

Overall, our results are of particular interest in improving the management of laboratory quality results and optimizing patient care with appropriate resampling. The European Federation of Clinical Chemistry and Laboratory Medicine states the practical recommendations for the management of test results in hemolyzed samples [10, 26]. Regarding the definition of HI cut-offs, Lippi et al. reported that ACL-based HI cut-off could be used for the purpose of annotating results indicating the direction of the variation, while both TCL- or RCV-based HI cut-off could be used to identify clinically significant interference that needs a cancellation of results [11]. The use of two cut-offs such as analytical and clinical cut-offs (RCV) allows a gradation of the interference to hemolysis, which could help clinicians: firstly, no interference when HI is below CV_a , “alert index” when HI comprises between CV_a and RCV, and an unacceptable index when HI is above RCV. In our study, only one cut-off was discussed, and the TCL considered as clinical cut-off appeared to be the most adapted. The use of one cut-off taking into account the analytical and biological variation is a good compromise and easy to implement in the laboratory information system. In addition, since we have tested hemolysis interference on several levels of analyte value, we can also establish a gradation in the management of hemolysis interference. For example, it is not necessary for a patient with an iron >30 $\mu\text{mol/L}$ to request another sample if the HI is <5.1 g/L, while for an iron <30 $\mu\text{mol/L}$, the allowable HI is 2.5 g/L. All troponin results should be deleted from the report in the presence of a HI of 2.5 g/L. However, following our results, for a troponin <20 ng/mL, with a HI between 1.4 and 1.8 g/L, a comment specifying an underestimation of 10%–15% may be added below the result, and for the troponin results between 20 and 60 ng/mL, the results must be deleted from the report when HI is >1.8 g/L.

Based on our data, the use of 10% Δ as a significant bias seems to be too general, the ACL is too restrictive and could be used to alert about the presence of hemolysis, and the RCV, if used alone, is not suitable to certain analytes such as hs-cTnT. To standardize the management of hemolysis interference for the analytes studied, our study confirms that the TCL is the most appropriate calculation method in practice for biochemistry and immunochemical parameters. Also, it is strongly advised on the other hand

to test the interference criteria on at least two values of analytes as suggested by Von Meyer et al. [26]. Following our results, the conditions for reporting results for several parameters will be broadened, particularly in the case of pathological values avoiding resampling.

Conclusions

In conclusion, the use of TCL-based HI cut-off would best serve the expectations of clinicians and the best compromise for making the decision to suppress the analyte result on the laboratory report when HI values measured on the samples exceed it. In addition, our results highlight different maximum allowable HI according to analyte value using Cobas 8000© from Roche Diagnostics. This own determination of HI appears essential to ensure patient safety and to avoid overestimating hemolysis and re-sampling resulting in delayed patient care. However, implementation in practice in each lab is difficult. Recently, the European Federation of Clinical Chemistry and Laboratory Medicine carried out a survey on the management by laboratories of hemolytic, icteric and lipemic samples, highlighting a fairly significant heterogeneity [27]. As a result, a general consensus in the field regarding comments or cancellation of the test results affected by hemolysis is expected.

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