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Short Communication

Clinical decision limits for interpretation of direct bilirubin — A CALIPER study of healthy multiethnic children and case report reviews

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ABSTRACT

Objective: Measurement of total and direct bilirubin is routinely performed for the differential diagnosis of hyperbilirubinemias. The diagnostic efficiency of a test is dependent on the chosen clinical decision limit. This study is designed to address the clinical decision limits for direct bilirubin.

Design and methods: Routine laboratory method was used to measure total and direct bilirubin in children up to the age of 18 years. Case study data and serum from a group of healthy children were analyzed and statistical exercise was performed to establish decision limits.

Results: The reference interval for total bilirubin was 1–12 μmol/L and for direct bilirubin 1–9 μmol/L with the median direct bilirubin of 3 μmol/L. In 17% of children with non-pathological jaundice, median total bilirubin was 173 μmol/L, median direct bilirubin was 8 μmol/L and median direct bilirubin percent was 49%. From birth direct bilirubin percentage decreased until total bilirubin was 41 μmol/L, then it remained at ≤10%. Albumin increased with age, and was on average 2.4 g/L higher when measured using bromocresol-green compared with bromocresol-purple. An increased amount of direct bilirubin was observed when albumin (detected using the bromocresol-purple method) was >35 g/L.

Conclusions: Direct bilirubin concentration of ≥10 μmol/L should be used to consider the presence of conjugated hyperbilirubinemia provided that total bilirubin is also above the reference interval. A high direct bilirubin percentage is unlikely to offer any clinical value when total bilirubin is not increased. It is, however, a useful diagnostic tool when there is a persistence of hyperbilirubinemia or when total bilirubin increases during times of stress with direct bilirubin >10%.

Introduction

A high level of measured serum total bilirubin (TBIL) establishes the presence of hyperbilirubinemia, and may help differentiate intrahepatic biliary obstruction from extrahepatic obstruction and in assessment and the prognosis of end stage liver disease. A disordered bilirubin metabolism can also result from increased production, impaired conjugation, reduced transport, and impaired excretion. Measurement of direct bilirubin (DBIL) is therefore a necessary and a useful diagnostic tool to elucidate the metabolic defect. The reference interval for TBIL is well-established [1–3] and is comprehensible to clinical professionals. Comparatively, the knowledge and interpretation of DBIL results by care-providers are not so inclusive [4,5]. The paucity of knowledge or the lack of appreciation of the clinical significance of DBIL measurement may ultimately be governed by how the result was conveyed to the care provider; that is, whether the DBIL result and its reference interval were expressed as a concentration and/or as a percentage of TBIL concentration. The CALIPER (Canadian Laboratory Initiative in Pediatric Reference Intervals) Project, a collaborative study among pediatric centers across Canada, has already addressed some critical gaps in pediatric reference intervals based on factors such as age, sex, and ethnicity [3]. In this study, we report on the nature of DBIL decision limits, and, how this should be better communicated for its effective clinical utility.

Methods

Blood samples were collected from 946 healthy children from birth to 18 years of age, centrifuged, separated, and serum aliquots kept

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frozen at −80 °C. A complete set of test results were obtained for 795 children (median age 9.8 years for 410 females, and 10 years for 385 males). Serum total bilirubin (TBIL), direct bilirubin (DBIL) and albumin were tested (Architect c8000, Abbott). Measurement of albumin employed the use of bromocresol-green (ALB-G, assay imprecision coefficient of variation <3%) and bromocresol-purple (ALB-P, assay imprecision coefficient of variation ≤4%) methodology. Processing of the data was in accordance with CLSI C28-A3 guidelines on defining, establishing, and verifying reference intervals in the clinical laboratory. Visual inspection and the Dixon test were used to remove outliers. In addition, age/sex partitions were determined by visual inspection of scatter plots, distribution plots, and examination of the overall trend. The partitions were assessed for normality of distribution using the skewness statistic, and the non-Gaussian distributed partitions were log-transformed. The Harris and Boyd method was used for evaluation of partitions; for partitions with a sample size greater than 120, the non-parametric rank method was used to calculate the reference interval. Non-parametric 95% reference interval (RI) and regression analysis of the paired data sets were performed using the Analyse-it software program for Microsoft Excel spreadsheet [6].

In addition, and because of the uncertainty of clinical diagnosis, blood specimens from three patients (Table 1) were tested to evaluate and illustrate the potential benefit of DBIL measurement. Two cases are included as uncertainty of their clinical diagnoses extended over a very prolonged period that included extensive but unnecessary clinical examinations. Bilirubin analysis was on the Beckman–Coulter UniCel DxC-800 Synchron clinical system. Urinary coproporphyrin-1 isomer (UCP1) measurement was performed by a national reference laboratory (Cardiff Porphyria Service, Cardiff, UK) using a high performance liquid chromatography system.

Results

The median TBIL (464 females, 426 males) was 5 (RI 1–12) and DBIL was 3 μmol/L (RI 1–9), respectively. The median DBIL% for all subjects was 49% (RI 4–94) (Fig. 1). When TBIL was greater than 12 μmol/L, the median DBIL% was 3 (RI 1–6) and only 2 subjects had DBIL% >10%. In 17% of all subjects with non-pathological jaundice, the median TBIL was 173 μmol/L (RI 35–296) and median DBIL was 8 μmol/L (RI 5–10). From birth, DBIL percentage decreased until TBIL was 41 μmol/L, then it remained at ≤10%.

From the overall group, albumin results were available for 538 children. The relationship of albumin measured with bromocresol-green compared with bromocresol-purple was ALB-G = 17.82 + 0.63 ALB-P (r^2 = 0.61, t = 28.7, p < 0.001). Albumin concentration increased gradually with age but had a low degree of correlation, that is, ALB-G = 42.28 + 0.19 year (R^2 = 0.45, t = 11.56, p < 0.001)); in children over 1-year of age, the lowest observed ALB-G was 40 g/L. With the bromocresol-purple method, the albumin correlation was ALB-p = 40.43 + 0.15 year (r^2 = 0.29); the lowest observed albumin concentration in children over one year of age was 35 g/L. Interestingly, DBIL in the ALB-G method was never >2 μmol/L when albumin was

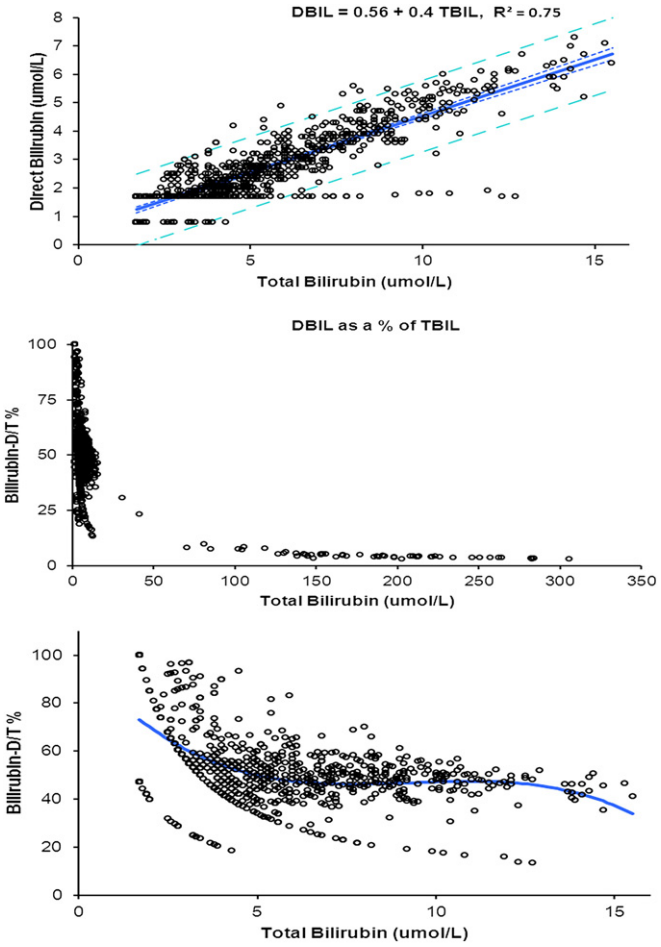

Fig. 1. Pediatric bilirubin: (top) within reference interval total bilirubin against direct bilirubin; (middle) direct bilirubin (Bilirubin-D/T%) as a percentage at all concentrations of total bilirubin and; (bottom) direct bilirubin as a percentage when total bilirubin is within the reference interval. (Bilirubin unit conversion equation: μmol/L × 0.0585 = mg/dL).

Table 1
Serum total bilirubin, direct bilirubin and urinary excretion of coproporphyrin isomer 1 in health and acute infection.

Case	Patient	Day post illness	Total bilirubin, μmol/L	Direct bilirubin, μmol/L	Direct/total bilirubin (%)	Urinary coproporphyrin isomer 1 (%)
1	Female, 90 year	In health	13	0	<10	43
		1	33	12	36	–
		6	22	11	50	–
		7	17	5	29	56
MRP2/ABCC2 mutation carrier: c.[821_822del] ie. p.Gln379Lys						
2	Male, 54 year	In health	54	22	41	–
		115	59	40	68	–
		498	96	67	70	80
		672	69	29	42	85
Compound heterozygote with MRP2/ABCC2 mutation: c.[821_822del]; [1135C > A] ie. p.Gln379Lys & p.Pro274ArgfsX19.						
3	Male, newborn	0	241	139	58	–
		4	203	114	56	–
		7	81	44	54	–
		15	30	16	53	–
		29	13	8	62	72
Current reference interval			<22	<8	<15	<33