Automated Total and Neonatal Bilirubin Values in Newborns: Is a Distinction Clinically Relevant?

Michael E. Langbaum,¹,² Sergio J. Farber,⁴ and Philip Rosenthal¹,³,⁵,⁶

Guidelines for managing hyperbilirubinemia in newborns were developed by using diazo methods that measure total and direct-reacting bilirubins and calculate an indirect fraction. The automated Kodak Ektachem system allows for measuring serum bilirubin by either of two dry-slide methods: TBIL, involving a modified diazo method, and NBIL, involving a dual-wavelength colorimetric method that fractionates and directly measures the unconjugated (Bu) and conjugated (Bc) bilirubins (Bu + Bc = neonatal bilirubin). The manufacturer recommends that NBIL be used in newborns <15 days old, which is impractical in a large, busy hospital laboratory. We compared NBIL and TBIL in 500 paired serum samples from infants <15 days old. We noted a statistically significant difference between TBIL and NBIL values (162.9, SD 70.4, vs 164.6, SD 69.2, μmol/L; P <0.0001), which was small and of no clinical significance. We conclude that TBIL values may be used with caution for newborn bilirubin screening. Furthermore, NBIL measurements are an acceptable alternative to diazo measurements for neonatal care, allowing the use of previously developed guidelines with NBIL values.

Additional Keyphrases: multilayer analysis · pediatric chemistry

Serum bilirubin determinations are often required in the routine management of newborns. Elevated serum unconjugated bilirubin concentrations are associated with the development of yellow staining of the skin, kernicterus, and brain injury resulting in spasticity, hearing loss, and mental retardation. Phototherapy and exchange transfusions are used to keep serum bilirubin values from exceeding 342 μmol/L. Recently, metalloporphyrin administration has shown promise in preventing exaggerated unconjugated hyperbilirubinemia by inhibiting heme oxygenase and bilirubin production.

Thus, accurate determination of serum bilirubin concentrations is important for the care of human newborns. High-performance liquid chromatography (HPLC) provides accurate quantitation and fractionation of serum bilirubin; however, it is expensive and the time required for sample preparation and analysis precludes its routine use (5, 6). Bilirubin determinations performed by the Ektachem 700 system (Eastman Kodak Co., Rochester, NY), an automated clinical-chemistry analyzer, correlate well with both HPLC and Jen-drassik-Grof diazo methods (7–9). The Ektachem system is rapid and uses a small sample size (10 μL).

The Ektachem system determines serum bilirubin concentrations by one of two dry-slide methods: a method reporting results as either total bilirubin (TBIL) or a method reporting results as neonatal bilirubin (NBIL). The TBIL slide measures total bilirubin by a modified diazo method (10). NBIL determinations involve the BuBc slide, which fractionates and directly measures the unconjugated (Bu) and conjugated (Bc) bilirubin fractions by a dual-wavelength colorimetric method. The sum of Bu and Bc is known as the neonatal bilirubin.

Another bilirubin fraction, delta (bilirubin – protein conjugates), is not measured directly by the Ektachem system but can be estimated: TBIL – (Bu + Bc) = delta bilirubin. Delta bilirubin concentrations are low in neonates (12).

Previous studies by the manufacturer showed that an intermediate layer in the NBIL slide minimizes the spectral interference from hemoglobin and prevents the detection of delta bilirubin (7). The manufacturer recommends that BuBc slides be used for serum bilirubin determinations in newborns <15 days old, whereas TBIL slides be used for ages >15 days. In a large and busy hospital laboratory, it is impractical, difficult, and time consuming to separate specimens by patient age.

Therefore, we compared NBIL and TBIL for human newborns <15 days old and determined if clinically significant differences exist between these two methods.

Materials and Methods

We analyzed 500 paired serum specimens from full-term and preterm neonates <15 days of age by both NBIL and TBIL with the Ektachem 700 system. The specimens had been submitted to the clinical laboratory at Cedars-Sinai Medical Center for routine serum bilirubin determination. We obtained 250 paired specimens.

¹ Ahmanson Pediatric Center, Divisions of ² Neonatology and ³ Pediatric Gastroenterology and Nutrition, and ⁴ Department of Pathology and Laboratory Medicine, Cedars-Sinai Medical Center, and the ⁵ Department of Pediatrics, University of California at Los Angeles School of Medicine, Los Angeles, CA. ⁶ Address correspondence to this author at Cedars-Sinai Medical Center, 8700 Beverly Blvd., Los Angeles, CA 90048-1869.

Received August 20, 1991; accepted February 27, 1992.
between October 1 and November 2, 1990 (group A) and another 250 specimens between January 20 and February 25, 1991 (group B). We used slides with different lot numbers for the two groups.

All phototherapy lamps, when in use for ongoing care of the infant, were turned off before specimen collection. In addition, samples were not exposed to any bright light after collection and were analyzed within 1 h after the blood was drawn. Grossly hemolyzed specimens, as detected by routine visual analysis, were excluded from the study because the TBIL slide is known to be adversely affected by hemolyzed specimens (10). Instrument calibration and operation were performed to manufacturer's specifications. Controls were run three times daily.

Data were analyzed by the Student's paired t-test, and correlation coefficients were analyzed for each data group. Data are presented as mean ± SD.

Results

Group A. Mean TBIL (164.6, SD 77.7, μmol/L) and NBIL (164.4, SD 75.0, μmol/L) values were virtually identical. TBIL values ranged from 25.7 to 408.7 μmol/L. NBIL values ranged from 17.1 to 393.3 μmol/L. Median patient age was 2 days (range 1–11 days). For TBIL (y) plotted vs NBIL (x) values, y = 1.089 x - 4.833 μmol/L, n = 250, Sxy = 1.121. Figure 1 depicts frequency histograms of the NBIL–TBIL differences (DIFBIL) at various intervals.

Group B. The mean TBIL value was 161.3, SD 62.3, μmol/L (range 46.2–442.9 μmol/L) and the mean NBIL was 164.8, SD 62.9, μmol/L (range 42.8–444.6 μmol/L). Median patient age was 2 days (range 1–12 days). For TBIL (y) plotted vs NBIL (x) values, y = 0.983 x - 0.821 μmol/L, n = 250, Sxy = 1.217. Figure 2 depicts frequency histograms of DIFBIL at various intervals.

Both groups combined. The mean TBIL value for all 500 samples was 162.9, SD 70.4, μmol/L and the mean NBIL value was 164.6, SD 69.2, μmol/L. Median patient age was 2 days. For TBIL (y) plotted vs NBIL (x) values, y = 1.011 x - 3.393, n = 500, Sxy = 1.118. Figure 3 displays a scattergram analysis of DIFBIL vs NBIL for the entire population ($r^2 = 0.008$). Table 1 depicts the mean TBIL–NBIL differences (DIFBIL) across the entire population.

Discussion

In 1952, Haia et al. (13) published their landmark work describing the relationship of hyperbilirubinemia to the development of kernicterus. In that study, bilirubin concentrations were measured in serum from 229 infants with erythroblastosis fetalis by a diazo method.
Table 1. DIFBIL Values (μmol/L) for Groups A and B Combined

<table>
<thead>
<tr>
<th>Bilirubin range, μmol/L</th>
<th>n</th>
<th>mean (SD)</th>
<th>Minimum</th>
<th>Maximum</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-85.5</td>
<td>51</td>
<td>1.1 (4.4)</td>
<td>-8.6</td>
<td>13.7</td>
<td>-0.1 to 2.3</td>
</tr>
<tr>
<td>85.5-171</td>
<td>267</td>
<td>3.0 (5.9)</td>
<td>-13.7</td>
<td>25.7</td>
<td>2.3 to 3.7</td>
</tr>
<tr>
<td>171-256.5</td>
<td>132</td>
<td>1.3 (9.1)</td>
<td>-32.5</td>
<td>22.2</td>
<td>-0.3 to 2.9</td>
</tr>
<tr>
<td>256.5-342</td>
<td>38</td>
<td>-3.1 (12.8)</td>
<td>-30.8</td>
<td>17.1</td>
<td>-7.4 to 1.2</td>
</tr>
<tr>
<td>&gt;342</td>
<td>14</td>
<td>-6.8 (16.5)</td>
<td>-32.5</td>
<td>25.7</td>
<td>-16.1 to 2.9</td>
</tr>
<tr>
<td>Total</td>
<td>500</td>
<td>1.7 (8.1)</td>
<td>-32.5</td>
<td>25.7</td>
<td>0.9 to 2.4</td>
</tr>
</tbody>
</table>

Subsequently, automated methods such as the Ektachem system were developed for rapid bilirubin determination. Recent studies have substantiated the accuracy of measurements involving this system (7, 8).

Kodak has recommended that different methods be used for bilirubin determinations, depending on the patient's age at the time of sampling. Newborns do not produce significant amounts of delta bilirubin and, therefore, serum bilirubin measurements at this age can be accomplished by using a slide that does not include the delta fraction (9). The division of specimens on the same automated system based on patient age causes problems for busy hospital laboratories. Personnel must determine patient age before selecting the appropriate slide and analyzing, calculating, and reporting the bilirubin results. If the proper information is not readily available or if the samples must be segregated for separate sample runs, this becomes inefficient and time consuming. Furthermore, confusion over interpreting different methods could occur if bilirubin concentrations were measured before and after age 15 days, e.g., if NBIL was used for age 15 days and TBIL for age 16 days.

In a recent abstract (14), Kodak researchers reported analysis of 83 paired samples of serum bilirubin by NBIL and TBIL. Kodak stated that "customers may elect to consistently use TBIL for total bilirubin and BuC for direct bilirubin to eliminate the need to monitor patient age when performing neonatal/pediatric bilirubin determinations." We sought to determine whether any clinically significant differences exist between the two methods for a larger sample size. We also sought to determine whether these results would be valid for two different groups of newborns, each analyzed by using slides from different lots. Our study demonstrates that although the NBIL and TBIL difference may be statistically significant at certain bilirubin concentrations, the difference is usually small and clinically insignificant.

Discussions with the manufacturer have indicated the potential for errors approaching 10% when using TBIL slides for neonatal specimens. The cause of these errors is unknown and, according to a Kodak representative, differs between TBIL slide lots. We did not encounter such differences in our study with two different TBIL lots. However, because of this potential for error, users may wish to establish a program for revalidating TBIL slides vs BuBc slides because of the manufacturer's concern for their inability to control between-lot differences for TBIL measurement of neonatal specimens.

Only seven of the data points are >25.65 μmol/L and none is >32.49 μmol/L (Figure 3). There is also no correlation between NBIL and DIFBIL values. At concentrations approaching 342 μmol/L, these NBIL–TBIL differences could lead to different therapeutic options; however, at such concentrations bilirubin fractionation must be carried out and Bu must be delineated, because the unconjugated (indirect) fraction of bilirubin is thought to be responsible for the devastating neurologic sequelae of hyperbilirubinemia. In addition, these extreme concentrations of hyperbilirubinemia are considered nonphysiologic and warrant further investigation, including measuring the direct-reacting fraction. For this reason NBIL must be used when bilirubin increase approaches concentrations indicating intervention.

In our own institution, as a result of this study, we routinely screen all bilirubin samples from newborns by using the TBIL slide. However, if there is a clinical concern about conjugated hyperbilirubinemia, or if the bilirubin concentration approaches values requiring intervention, we also use the NBIL slide for bilirubin fractionation.

A recent study found that determining bilirubin directly may not be cost effective for screening neonates because most hyperbilirubinemia in term newborns involves only the unconjugated fraction (15). By not fractionating bilirubin, a considerable cost savings may be realized. Our data indicate that TBIL is an acceptable screening tool for both inpatient and outpatient newborns. However, bilirubin fractionation is always necessary if conjugated hyperbilirubinemia is suspected, if persistent jaundice is noted, or if bilirubin concentration approaches that requiring therapeutic intervention. In such cases, BuBc slides, with serum bilirubin fractionation, would provide important information.

Because therapeutic intervention has been based upon values obtained by the diazo method described by Hsia et al. (13), it is also important to ascertain whether NBIL is comparable with the diazo-obtained TBIL in infants with clinically significant unconjugated hyperbilirubinemia. Ektachem NBIL determines serum bilirubin by an entirely different colorimetric method. Our results suggest that NBIL values are clinically similar to diazo-derived TBIL values.

We did not attempt to validate which of these two methods is superior for measuring serum bilirubin. Previous studies comparing these methods showed that reasonable estimates were made for routine clinical use (5, 7). We designed this study to closely approximate the methods of routine blood drawing and bilirubin analysis as they occur daily in a busy hospital. Samples were collected in reduced ambient lighting to duplicate conditions usually found in outpatient laboratories and hospital nurseries. Hemolysis was determined visually just as it would be done routinely. We did not attempt to measure in vivo photolysis as an effect of lighting, nor
did we attempt to measure hemolysis quantitatively, because these maneuvers would not be practical in a hospital laboratory.

In summary, our results indicate that TBIL is an appropriate screening method for evaluating hyperbilirubinemia in newborns. The potential confusion caused by separating specimens by age is not warranted, based on the similarity of TBIL and NBIL values for paired specimens. Laboratories need not take the time and effort to separate most bilirubin specimens on the basis of patient age. However, because this procedure is not officially endorsed by the manufacturer and because the manufacturer cannot guarantee that differences between lots will not result in errors when TBIL slides are used for neonatal specimens, individual laboratories may desire to have an ongoing validation of TBIL values against BuBc slides. As new lots are used, laboratory personnel may wish to compare TBIL and NBIL results for paired specimens until they feel comfortable that the differences are not clinically significant. If fractionation of bilirubin becomes necessary, NBIL may be used because the values obtained are clinically comparable with the previously referenced diazo-obtained values.

References