Clinica Chimica Acta 480 (2018) 71–78

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Lipemic interference of ceruloplasmin assays – An evaluation of lipid removal methods

Joshua J.H. Hunsaker[a], Sonia L. La'ulu[a], Sara P. Wyness[a], Jonathan R. Genzen[a,b,*]

[a] ARUP Institute for Clinical and Experimental Pathology, 500 Chipeta Way, Salt Lake City, UT 84108, USA
[b] Department of Pathology, University of Utah, 500 Chipeta Way, Salt Lake City, UT, 84108, USA

ARTICLE INFO

Keywords:
Ceruloplasmin
Lipemia
Interference
Lipoclear
Ultracentrifugation
Wilson disease

ABSTRACT

Background: The present studies were conducted to characterize lipemic interference across three FDA-cleared ceruloplasmin (CERU) assays and to evaluate procedures designed to remove lipemic interference.
Methods: CERU assays on the Abbott ARCHITECT *ci*8200, Beckman AU5800, and Roche cobas Integra 400 Plus were evaluated. Precision, linearity with dilution, lipemic interference, and three methods for removing lipemia were assessed on each platform: ultracentrifugation (UC), lipemia-clearing reagent LipoClear (LC), and 1:5 dilution (DIL). Lipemia-index (L-index) thresholds were established using endogenously lipemic specimens and sera spiked with human-derived triglyceride-rich lipoproteins.
Results: The *ci*8200 showed greater susceptibility to endogenous lipemic interference than would be expected based on vendor-derived limits established with Intralipid. Endogenous lipemia causes a negative interference on the *ci*8200 and a positive interference on the Integra. UC was generally the most reliable method of removing lipemic interference without impacting baseline CERU results.
Conclusions: CERU assays on different platforms have varying susceptibility to lipemic interference. L-index thresholds derived using Intralipid may not accurately represent interference caused by endogenous lipemia.

1. Introduction

Ceruloplasmin (CERU) is the major copper-containing protein in human plasma [1]. As a multi-copper oxidase, the primary function of CERU is to oxidize iron (Fe) from the Fe^{2+} to Fe^{3+} state, thus allowing Fe to bind to transferrin and be transported in the circulation [2,3]. Decreased CERU is a characteristic finding in Wilson disease (WD) [4], an inherited disorder associated with mutations in the *ATP7B* gene. The *ATP7B* protein is a P-type ATPase involved in copper transport [5]. Mutations in *ATP7B* can therefore lead to copper accumulation, particularly in the liver and nervous system which may result in hepatic, neurologic, and/or psychiatric symptoms [6,7]. Other disorders that are associated with decreased CERU concentrations include Menkes Disease (due to mutations in the *ATP7A* gene) [8], as well as mutations in the gene for CERU, as seen in aceruloplasminemia [9]. Conversely, increased CERU concentrations may be associated with acute phase reactions and clinical scenarios of increased estrogens, such as pregnancy or use of oral contraceptives [10,11].

CERU is typically measured on automated chemistry analyzers and/or immunoanalyzers using nephelometric or turbidimetric methods. Interpretation of results in the context of suspected WD requires corresponding measurement of serum free copper or urine copper. Molecular testing for *ATP7B* mutations is available, although given its expense molecular testing is generally only used to confirm diagnoses and/or to conduct testing of family members in confirmed cases.

As nephelometric and turbidimetric methods are dependent on light scattering, potential interference due to specimen lipemia (e.g. turbidity with increased lipids) is widely recognized [12–14]. Diagnostic manufacturers are required to conduct interference testing for clinical laboratory assays and define acceptable limits for which potential interferents do not adversely affect patient results [15]. However, approaches to assessing and describing lipemic interference vary widely across manufacturers and often use Intralipid – an emulsion derived from soybean oil [16]. Lipemic interference may be reduced by applying techniques such as ultracentrifugation (UC), lipid clearing reagents such as StatSpin LipoClear® (LC) (IRIS International; Chatsworth, CA), or dilution (DIL) [13,17,18]. It is critical, however, to validate that a chosen interference-reduction technique does not adversely affect the baseline concentration of analyte being measured.

The potential for lipemic interference in CERU assays has previously

* Corresponding author at: University of Utah, Department of Pathology, ARUP Laboratories, 500 Chipeta Way, Mail Code 115, Salt Lake City, UT 84108, USA.
E-mail addresses: joshua.hunsaker@aruplab.com (J.J.H. Hunsaker), sonia.laulu@aruplab.com (S.L. La'ulu), sara.wyness@aruplab.com (S.P. Wyness), jonathan.genzen@path.utah.edu (J.R. Genzen).

https://doi.org/10.1016/j.cca.2018.01.037
Received 29 October 2017; Received in revised form 21 December 2017; Accepted 20 January 2018
Available online 31 January 2018
0009-8981/ © 2018 Elsevier B.V. All rights reserved.

been described on Modular Analytic P (Roche Diagnostics; Indianapolis, IN) [19] and BN ProSpec (Siemens; Tarrytown, NY) [14] analyzers. These studies were conducted using Intralipid [14,19] and/or endogenously lipemic patient samples [19]. It is unknown whether CERU assays from different manufacturers have different susceptibility to lipemic interference, or if methods to remove such interference (e.g. UC, LC, and/or DIL) are effective when applied to specimens across different platforms. The present study was therefore designed to characterize lipemic interference among three FDA-cleared CERU assays. Procedures designed to address lipemia were also evaluated to determine their effectiveness at reducing lipemia and to understand whether any of these methods may adversely affect baseline CERU concentrations in non-lipemic specimens.

2. Materials and methods

2.1. General

For studies involving patient specimens, residual clinical specimens were obtained from −20 °C storage and de-identified according to an Institutional Review Board (IRB)-approved protocol (University of Utah IRB Protocol #0007275). Experiments were conducted using three different instruments: ARCHITECT *ci*8200 (Abbott; Abbott Park, IL), AU5800 (Beckman Coulter; Brea, CA), and cobas Integra 400 Plus (Roche Diagnostics) using CERU assays from their corresponding instrument manufacturers [20–22].

2.2. Serum indices and vendor criteria

Serum indices – hemolysis (H), icterus (I), and lipemia (L) – were performed on each instrument for all specimens to obtain the lipemic index (L-index) according to each manufacturer's method and scale. The AU5800 reports L-index on a semi-quantitative scale corresponding to concentration of Intralipid (in approximate mg/dL units): *N* (e.g. none), < 40; *+1*, 40–99; *+2*, 100–199; *+3*, 200–299; *+4*, 300–500; *+5*, > 500 [23]. Abnormal (ABN) high L-index error occurs with markedly lipemic specimens when the "the mathematical logic in determining the amount of interference failed one or more internal evaluations" [24]. The manufacturer states these may be considered consistent with *+5* L-index results when severe turbidity is present [23]. ABN high L-index results in this study were therefore grouped into the *+5* category for subsequent analysis. The *ci*8200 and Integra 400 Plus report numeric L-index results displayed in approximate mg/dL units [25–27]. It should be emphasized that L-index results are not meant to provide accurate quantitative measurements of triglycerides [25] and are therefore not displayed with mg/dL units throughout this manuscript.

Lipemic interference thresholds for CERU assays are included in their respective package inserts (PIs). The *ci*8200 CERU PI describes interference studies with "acceptance criteria ± 10% deviation from target value" and an interference threshold of 1000 mg/dL Intralipid [20]. The AU5800 CERU PI states that there is "no significant interference up to 1000 mg/dL Intralipid" [21]. The Integra CERU PI states that there is "no significant interference up to an L-index of 50" also citing use of Intralipid for interference studies [22]. Roche literature describes lipemic interference on the Integra as "increasing" CERU results [27].

Hemolysis-index (H-index) and icteric-index (I-index) on the *ci*8200 and Integra roughly correlate to mg/dL hemoglobin and mg/dL bilirubin, respectively [25–27]. H-index and I-index scales on the AU5800 are semi-quantitative and are defined as: hemolysis, *N* (e.g. none), < 50; *+1*, 50–99; *+2*, 100–199; *+3*, 200–299; *+4*, 300–500; *+5*, > 500, and icterus, *N* (e.g. none), < 2.5; *+1*, 2.5–4.9; *+2*, 5.0–9.9; *+3*, 10–19.9; *+4*, 20–40; *+5*, > 40 [23].

2.3. Precision

To evaluate assay imprecision, experiments were conducted over 5 days with 2 runs daily, and a minimum of 2 h separating each run. Two levels of Thermo Scientific™ MAS™ Omni·CORE QC material (levels 1 and 3; low and high) were run in duplicate for each run. Multiple bottles of each level of QC were prepared following manufacturer's instructions, pooled together, aliquoted for single use, and stored at 2–8 °C prior to analysis.

2.4. Diluent evaluation

A stock solution of CERU in human serum was created by enriching human AB sera (Mediatech; Manassa, VA) with lyophilized human CERU (#C4519, Sigma-Aldrich; St. Louis, MO) to yield a standard serum solution with a CERU concentration of approximately 40–50 mg/dL. Serial dilutions of this stock solution were then made using diluents recommended in the PIs across each assay: distilled water (dH_2O), 0.85% NaCl, and 9% NaCl. Three sets of serial dilutions were prepared for each diluent and tested on all platforms. Linearity and average % recoveries were then evaluated for each diluent.

2.5. Patient specimens and exclusions

Residual patient serum specimens (n = 75) with L-index results ranging from 0 to 2000, as previously determined on a cobas 8000 system (Roche), were retrieved from −20 °C storage and de-identified. 5 specimens were excluded from all subsequent analyses: *n* = 3 (AU5800 baseline produced a negative CERU result); *n* = 1 (Integra outlier with DIL treatment, insufficient quantity to repeat testing); n = 1 (*ci*8200 did not result due to lipemic interference). The 70 specimens analyzed had baseline L-index values ranging from non-lipemic to markedly lipemic (average ± SD, min-max): *ci*8200 (335.5 ± 386.7, 1–1673), AU5800 (3.0 ± 3.0, 0–5), Integra (490.3 ± 497.9, 15–2150). Specimens were generally non-hemolyzed [H-indices: *ci*8200 (7.5 ± 21.7, 0–144), AU5800 (0.1 ± 0.4, 0–2), Integra (5.8 ± 19.8, 0–113) and non-icteric [I-indices: *ci*8200 (0.5 ± 1.8, 0–15.2), AU5800 (0.1 ± 0.4, 0–3), Integra (0.4 ± 1.9, 0–16)]. Lastly, 11 specimens were excluded only from the DIL data for the Integra, as diluted specimen results were below the assay analytical measuring range (AMR) and accurate calculation of % difference could not be calculated for these specimens.

2.6. Methods of eliminating lipemic interference

Patient serum specimens were split into an aliquot to remain untreated, and three additional aliquots were made to evaluate methods to minimize lipemia: 1) UC, 2) LC, and 3) 1:5 DIL in dH_2O. UC was performed using an Airfuge Ultracentrifuge (Beckman Coulter; Brea, CA) with an A-95 rotor for 10 min (maximum speed, 95,000 rpm; maximum relative centrifugal field, 178,000 ×*g*). LC treatment was performed according to the PI protocol where a dilution of 0.1 mL reagent to 0.5 mL of each specimen was prepared and allowed to incubate at room temperature for 5 min, followed by centrifugation at 2000 ×*g* for 20 min using a Beckman Allegra X-12 benchtop centrifuge (Beckman Coulter; Brea CA) [28]. Following centrifugation, cleared samples were aliquoted into fresh tubes.

After lipid removal procedures were performed, all untreated and treated aliquots were then tested on each platform for CERU and serum indices. For LC studies, results from treated specimens were multiplied by 1.2 to adjust for dilution by the LC reagent [28]. For 1:5 DIL studies, results after testing were adjusted to account for the dilution factor.

The effectiveness of each lipemia removal strategy was assessed by calculating the L-index change for each specimen (on each analyzer), which was defined as:

Download English Version:

https://daneshyari.com/en/article/8309639

Download Persian Version:

https://daneshyari.com/article/8309639

Daneshyari.com