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Frequency of icteric interference in clinical chemistry laboratory tests and causes of severe icterus

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ABSTRACT

Objectives: The aims of this study were to identify the causes of severe icterus in an academic medical center patient population and to assess the impact of icterus on clinical chemistry testing using assay package insert thresholds.

Design: and Methods: In this retrospective study at an academic medical center core clinical laboratory, icteric, hemolysis, and lipemia indices were available for all serum and plasma chemistry specimens analyzed on Roche Diagnostics cobas 8000 analyzers over a 12-month period, encompassing 414,502 specimens from 94,081 unique patients (51,851 females; 42,230 males) including children, inpatient, outpatient, and emergency department patients. Extensive chart review was done for all 57 patients (4 pediatric, 53 adult; 534 total specimens) who had one or more samples with an icteric index of 40 or higher (defined as severe icterus).

Results: Specimen icteric index exceeded package insert icteric index thresholds in 0.14% of clinical chemistry assays, with the highest number of instances for creatinine (1358 samples, 0.6% of total tests), total protein (1194 samples, 2.2%), and ammonia (161 samples, 3.9%). The 57 patients with an icteric index of 40 or higher accounted for 49.7% of all instances where the icteric index exceeded the specific assay package insert limit. The most common etiologies of this group of 57 patients were alcohol-related liver disease (34 patients), biliary tract disease (7 patients), and neoplasms (6 patients).

Conclusions: Approximately half of all instances where specimen icteric index exceeded assay package insert thresholds occurred in a small cohort of patients with severe liver/biliary tract disease.

1. Introduction

Endogenous interferences comprise a significant source of error for clinical laboratory testing [1–5]. Common endogenous interferences include hemolysis, icterus, and lipemia [1,6–9]. Icterus is caused by elevated concentrations of bilirubin, which can occur in a variety of physiologic states and disease conditions in children and adults [10,11].

The presence of bilirubin, and potentially also breakdown products such as biliverdin, can interfere with laboratory analysis by

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several mechanisms [3,12,13]. At a broad level, the two main mechanisms for icteric interference are spectral interference or chemical reactivity with assay reagents [13,14]. The spectral properties of bilirubin can cause interference with oximetry, co-oximetry, and methemoglobin measurements [3]. These interferences are related to the presence of bilirubin and/or biliverdin. In terms of chemical reaction interferences, bilirubin is well-known to interfere with peroxidase-coupled reactions used for assays such as total cholesterol, triglycerides, and uric acid [14]. Bilirubin also interferes with Jaffe and enzymatic creatinine methods [15–17]. Specimen icterus associated with total bilirubin concentrations of less than 10 g/dL usually does not significantly impact clinical laboratory testing. However, the presence of higher levels of icterus is more likely to affect laboratory analysis depending on vulnerabilities of specific assays to icteric interference [1,3,5]. As such, there is interest in identifying which patient factors are most likely to be associated with levels of icterus that can significantly impact clinical laboratory analysis. We were unable to find any consistent definition in the published literature of levels of icterus. For the current study, we defined 'severe icterus' as a specimen with icteric index of 40 or higher. We chose icteric index of 40 as severe based on preliminary studies on our patient population that this degree of icterus was only found in less than 1% of patients and also exceeded the package insert thresholds for icterus for over 30 chemistry assays on our test menu.

Hyperbilirubinemia may be caused by a variety of conditions [10,11]. Increased bilirubin production is seen in conditions such as hemolysis, ineffective erythropoiesis, and skeletal muscle damage. These conditions tend to have unconjugated (indirect) hyperbilirubinemia. Hyperbilirubinemia due to decreased bilirubin conjugation occurs in physiologic jaundice of the newborn, Gilbert syndrome, and the rare Crigler-Najjar syndromes. Hyperbilirubinemia due to decreased excretion may be seen in a variety of diseases with impaired liver and/or biliary tract function including hepatitis, cirrhosis (many possible etiologies), obstruction of biliary drainage, and rare congenital defects in biliary excretion. These conditions will lead to a predominantly conjugated (direct) hyperbilirubinemia.

There are a variety of methods to assess icterus in laboratory samples [2–5,12,13,18–21]. The simplest is visual inspection of the specimen; however, there is significant inaccuracy and inter-individual variation in this type of assessment [3]. A second approach is to measure total and sometimes also conjugated bilirubin. As a third option, most clinical chemistry platforms possess the ability to quickly determine an icteric index [4–6,14,18,19,22,23]. This may be done as part of automated protocols that first dilute the patient's sample in saline or buffer and then measure specific wavelengths for determination of indices for hemolysis, icteric, and lipemia (HIL). Different manufacturers use various wavelengths to detect icteric index. For example, Roche Diagnostics cobas and Siemens Advia chemistry analyzers use very similar primary and secondary wavelengths (480/505 nm and 478/505 nm, respectively), while Abbott Architect and Ortho Vitros analyzers use different sets of wavelengths [5]. There are advantages and disadvantages of automatic detection of HIL indices [5,14,19,24,25]. Low cost, high-throughput, rapid analysis time, and excellent reproducibility are some of the advantages of automatic detection, whereas some of the disadvantages are lack of standardization among manufacturers in reporting indices [18,19,24,25]. This may be further complicated when reagents produced by one manufacturer are used on instrumentation from another manufacturer. The performance of icteric indices might also be sub-optimal in patient conditions that result in predominantly conjugated hyperbilirubinemia [20], a finding also supported by limited data from preanalytical surveys comparing HIL indices across platforms (see discussion in Ref. [5]). In addition, other constituents of patient samples can potentially cause absorption at the wavelengths used for determination of the indices [3,26].

The aim of this study was to assess the causes of severe icterus and additionally the impact of icterus on clinical chemistry testing using assay package insert thresholds. To this end, we utilized a large body of retrospective data from the centralized laboratory at an academic medical center. We focused our chart review on patients who had specimens with markedly icteric indices, reasoning that specimens from these patients have the highest likelihood of causing clinically significant interference. The icteric index data was drawn from all clinical chemistry testing performed during a 12-month period at an academic medical center core clinical laboratory.

2. Materials and methods

The study was conducted at the University of Iowa Hospitals and Clinics (UIHC), an 811-bed tertiary/quaternary care academic medical center with inpatient and outpatient services. The electronic health record (EHR) for UIHC since May 2009 has been Epic (Epic systems, Inc., Madison, WI, USA). The data in this study is from the central (core) laboratory clinical chemistry section and was collected as part of a retrospective study approved by the University of Iowa Institutional Review Board (protocol # 201907707) covering the time period from January 1, 2018 to December 31, 2018. This study was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki). As described in our previous studies [27], Reporting Workbench functionality within Epic allowed for retrieval of past laboratory results from the EHR database. Interfacing throughout the laboratory from instruments to the laboratory information system (Epic Beaker) is provided by Middleware software (Instrument Manager) from Data Innovations (Burlington, VA) [28,29]. HIL indices were extracted from Instrument Manager data in the time period of January 1, 2018 to December 31, 2018 using specimen accession numbers, allowing for linkage to patient data and the laboratory testing ordered on the accession number [30].

The main automated clinical chemistry instrumentation was from Roche Diagnostics (Indianapolis, IN, USA), a cobas 8000 system with two c702, three c502, and five e602 analyzers. The present study includes data for a total of 114 chemistry assays run on the cobas 8000 system, 105 assays using Roche Diagnostics reagents and 9 assays using reagents from other manufacturers. All serum and plasma specimens run on the Roche analyzers use spectrophotometry to determine HIL indices, which are used in autoverification rules for all chemistry tests [28]. The analyzers take an aliquot of the patient specimen and dilute in 0.9% sodium chloride saline to measure the absorbance for icterus at 480 nm (primary wavelength) and 505 nm (secondary wavelength), for hemolysis at 570 nm (primary wavelength) and 600 nm (secondary wavelength), and lipemia at 660 nm (primary wavelength) and 700 nm (secondary wavelength)

[5]. Four assays (angiotensin converting enzyme assay from Trinity Biotech; direct bilirubin, total bilirubin, and plasma hemoglobin assays from Roche Diagnostics) did not have an icteric index threshold stated in their respective package inserts. At time of instrument validation, verification of HIL indices was performed by comparison of hemolysis index to hemolysate added to patient pools, comparison of icteric index to total and direct bilirubin measurements, and comparison of lipemic index to intralipid added to patient pools. This follows recommendations from Clinical and Laboratory Standards Institute [14]. The laboratory also performs ongoing comparisons of HIL indices across the different Roche analyzers.

3. Results

3.1. Icteric index data distribution

Data for HIL indices from the UIHC core clinical chemistry laboratory over a 12-month period were available for 414,502 specimens from 94,081 unique patients (51,851 females and 42,230 males). Detailed chart review was performed for all patients in the data set who had one or more samples with icteric index of 40 or higher, which included 57 unique patients (0.06% of all unique patients). Only 534 specimens (0.13%) from these 57 patients had an icteric index of 40 or higher. There were only 96 specimens from 9 unique patients with an icteric index of 60 or higher. Table 1 has a summary of the patient demographics. Fig. 1 has a flow diagram for the specimens and patients included in the retrospective study. Fig. 2 shows the distribution of icteric indices for specimens using a logarithmic scale. Of the 414,502 total specimens, 400,291 (96.6%) had an icteric index less than 5 and 408,426 (98.5%) had an icteric index less than 10. Of the chemistry assays analyzed in the present study, an icteric index of 10 was the lowest icteric index threshold in the package insert for any assay except for an infrequently ordered enzymatic assay for ethylene glycol (package insert icteric index threshold of 2.2; no specimens tested in the present study for ethylene glycol exceeded that threshold).

Specimens with higher icteric index were more common in inpatient units. For 408,626 specimens with an icteric index of 9 or lower, 211,876 (51.9%) were collected from inpatient locations, while 42,269 (10.3%) were from the emergency department and 154,281 (37.8%) were from outpatient locations (including phlebotomy sites). In contrast, for 6076 specimens with icteric index of 10 or higher, 5131 (84.4%) were collected from inpatient locations, while 427 (7.0%) and 518 (8.5%) were from emergency department or outpatient locations, respectively. For 534 specimens with icteric index of 40 or higher, the trend was even more pronounced towards inpatient location: 479 specimens (89.7%) from inpatient units, 41 (7.7%) specimens from the emergency department, and only 14 specimens (2.6%) from outpatient locations.

3.2. Association between icterus, other indices, and bilirubin fractions

Fig. 3 shows the relationship between icteric index and hemolysis index (Fig. 3A) or lipemia index (Fig. 3B), with the indices grouped into categories and classified as percentages of the total within an icteric index category. Interestingly, specimens with icteric indices between 5 and 29 had a higher proportion of specimens showing mild hemolysis (H index 31–100), moderate hemolysis (H index 101–300), or severe hemolysis (H index >300) (Fig. 3A). However, at icteric indices of 30 or higher, this relationship was much less apparent. With respect to lipemia index, specimens with icteric indices of 20 or higher showed a higher proportion of mild lipemia (L index 31–120) (Fig. 3B). For the 96 specimens with an icteric index of 60 or higher, 48 (50%) showed an L index of at least 31 compared to only 52,523 of 408,426 (12.9%) specimens with an icteric index of less than 10.

Fig. 4 shows the relationship between icteric index and direct (conjugated) bilirubin (Fig. 4A) and indirect (unconjugated) bilirubin (Fig. 4B) for specimens in which direct and total bilirubin were both determined (4172 total specimens). Indirect bilirubin was calculated by subtracting direct from total bilirubin. Specimens with icteric index exceeding 20 were predominantly ones where direct bilirubin was the major fraction (Fig. 4A). Total bilirubin (measured in mg/dL) showed an overall linear relationship across the entire span of icteric indices (R^2 0.992) with a slight negative proportional bias (slope 0.73).

3.3. Impact of icterus on specific clinical chemistry assays

Of the 114 different chemistry assays analyzed in the retrospective timeframe, 58 assays had at least one specimen with an icteric index that exceeded the package insert threshold, and 29 assays had 10 or more specimens exceeding icteric index threshold (Table 2). As mentioned above, 4 assays did not list an icteric index threshold in the package insert. By absolute number of occurrences, the assays with the most instances exceeding the icteric index threshold were enzymatic creatinine (1358 samples, 0.6% of total creatinine tests), total protein (1194 samples, 2.2%), and ammonia (161 samples, 3.9%). In addition to total protein and ammonia, there were 4 other assays where 1.0% or more of the specimens tested for that assay exceeded the icteric threshold: hepatitis B core IgM antibody (16 specimens, 1.8%), hepatitis B core total antibodies (51 specimens, 1.2%), acetaminophen drug level (47 specimens, 1.1%), and beta-

Table 1
Patient demographics.

	N Unique Patients (females/males)	Average Age ± SD	Median Age	Age Range
All patients	94,081 (51,851/42,230)	44.4 ± 23.6	47	Newborn to > 89
Patients with one or more samples with icteric index 40 or higher	57 (17/40)	47.0 ± 17.7	49	Newborn to 84

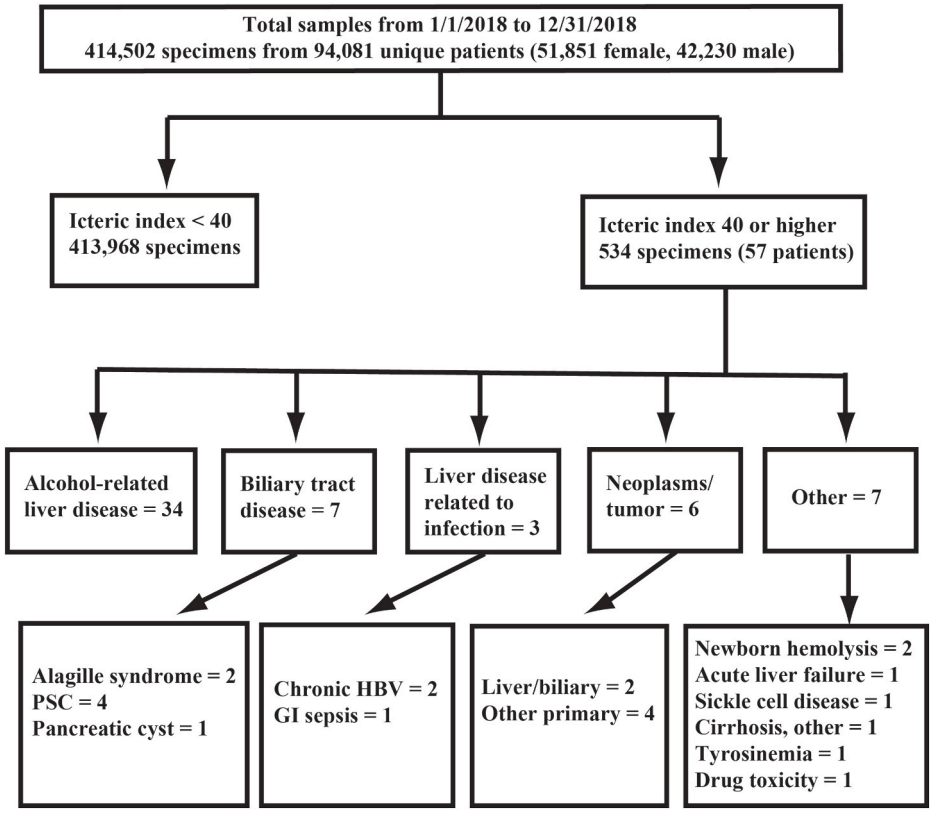

Fig. 1. Flow chart showing the breakdown of icteric indices and the underlying diseases in those with one or more specimens with an icteric index of 40 or higher. Abbreviations: HBV, hepatitis B virus; PSC, primary sclerosing cholangitis.

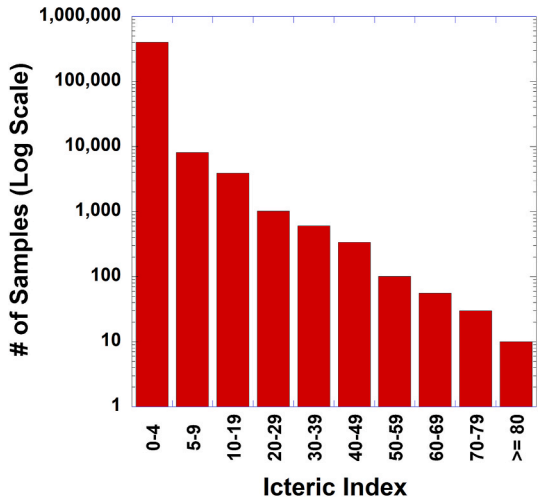

Fig. 2. Distribution of icteric index of 414,502 specimens. The number of specimens is plotted on a logarithmic scale.

hydroxybutyrate (27 specimens, 1.0%). In total, out of 2,791,591 discrete assays tested (including components of panels), 3888 (0.14%) were on specimens that had an icteric index exceeding the assay package insert threshold for icterus.

3.4. Patient cohort with one or more specimens with icteric index of 40 or higher

A total of 57 unique patients had at least one specimen with an icteric index of 40 or higher. These 57 patients accounted for all 534

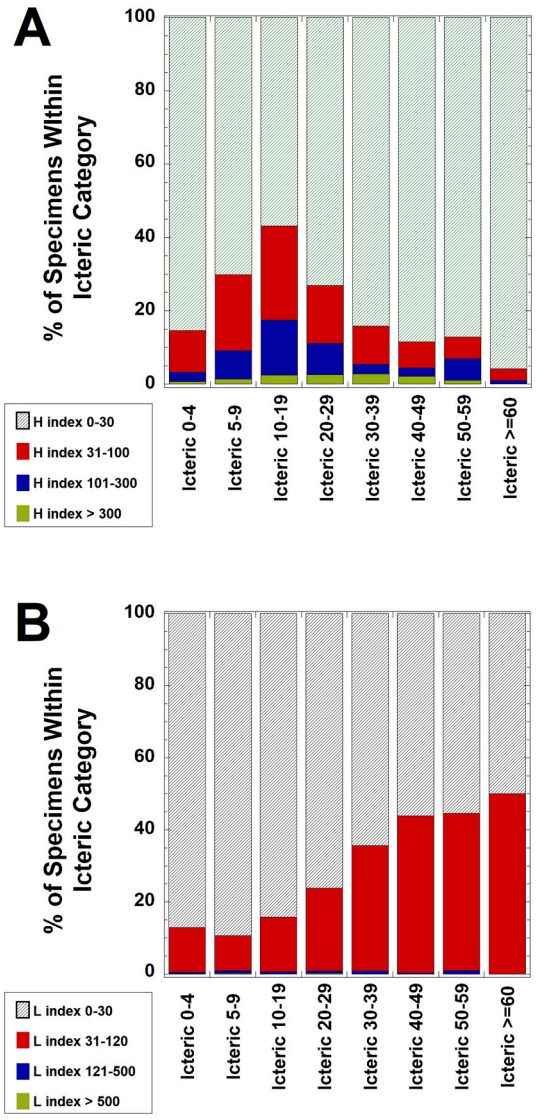

Fig. 3. The bar graph details the frequency of hemolysis (A) and lipemia (B) within ranges of icteric index. Overall, specimens with icteric indices of 5–29 have higher rates of hemolysis compared to specimens with icteric indices outside of this range. Lipemia indices of 31–120 ('mild lipemia') was increasingly more common for higher icteric index categories starting at icteric index of 20.

specimens with an icteric index of 40 or higher, and 1375 out of 6076 (22.6%) specimens with an icteric index of 10 or higher. This cohort further accounted for 1934 out of 3888 (49.7%) total instances where the specimen icteric index exceeded the package insert threshold for individual assays.

From detailed chart review, the most common disease etiologies likely underlying the icterus for this group of patients were: alcohol-related liver disease (34 patients), biliary tract disease [7 patients, including Alagille syndrome (2 patients), primary sclerosing cholangitis (4 patients), and pancreatic cyst blocking biliary flow (1 patient)], liver disease related to infection [including chronic hepatitis B virus infection (2 patients) and gastrointestinal sepsis (1 patient)], and neoplasms impacting the liver and biliary tract (2 patients with primary tumors in the liver/biliary tract and 4 patients with aggressive, metastatic tumors with primary either unknown or originating outside the liver/biliary tract). There were 2 neonates with hemolytic disease of the newborn. The remaining 5 patients had the following underlying diseases: acute liver failure (which resolved), cirrhosis of unknown etiology, drug toxicity (suspected cholestatic hepatitis from leflunomide), sickle cell disease, and tyrosinemia type 1 (Fig. 1).

The group of 57 patients with very high icterus had high mortality, with 15 patients (26.3%) expiring during the inpatient admission where laboratory testing with the high icteric index was performed or directly afterwards following discharge to hospice or home care. Another 15 patients (for a total of 30 or 52.6% of total) expired within 12 months following the first specimen to have an icteric index of 40 or higher in 2018, and an additional 6 patients expired between 1 and 3 years after the first specimen to have an icteric index of 40 or higher. Only 1 of the 4 pediatric patients with an icteric index of 40 or higher expired within 3 years of testing.

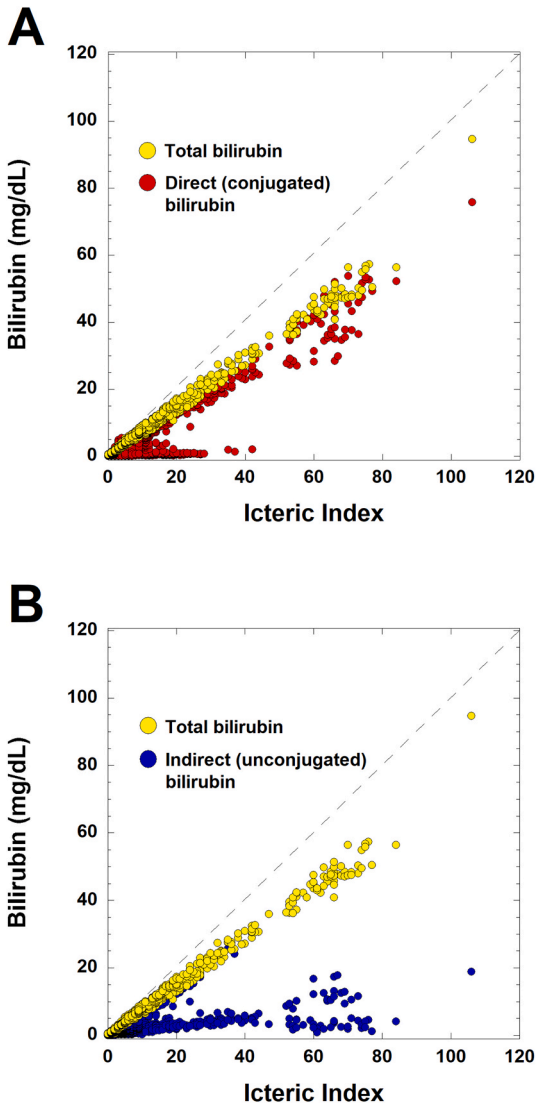

Fig. 4. The relationship between direct bilirubin (A), indirect bilirubin (B), and total bilirubin (both A and B) relative to icteric index. Bilirubin is measured in mg/dL. Direct (conjugated) bilirubin was the main fraction of bilirubin for specimens with icteric index of 25 or higher. The linear regression equation for total bilirubin vs. icteric index was $y = -0.026 + 0.73$ x ($R^2 = 0.992$). The dashed line is the line of 1:1 relationship between bilirubin and icteric index.

This pediatric patient was 17 years old at the time of the first specimen with high icteric index but died after turning 18 from long-standing multi-organ complications of Alagille syndrome.

4. Discussion

In this retrospective study at an academic medical center, we analyzed a large data set for icteric index, which was measured routinely as part of HIL indices performed on all plasma and serum samples by the automated clinical chemistry system. Published literature has demonstrated interference by icterus predominantly occurs through spectral properties or chemical reaction with enzymatic methods [13,14]. In contrast to hemolysis, high levels of bilirubin are related to patient disease conditions as opposed to pre-analytical factors such as phlebotomy technique, specimen transport, and specimen processing [18,31]. In this sense, icterus has more similarity to lipemia, for which disease conditions such as diabetes and dyslipidemias underlie many cases leading to severe lipemia [3,30]. However, unlike lipemia, icterus is not strongly affected by diet status (e.g., fasting or non-fasting) or medications/treatments such as how lipid emulsions or propofol might temporarily impact lipemia index [27,32,33].

One important limitation impacting studies of both icteric and lipemic interference is that there are very limited published data comparing these two indices across different chemistry platforms. Other than a reference to unpublished preanalytical survey data (see

Table 2
Assay details and data on icteric interference.

Assay name[a]	Package insert icteric index limit	Total tests performed in retrospective analysis period	Number of results that exceed package insert icteric index limit (%)	Number of results that exceed package insert icteric index limit by patients who had one or more icteric indices of 40 or higher
Creatinine, Enzymatic	15	242,553	1358 (0.6%)	550
Total Protein	20	53,920	1194 (2.2%)	198
Ammonia, Plasma	30	4139	161 (3.9%)	143
Potassium	60	239,930	75 (<0.1%)	75
		189,723	71 (<0.1%)	71
Sodium	60	199,506	71 (<0.1%)	71
Chloride	60	218,324	70 (<0.1%)	70
Blood Urea Nitrogen (BUN)	60	223,090	57 (<0.1%)	57
Glucose	60	204,961	57 (<0.1%)	57
Calcium	60	185,556	56 (<0.1%)	56
Hepatitis B Core Antibody Total	25	4169	51 (1.2%)	33
Albumin	60	63,754	49 (0.1%)	49
Acetaminophen Drug Level	13	4182	47 (1.1%)	16
Alanine Aminotransferase (ALT)	60	69,855	47 (0.1%)	47
Alkaline Phosphatase	60	55,794	47 (0.1%)	47
Aspartate Aminotransferase (AST)	60	69,333	47 (0.1%)	47
Hepatitis B Surface Antigen	30	8686	45 (0.5%)	37
Troponin T	27	26,554	45 (0.1%)	34
Magnesium	60	112,901	42 (<0.1%)	42
Phosphorus	60	92,759	39 (<0.1%)	39
Hepatitis B Surface Antibody	30	5602	32 (0.6%)	25
Beta-Hydroxybutyrate[a]	10	2626	27 (1.0%)	11
Cortisol, Plasma	25	4522	25 (<0.1%)	15
Gamma Glutamyltranspeptidase (GGT)	50	25,395	21 (0.1%)	21
Folate	29	3981	19 (0.4%)	17
Hepatitis B Core Antibody-IgM	25	889	16 (1.8%)	14
D-Dimer	20	2508	15 (0.6%)	6
NT-ProBNP	25	10,316	15 (0.1%)	13
Salicylate Drug Level	23	3693	12 (0.3%)	12

[a] All assays are from Roche Diagnostics except beta-hydroxybutyrate which is from Stanbio. Data includes assays that had 10 or more occurrences of a patient specimen exceeding the package insert icteric index threshold in the retrospective timeframe (January 1, 2018 to December 31, 2018). An additional 29 assays not shown had 1-9 occurrences exceeding package insert limit, 52 assays had no occurrences, and 4 assays had no icteric index limit stated in the package insert.

discussion in Ref. [5]), we were unable to locate any literature describing comparison of Roche I indices to icteric indices in other chemistry platforms. This contrasts with hemolysis interference, for which data across chemistry platforms has been published [34].

In our study analyzing 114 assays tested on Roche Diagnostics chemistry platforms, the lowest icteric index threshold in the assay package insert for all but one assay (enzymatic ethylene glycol) was 10; approximately two-thirds (77 of 114) of the assays analyzed had a package insert icteric index threshold of 40 or higher. This means that significant icteric interference (if using package insert limits) will be on a relatively small group of patients who have disease conditions leading to very high bilirubin levels. In the 12-month retrospective timeframe, just 57 of 94,081 unique patients accounted for all instances where a specimen had an icteric index of 40 or higher and further comprised 22.6% of all specimens with an icteric index of 10 or higher. Not surprisingly, this limited group accounted for almost 50% of all instances where the icteric index of a specimen exceeded the package insert threshold for a specific assay ordered on that specimen. From chart review, this group had high mortality; 15 of 57 patients expired during hospital admission or shortly afterwards at home or hospice care and a further 15 patients expired within 12 months. In examining the disease mechanisms underlying severe icterus in this cohort of 57 patients, many had end-stage liver and/or biliary tract disease from chronic conditions such as alcohol-related liver disease, Alagille syndrome, primarily biliary sclerosis, or neoplasms (often aggressive and sometimes undifferentiated cancers with unknown primary) that were either untreatable or require liver transplantation. There was only a handful of patients who had either time-limited disease (hemolytic disease of the newborn, acute liver injury that resolved) or a disease amenable to specific therapies that could alter disease course (pancreatic cyst causing biliary obstruction, tyrosinemia type 1).

In terms of how to report results from specimens whose icteric index exceeds the package insert or separately validated icteric index threshold by the laboratory, there are four broad approaches [3,9,21]. The first approach is simply to cancel these results (i.e., not report the assay result), ideally with verbiage that indicates the reason for cancellation. The general challenge with canceling results is that the patient disease condition may not resolve in the near- or even long-term, leading to more cancellations when the same test is ordered in the future. This certainly applies to the limited group of patients in our cohort who had multiple specimens with high icteric

index. The second approach is to report the results from the assay but have a footnote/comment or other result indication that the specimen was icteric to a degree that could impact the assay results. The third and more complicated approach would be to define ranges where the assay result could be reported but cancel results if the icteric index is beyond a certain threshold where the interference is likely to be extreme. This would typically require additional studies by the laboratory to better define impact of icterus on specific assays. A third approach is to use specimen dilution to limit icteric interference, as has been described for Roche Diagnostics creatinine [35] and ammonia assays [36]. And finally, laboratories can perform the effort to validate their own thresholds for icterus. If assay values are reported despite potential icteric interference, documentation would ideally indicate the effect of icterus interference for that assay (e.g., decrease or increase) and an estimated degree of interference. The challenge here is that interference may be influenced by a range of factors including ratio of conjugated to unconjugated bilirubin, interference by bilirubin breakdown products, the quantitative result of the assay being tested, and presence of other interferents [19,20,25].

We observed that specimens with an icteric index between 5 and 29 had a higher proportion of samples with hemolysis compared to specimens with an icteric index less than 5 or those with an index of 30 or higher. This may relate in part to common disease mechanisms that can produce both hemolysis and unconjugated hyperbilirubinemia [10,11]. In the specimens with icteric indices of 30 or higher, conjugated hyperbilirubinemia predominated. Conjugated hyperbilirubinemia would not be clearly associated with a common disease mechanism producing hemolysis [10,11]. In addition, lipemia was more common in specimens with icteric indices of 20 or higher. This may relate to a variety of factors including diet, inpatient status, medications, and underlying disease [3,30]. The presence of more than one endogenous interference can lead to complex effects on assays [2,3].

At our institution, after extensive discussion with the clinical service leadership (especially those with experience in hepatology and critical care), we have elected to 'dual result' the assay result (if obtainable) along with a result of 'Icteric', combined with education on the effects of endogenous interferences on laboratory testing. 'Icteric' gets resulted for a laboratory test when the specimen I index (determined as part of the routine HIL indices assessed for plasma or serum samples) exceeds the I index threshold for that particular test. This takes advantage of functionality in the laboratory information system and EHR that allows for 2 different components per test name. If the I index for the specimen does not exceed the threshold for a test, then a second component is not displayed in the chart. We chose this approach since 'Icteric' will then be easily visible in multiple result viewing formats in the EHR and not hidden as a comment. A previous practice at our institution had been to cancel all results exceeding the icteric index threshold but to allow clinicians to request by phone call that the cancellation be overridden for certain results. However, this was a significant customer service dissatisfier and led to complaints that this practice was burdensome and frustrating, especially given that this primarily involved a relatively small group of patients with severe disease who often required intensive treatments and frequent laboratory monitoring.

Educational efforts related to HIL interferences have included lectures in the medical student curriculum and in hospital-wide housestaff orientation that include information on specimen interferences. The institutional online clinical laboratory handbook specifies the I index threshold for the chemistry tests reported in the current study and what approximate bilirubin concentrations these apply to. The clinical laboratory call center will generally refer questions on specimen interferences to the clinical pathology resident on-call or to clinical chemistry faculty. There is ongoing work at our medical center in education and in optimizing EHR alerts on specimen interferences.

5. Conclusions

In conclusion, icteric interference exceeding package insert icteric index thresholds occurred in only 0.14% of clinical chemistry assays performed at a busy academic medical center. A limited group of mostly adult inpatients with severe liver and/or biliary tract disease accounted for almost 50% of all instances where icteric index exceeded the threshold for a specific assay. At the highest icteric index values, the most common underlying causes were end-stage liver and/or biliary tract disease (especially alcohol-related liver disease) resulting in a predominantly conjugated hyperbilirubinemia. Targeted education and collaboration with clinical services can help devise effective reporting strategies for specimens with high levels of icterus. The data presented also indicate that development of more icteric-resistant assays for creatinine, ammonia, and total protein by *in vitro* diagnostics manufacturers would be valuable.

CRediT author statement

Sandhya Mainali: Formal analysis, Writing – Review & Editing. **Anna Merrill:** Formal analysis, Conceptualization, Writing – Original Draft, Writing – Review & Editing, Methodology. **Matthew D. Krasowski:** Formal analysis, Conceptualization, Writing – Original Draft, Writing – Review & Editing, Methodology, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plabm.2021.e00259.

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