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Analytical interference of HBOC-201 (Hemopure, a synthetic hemoglobin-based oxygen carrier) on four common clinical chemistry platforms

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ABSTRACT

Background: There are 13 million blood transfusions each year in the US. Limitations in the donor pool, storage capabilities, mass casualties, access in remote locations and reactivity of donors all limit the availability of transfusable blood products to patients. HBOC-201 (Hemopure®) is a second-generation glutaraldehyde-polymer of bovine hemoglobin, which can serve as an "oxygen bridge" to maintain oxygen carrying capacity while transfusion products are unavailable. Hemopure presents the advantages of extended shelf life, ambient storage, and limited reactive potential, but its extracellular location can also cause significant interference in modern laboratory analyzers similar to severe hemolysis.

Methods: Observed error in 26 commonly measured analytes was determined on 4 different analytical platforms in plasma from a patient therapeutically transfused Hemopure as well as donor blood spiked with Hemopure at a level equivalent to the therapeutic loading dose (10% v/v).

Results: Significant negative error ratios > 50% of the total allowable error (> 0.5tAE) were reported in 23/104 assays (22.1%), positive bias of > 0.5tAE in 26/104 assays (25.0%), and acceptable bias between −0.5tAE and 0.5tAE error ratio was reported in 44/104 (42.3%). Analysis failed in the presence of Hemopure in 11/104 (10.6%). Observed error is further subdivided by platform, wavelength, dilution and reaction method.

Conclusion: Administration of Hemopure (or other hemoglobin-based oxygen carriers) presents a challenge to laboratorians tasked with analyzing patient specimens. We provide laboratorians with a reference to evaluate patient samples, select optimal analytical platforms for specific analytes, and predict possible bias beyond the 4 analytical platforms included in this study.

1. Introduction

Over 13 million transfusions are administered each year in the U.S [1]. General medicine services are the primary consumers of donor red blood cells (RBCs) with 28.5% of the total volume, followed by Surgery and associated Subspecialties (19.8%), Oncology (19.2%), ICU (12.5%), and Trauma (9.5%) [1]. Demand for blood products can exceed supply in the case of mass casualties, transport of blood products is not without risk, and collected units are subject to finite shelf life and mandatory preparation time before administration. Not all facilities have blood banks on site, traumas may occur far from hospitals where first responders do not possess readily available blood products for transfusion on the scene, and an aging population is expected to require blood-intensive surgical and oncologic interventions at an ever-increasing rate.

Further, blood products must be matched to a patient's serology and patients with uncommon blood types were at one time severely limited in availability of transfusable units. In 1998, the American Red Cross (ARC) and the American Association of Blood Banks (AABB) collaborated to form the American Rare Donor Program (ARDP) to serve the needs of patients expressing antibodies to high-frequency antigens or with multiple common antibodies. The ARDP maintains a database (REGGI) with > 59,000 active rare donors and mediates transfusion requests from providers. Even with this network of rare donors in place, 7.9% of requests are left unfilled due to inadequate numbers of identified donors or inadequate retention of donors [2]. Regardless of the reason requests are left unfilled, patients with rare blood types are left with few options. Thus, there is a clear need for additional options to

Abbreviations: HBOC, Hemoglobin-based oxygen carrier; ARC, American Red Cross; AABB, American Association of Blood Banks; ARDP, American Rare Donor Program; tAE, Total Allowable Error (Ratio)

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meet the demand for transfusion products.

Hemoglobin-based oxygen carriers (HBOC) have been a target of study and potential therapy since the work of Amberson and others 80 years ago [3]. Briefly, purified hemoglobin was administered to animals and later patients in an attempt to avoid transfusion reactions involving cell surface antigens. First-generation HBOCs were generally smaller molecules, which resulted in extravasation from the vessel and nephrotoxicity. Second generation HBOCs are larger, polymerized molecules to reduce these effects. HBOC-201 (Hemopure®, Hemoglobin Oxygen Therapeutics) is a second-generation glutaraldehyde-polymer of bovine hemoglobin which can serve as an "oxygen bridge" to maintain oxygen carrying capacity while transfusion products are unavailable due to location, demand or recipient reactivity. A similar formulation named Oxyglobin® was approved by the FDA in 1998 to treat anemia in canines, but Hemopure has never achieved FDA approval for use in humans. Hemopure has the distinct advantages over packed red blood cells of a 3-y shelf life, ambient temperature storage (2–30 °C), and no risk of immune-mediated hemolytic anemia seen in unmatched red cells due to the cell-free format of Hemopure. The polymerized hemoglobin is prepared at 13 ± 1 g/dL and remains isosmotic to serum (300 ± 15 mOsm/Kg) in lactated Ringer's solution (4 mmol/L Potassium Chloride, 11 mmol/L Sodium Hydroxide, 27 mmol/L Sodium Lactate, 1.6 mmol/L Calcium Chloride, 114 mmol/L Sodium Chloride, 200 mg/dL *N*-acetyl-L-cysteine). The downside is the significantly shortened half-life in blood of 13–24 h compared to transfused RBCs which typically survive 90 days or more [4–6]. Further, the extracellular nature of Hemopure in patient serum results in an appearance of severe hemolysis in serum or plasma, potentially complicating many routine assays used to assess the physiologic state of a critically ill patient. Fig. 1 provides an example of separated human plasma both without (A) and with (B) a 10% spike of Hemopure as well as serum without (C) and with (D) a 10% spike of Hemopure.

A limited set of publications described the effect of Hemopure on chemistry analyzers at the time Hemopure was first introduced over 2 decades ago. In 1997, Callas et al. spiked plasma, serum and whole blood over a range of Hemopure concentrations thought to include the common therapeutic range and subsequently assessed the bias in reported values for chemistry, coagulation and complete blood counts and/or therapeutic drugs on a variety of analyzers commonly used at the time [4]. Callas described absorbance peaks at 415, 540 and 576 nm, which complicated colorimetric assays, while ion-selective electrode (ISE) methods were largely unaffected. Turbidometry, radiometry, fluorescent and amperometric methods varied in their response in the presence of Hemopure. Interestingly, gentamycin and vancomycin exhibited significantly greater interference from Hemopure compared to other therapeutic drugs even though they shared the same method (fluorescence polarization) and platform as the unaffected drugs. The authors postulated that this could be "due to unique and complex interactions with some component(s) of the reaction product", which offers the potential for unique interferences in any modern assay. In the same issue as Callas, Ma et al. published bias induced by Hemopure and an additional blood substitute on several platforms and established guidelines for institutional management of Hemopure samples from patients [7]. Two years later, Wolthuis et al. showed significant negative effects of spiked Hemopure in the measurement of creatinine kinase MB fraction (CK-MB) and total creatinine kinase (CK) on a Cobas Integra (Roche), interferences which were not seen in the systems utilized by Callas in their 1997 study [8]. Neither study utilized samples obtained from patients following administration of Hemopure. In the 20 years following these initial studies, a handful of additional studies have described the effects of Hemopure (or the related animal version, Oxyglobin) on chemistry analysis following its in vivo administration to swine and several others have updated earlier studies using spiked human samples looking at specific analytes, but to our knowledge, no comprehensive study to date has assessed variability in chemistry analyses in human samples following in vivo administration of Hemopure [6,7,9–12]. Therefore, the objective of the current study was to assess the interference and its direction on common clinical chemistry tests currently used in clinical laboratories.

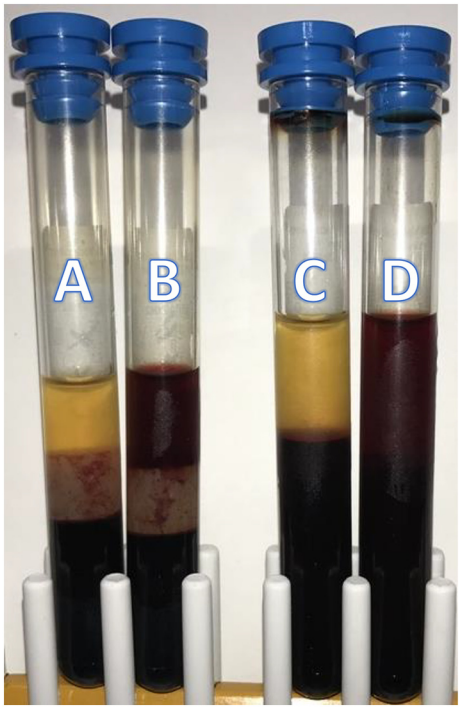

Fig. 1. An example of separated human lithium-heparin plasma both without (A) and with (B) a 10% spike of Hemopure as well as serum without (C) and with (D) a 10% spike of Hemopure. (Beckman Coulter IDS plug caps do not denote a specific additive.) (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2. Methods

HBOC-201 (Hemopure Lot H11T04) was obtained free of charge from the manufacturer through a compassionate use agreement and the Food and Drug Administration (FDA) likewise approved administration of the unapproved therapeutic for compassionate use.

Plasma samples were obtained from remnant volume samples from physician-ordered routine testing at the first opportunity to collect sufficient volumes, which was 36 h post infusion. The initial patient sample was drawn 12 h before infusion, thus the in vivo comparison data represents the amount of Hemopure remaining after 36 h in addition to a total of 48 h of biological variation. The in vivo samples were aliquoted into 0.5 mL volumes and maintained frozen at −70 °C or on dry ice until all facilities were able to analyze the samples on the same date and time to prevent variation due to unequal storage time. Freshly drawn donor whole blood was spiked with 10% Hemopure (v/v) to approximate the final concentration in the patient's blood following administration of 3 units of Hemopure. The in vitro samples were immediately separated by centrifugation (5 min at 2400 × *g*) in Vacutainer Lithium-Heparin with Gel plasma separators (Becton Dickinson), the standard method in our laboratory, then aliquoted and frozen alongside the in vivo samples.

All samples were processed on the respective systems as routine patient samples, with the exception that values would be reported regardless of high hemolysis index. Clinical platform comparisons included the UniCel DxC 800 (Beckman Coulter), Advia 1800 (Siemens Healthineers), Cobas 8000 with c702, c502, and e602 modules (Roche Diagnostics), and the Vitros 5600 (Ortho Clinical Diagnostics). All systems were in routine clinical use at the facility for all analytes included in the study. Proper calibration, quality control, preventative