Hour-Specific Bilirubin Nomogram in Infants With ABO Incompatibility and Direct Coombs-Positive Results

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Objective: To determine the usefulness of the hour-specific Bhutani et al bilirubin nomogram when applied to infants with Coombs-positive test results.

Design: Retrospective chart review.

Setting: Term nursery and neonatal intensive care unit of a university-affiliated hospital.

Patients: All infants with A+ or B+ blood type born in our center from September 1, 2006, through August 31, 2008, to mothers with O+ blood.

Outcomes: Proportion of infants with Coombs-positive results from the nomogram zones who required phototherapy and comparison of the percentage of infants with Coombs-positive results in each zone with the percentage of those with Coombs-negative results in each zone.

Results: A total of 240 infants with Coombs-positive and 460 with Coombs-negative results having a gestational age of 35 weeks or older were evaluated. Sensitivity and specificity of data for infants with direct Coombs-positive results in zone 4 (high risk; 74.2% and 97.1%) and those for infants in zones 3 (high-intermediate risk) and 4 combined (96.7% and 83.7%) compared favorably with the data from the Bhutani et al cohort, which had direct Coombs-negative results (54.0% and 96.2% for zone 4; 90.3% and 84.7% for zones 3 and 4 combined). The likelihood ratio for infants with direct Coombs-positive results in zone 4, 25.8 (95% confidence interval, 11.4-58.4), was twice that of the Bhutani et al cohort, 14.1 (11.0-18.1). The nomogram performed well in directing the timing of bilirubin level follow-up. All infants in zones 3 and 4 with Coombs-positive results were followed up after hospital discharge. None required an exchange transfusion or developed bilirubin encephalopathy.

Conclusions: The Bhutani et al bilirubin nomogram reliably identified infants at gestational age of older than 35 weeks with direct Coombs-positive results who were at risk for significant hyperbilirubinemia and directed the timing of follow-up for these infants. This finding has direct clinical applicability to the health care professional practicing in the newborn nursery.

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Hyperbilirubinemia is a universal problem in the assessment and management of the infant in the well-infant care nursery. In 1999 Bhutani et al published their seminal article relating the hour-specific bilirubin level of an infant to the risk for needing treatment for hyperbilirubinemia. The Bhutani et al–based nomogram has been widely adopted. Use of the nomogram in the term nursery has allowed easily targeted intervention and follow-up of those infants at risk for developing hyperbilirubinemia. However, the study by Bhutani et al excluded infants who had direct Coombs-positive test results. Therefore, the applicability of the Bhutani et al nomogram is limited to infants with direct Coombs-negative results. The lack of a bilirubin algorithm for infants with direct Coombs-positive results is problematic. Infants who have direct Coombs-positive results are at higher risk for significant jaundice compared with those with direct Coombs-negative results. The most recent clinical practice guideline from the American Academy of Pediatrics (AAP) Subcommittee on Hyperbilirubinemia recommended initiating phototherapy at a lower level in infants with direct Coombs-positive results than in those with direct Coombs-negative results. Having a nomogram to predict the rate of rise of bilirubin level in infants with direct Coombs-positive results would greatly aid in the clinical management of those infants. This is an important area of study with direct clinical applicability for health care professionals.
in the newborn nursery. The objective of our study was to evaluate the usefulness of the Bhutani et al1 bilirubin nomogram in infants with direct Coombs-positive results.

**METHODS**

We conducted a retrospective review of the records of all infants born in our hospital during a 2-year period, from September 1, 2006, through August 31, 2008. The setting was a university-affiliated hospital with approximately 2800 births per year that served a minority population who were predominantly publicly insured. The study population included all infants with a gestational age (GA) of 33 weeks or more whose blood type was A or B and whose mother’s blood type was O+.

For all infants whose mothers had type O or Rh-negative blood, hospital routine included sending cord blood to the laboratory for typing procedures and performing the Coombs test from the delivery room. All infants who were found to have direct Coombs-positive results immediately had complete blood cell count, reticulocyte count, and serum bilirubin assays sent to the laboratory. A serum bilirubin level assay was repeated every 8 hours until the level stabilized or phototherapy was started. Hospital routine for all infants regardless of direct Coombs test results included drawing a serum bilirubin specimen at the time of the newborn metabolic screening, shortly after midnight on the day of hospital discharge. This screening bilirubin level was plotted on the Bhutani et al1 hour-specific nomogram. Infants in the low-risk group (zone 1) were scheduled for routine newborn follow-up. Infants in the low-intermediate–risk zone (zone 2) were scheduled for follow-up assay of their bilirubin level 48 hours after discharge, while infants in the high-intermediate–risk zone (zone 3) were scheduled for a bilirubin level assay 24 hours after discharge. Infants in the high-risk zone (zone 4) had their discharge delayed or phototherapy started (Figure), using the criteria of the AAP Subcommittee on Hyperbilirubinemia clinical practice guideline regarding phototherapy.9

The serum bilirubin assay was performed using a modification of the diazocaffeine/benzoate coupling with blank method7 on a Siemens Dimension RXL Max machine (Siemens AG, Berlin, Germany) in the Albert Einstein Medical Center laboratory. The coefficient of variation for low results was 2.5%; for high results, it was 2.2%. This assay is performed on a daily basis on all shifts. The Coombs direct antiglobulin test was performed using a monospecific antihuman globulin. The reagent was tested daily for reactivity.

Demographic data were collected for all the infants. The mother assigned the infant's ethnicity at the time she filled out birth certificate information. The infants' screening bilirubin level and the corresponding zone on the hour-specific nomogram were recorded. For those infants who had more than 1 bilirubin level recorded, the highest bilirubin level while in the hospital or the highest level before the start of phototherapy was plotted onto the nomogram and the corresponding nomogram zone was recorded. After initial discharge, the need for rehospitalization for jaundice was ascertained via 2 methods. For those infants followed up in our outpatient clinic, their outpatient records were reviewed for evidence of rehospitalization for jaundice. Follow-up telephone calls were attempted to the parents of those infants not followed up in our clinic. A total of 135 of the 240 (56.3%) infants in the study had documentation of the need for rehospitalization ascertained via 1 of these 2 methods.

We applied the various zones of the Bhutani et al nomogram to our cohort of infants with direct Coombs-positive results and compared the sensitivity, specificity, and positive and negative predictive values of the zones with those of the Bhutani et al cohort (which had direct Coombs-negative results). We also compared the likelihood ratios of the nomogram zones when applied to our cohort and that of Bhutani et al. We compared the demographic data of our infants with those from the Bhutani et al cohort, and also compared the percentage of infants in each zone of the Bhutani et al nomogram with that in the each zone for our cohort.

Statistical analyses were performed using the t test for continuous data and the Fisher exact test for categorical data. The Institutional Review Board of the Albert Einstein Medical Center approved this study.
Of the 5719 infants born during the time frame of the study, 240 had direct Coombs-positive results: 134 (55.8%) were shown to be A+ and 106 (44.2%) type B+. 460 infants had direct Coombs-negative results: 267 (58.0%) were type A+ and 193 (42.0%) type B+. Approximately two-thirds (69.6%) of our infants with direct Coombs-positive results were African American compared with approximately two-fifths (41.2%) of the Bhutani et al cohort, which is consistent with a different population base (Table 1). The demographic distribution of our infants with direct Coombs-positive and direct Coombs-negative results was similar to the distribution of our entire birth cohort for the 2 years of the study. The percentage of our infants delivered by cesarean section and the percentage exclusively breastfed differed substantially from those of the Bhutani et al cohort (Table 1).

The percentage of our infants in the low-risk zone (zone 1) of the nomogram, the low-intermediate–risk zone (zone 2), the high-intermediate–risk zone (zone 3), and the high-risk zone (zone 4) are enumerated in Table 2. More than three-fourths (79.3%) of our infants with direct Coombs-positive results in zone 4 met the AAP Subcommittee on Hyperbilirubinemia phototherapy criteria, while one-fifth (20.0%) of our infants with direct Coombs-positive results in zone 3 met phototherapy criteria. A total of 20.0% of our infants with direct Coombs-negative results in zones 3 and 4 combined met phototherapy criteria (Table 3). Phototherapy was initiated with all infants who met phototherapy criteria prior to discharge.

The sensitivity and positive predictive values for zone 4 and for zones 3 and 4 combined for our cohort infants were higher than those of the Bhutani et al cohort, while the specificity and negative predictive values were similar between the 2 groups in all zones (Table 4). The likelihood of an infant with direct Coombs-positive results in zone 4 requiring phototherapy was much greater than for infants in the Bhutani et al zone 4 (Table 5). However, the Bhutani et al cohort infants in zones 2 and 3 were slightly more likely to require phototherapy than our infants with Coombs-positive results.

We achieved 100% follow-up after hospital discharge for the 64 infants with direct Coombs-positive results with values in zones 3 and 4. Of these, 1 infant in zone 4 required rehospitalization for phototherapy after discharge and 1 infant from zone 3 required readmis-
sion for jaundice after discharge. Of the 176 infants with direct Coombs-positive results whose bilirubin level categorized them in zones 1 and 2, a total of 71 (40.3%) were reached after discharge. Among these, only 1 infant from zone 2 required readmission for jaundice after discharge. None of these infants, for whom follow-up was ascertained, required an exchange transfusion, received intravenous immunoglobulin, or developed bilirubin encephalopathy.

**COMMENT**

This study demonstrates that the Bhutani et al\(^1\) nomogram can be applied reliably in infants with ABO incompatibility who have direct Coombs-positive results. The sensitivity of the nomogram was better in infants with direct Coombs-positive results at identifying those at risk for requiring phototherapy than in infants from the Bhutani et al cohort, all of whom had Coombs-negative results. Similarly, the likelihood ratio for requiring phototherapy for infants in zones 3 and 4 of the nomogram was higher in infants with Coombs-positive results than in those from the Bhutani et al cohort. Lastly, infants with Coombs-positive results in zones 1 and 2 were at extremely low risk for meeting phototherapy criteria. Only 1 of the 71 infants in the 2 groups we were able to follow up subsequently required readmission for phototherapy. Therefore, the nomogram can be helpful in directing the timing of follow-up bilirubin level assays after discharge for those infants.

The sensitivity and specificity of the data for infants with direct Coombs-positive results for the various zones of the nomogram was similar to the predictive characteristics of the Bhutani et al\(^1\) cohort, which had direct Coombs-negative results. Our levels of sensitivity, 74.2% for zone 4 and 96.7% for zones 3 and 4, meant that 74.2% of all infants who required phototherapy were categorized in zone 4 and 96.7% were in zones 3 and 4 combined. Because zones 3 and 4 of the nomogram are also much more sensitive for infants with direct Coombs-positive results than for those with direct Coombs-negative results, the likelihood ratio for infants with direct Coombs-positive results in zone 4 (25.84; 95% CI, 11.43-58.41) was also much greater than that of the Bhutani et al\(^1\) cohort infants (14.08; 11.00-18.05). Our likelihood ratio of 25.84 indicated that an infant who had direct Coombs-positive results in zone 4 was almost twice as likely as an infant with direct Coombs-negative results from the same zone, a likelihood ratio of 14.08, to require phototherapy. These results were not surprising because infants with direct Coombs-positive results overall have higher rates of hemolysis and bilirubin production than those with

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**Table 3. Infants Meeting Phototherapy Criteria\(^a\)**

<table>
<thead>
<tr>
<th>Zone</th>
<th>Coombs Positive (n=240)</th>
<th>Met Criteria (n=31)</th>
<th>Coombs Negative (n=460)</th>
<th>Met Criteria (n=8)</th>
<th>Total (N=2840)</th>
<th>Met Criteria (n=117)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>129</td>
<td>0</td>
<td>343</td>
<td>0</td>
<td>1756</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>47</td>
<td>1 (3.2)</td>
<td>77</td>
<td>0</td>
<td>556</td>
<td>9 (7.7)</td>
</tr>
<tr>
<td>3</td>
<td>35</td>
<td>7 (22.6)</td>
<td>31</td>
<td>2 (25.0)</td>
<td>356</td>
<td>43 (36.8)</td>
</tr>
<tr>
<td>4</td>
<td>29</td>
<td>23 (74.2)</td>
<td>9</td>
<td>6 (75.0)</td>
<td>172</td>
<td>65 (55.6)</td>
</tr>
</tbody>
</table>

\(^a\)All infants in the Bhutani et al cohort had direct Coombs-negative results. Percentages may not total 100 because of rounding. See the “Methods” section of the text for zone-designation information.

**Table 4. Predictability of Bilirubin Nomogram Findings in Infants With Direct Coombs-Positive Results Compared With Those From the Bhutani et al Cohort\(^a\)**

<table>
<thead>
<tr>
<th>Factor</th>
<th>Zone 4 Coombs Positive</th>
<th>Zone 4 Bhutani et al(^1)</th>
<th>Zone 3 and 4 Coombs Positive</th>
<th>Zone 3 and 4 Bhutani et al</th>
<th>Zone 2-4 Coombs Positive</th>
<th>Zone 2-4 Bhutani et al</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>74.2</td>
<td>54.0</td>
<td>96.7</td>
<td>90.5</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Specificity</td>
<td>97.1</td>
<td>96.2</td>
<td>83.7</td>
<td>84.7</td>
<td>61.7</td>
<td>64.7</td>
</tr>
<tr>
<td>PPV</td>
<td>79.3</td>
<td>39.5</td>
<td>46.9</td>
<td>21.6</td>
<td>27.9</td>
<td>11.6</td>
</tr>
<tr>
<td>NPV</td>
<td>96.2</td>
<td>97.8</td>
<td>99.4</td>
<td>99.5</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Abbreviations: NPV, negative predictive value; PPV, positive predictive value.

\(^a\)All infants in the Bhutani et al cohort had Coombs-negative results. See the “Methods” section of the text for zone-designation information.

**Table 5. Similarity of Likelihood Ratiosa**

<table>
<thead>
<tr>
<th>Zone</th>
<th>Coombs Positive (95% CI)</th>
<th>Bhutani et al(^1) (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0.15 (0.02-1.02)</td>
<td>0.48 (0.28-0.82)</td>
</tr>
<tr>
<td>3</td>
<td>1.68 (0.81-3.52)</td>
<td>3.20 (2.48-4.11)</td>
</tr>
<tr>
<td>4</td>
<td>25.84 (11.43-58.41)</td>
<td>14.08 (10.99-18.05)</td>
</tr>
</tbody>
</table>

Abbreviation: CI, confidence interval.

\(^a\)All infants in the Bhutani et al cohort had Coombs-negative results. See the “Methods” section of the text for zone-designation information.
direct Coombs-negative results. In fact, there were roughly similar percentages of infants from the 2 groups in zones 2 and 3. The lower percentage of infants with direct Coombs-positive results in zone 1 (8.0% less than the infants in the Bhutani et al cohort, all of whom had direct Coombs-negative results, in that zone) was almost equal to the higher percentage in zone 4 (5.9% greater than the infants from the Bhutani et al cohort in that zone).

The Bhutani et al nomogram is effective in directing the timing of follow-up bilirubin level assays for infants with direct Coombs-positive results after discharge. In part to screen for significant hyperbilirubinemia, the AAP Subcommittee on Hyperbilirubinemia has suggested that a qualified health care provider follow-up all infants within 48 to 72 hours after discharge. However, particularly for an inner-city population without significant ties to an individual primary care professional, there is a risk that these infants may not be followed up in a timely fashion. This has, unfortunately, led to the reoccurrence of kernicterus in a few infants. In accordance with the recommendations of Bhutani et al for 48-hour follow-up testing of bilirubin levels in infants in zone 2 and 24-hour follow-up testing of those in zone 3, infants with direct Coombs-positive results could be followed up as outpatients at the same frequency as those with direct Coombs-negative results without requiring an exchange transfusion or developing bilirubin encephalopathy. Since none of the infants in zone 1 of the Bhutani et al study ever needed treatment, those infants typically were not scheduled for a follow-up visit specifically directed at assessing for jaundice. The usefulness of the nomogram as a tool for outpatient follow-up can be seen by the fact that only 3 infants from this high-risk group with direct Coombs-positive results required rehospitalization for phototherapy. In addition, by using this follow-up algorithm, none of our infants with direct Coombs-positive results required an exchange transfusion or developed evidence of bilirubin encephalopathy.

An interesting finding from our study was that results from the positive direct Coombs test did not appear to add any value to the clinical management of the infants in question. Even in a cohort of infants deemed at high risk for significant jaundice, a single bilirubin level obtained before discharge was sufficient to direct the appropriate and safe clinical management and follow-up of these infants. This finding is similar to that of Madan et al, who found that a routine direct Coombs assay did not add materially to the clinical treatment of their cohort infants. Similarly, it may also be possible to eliminate the routine collection of blood-type information in infants born to mothers with type O blood. Infants with ABO incompatibility who had direct Coombs-negative results were represented in the original Bhutani et al cohort but were not separately evaluated. More of our infants with ABO incompatibility and direct Coombs-negative results were categorized as belonging in zone 1 of the nomogram compared with the Bhutani et al cohort, while fewer were categorized in zones 2 through 4. In addition, a smaller percentage of these infants met AAP Subcommittee on Hyperbilirubinemia phototherapy criteria compared with those of Bhutani et al. This finding supports the usefulness of the nomogram in managing these infants. This finding is similar to that of Serrao and Modanlou, who found that infants with ABO incompatibility who had direct Coombs-negative results were not significantly different from those who had ABO compatibility regarding the risk for hyperbilirubinemia. Elimination of routine blood typing and direct Coombs testing would be expected to provide a significant cost savings.

After the publication of the Bhutani et al hour-specific bilirubin nomogram, other attempts have been made to stratify infants at high risk for needing treatment for hyperbilirubinemia. Sarici et al evaluated 136 term newborns with ABO incompatibility, 6 of whom had direct Coombs-positive results. They determined that, at the postnatal age of 6 hours, a serum bilirubin level greater than 4 mg/dL (to convert to micromoles per liter, multiply by 17.104) was a strong predictor of significant hyperbilirubinemia, ie, that the infant would require phototherapy. They found that a serum bilirubin level greater than 6 mg/dL at the postnatal age of 6 hours was a good predictor of severe hemolytic disease, ie, that the infant would require additional modalities such as intensive phototherapy, intravenous immunoglobulin, or exchange transfusion. Keren et al evaluated 823 term and near-term infants, at least some of whom had direct Coombs-positive results. They found that adding GA to the Bhutani et al nomogram significantly improved the ability of the nomogram to predict jaundice that would require treatment.

Probably the most important parameter to the health care professional managing jaundice in newborns is the positive predictive value (PPV) of a screening test to predict the need for phototherapy. In our cohort of infants with direct Coombs-positive results, placement in zone 4, the high-risk zone, of the Bhutani et al nomogram had a PPV of 79.3%, indicating an extremely high likelihood for needing phototherapy. While the PPV of our infants in zone 3 was only 20.0%, this result still reflected a substantial risk that required close follow-up after discharge. Keren et al found that adding GA, specifically that younger than 38 weeks, to their infants’ risk assessment by nomogram zone improved the PPV substantially. We found no such improvement in our cohort. While the PPV of GA younger than 38 weeks in zone 4 was 100% in our cohort, the PPV of GAs of 38 to 39 weeks and older than 39 weeks in our cohort were each 75.0%. This finding was not significantly different than that of Keren et al (P = .22). Even for our infants in zone 3, for whom the overall PPV was 20.0%, there was no significant difference in PPV when GA was added to the risk factors for each bilirubin zone. In zone 3 the PPV was 50.0% for infants with a GA younger than 38 weeks (P = .19 compared with the entire cohort of zone 3); the PPV was 15.4% for infants with a GA of 38 and 39 weeks (P = .18), and the PPV was 16.7% for infants with a GA older than 39 weeks (P = .19). Gestational age most likely had no significant effect on the ability of zone 4 to predict the need for phototherapy because the PPV was already so high.
The identification and management of newborns at risk for significant hyperbilirubinemia is one of the major tasks of the health care professional in the term nursery. The use of an hour-specific bilirubin nomogram has aided this task considerably by demonstrating that a single serum bilirubin level before discharge can identify infants at high risk for hyperbilirubinemia and direct the timing of their follow-up. We have shown that although it was not specifically developed for infants with Coombs-positive results, the Bhutani et al nomogram can be applied in these infants. This finding has important clinical applicability for health care professionals who care for newborns.

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Author Contributions: Dr Schutzman had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Acquisition of data: Schutzman, Sekhon, and Hundalani. Analysis and interpretation of data: Schutzman, Sekhon, and Hundalani. Critical revision of the manuscript for important intellectual content: Schutzman, Sekhon, and Hundalani. Statistical analysis: Schutzman, Sekhon, and Hundalani.

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CONCLUSIONS

There were several limitations to our study, the first of which is the cohort size. Although 240 is a reasonable number of infants, a larger cohort would enable more confidence in these findings. Second, there were significant differences in demographics, breastfeeding, and cesarean section rates between our infants and those of the Bhutani et al cohort. However, other investigators have demonstrated the usefulness of the nomogram in other populations with direct Coombs-negative results, so the difference in our demographics should not have had a serious effect on our results. Finally, we might have missed some infants who required an exchange transfusion or developed bilirubin encephalopathy after discharge. This is unlikely because we were able to follow up all infants in the high-risk zones. A larger multicenter study equipped to follow up all infants for 1 to 2 weeks after discharge would be helpful in confirming the usefulness of this hour-specific bilirubin nomogram in managing infants with direct Coombs-positive results.

REFERENCES


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Poetry in Pediatrics

Ode to the Navicular

Navicular, navicular,
Why are you so particular?
You do not tolerate much stress at all,
Especially during 21 games of basketball.
Strength and blood supply are what you lack.
But really, couldn’t you cut an athlete some slack?
Your cartilaginous tendencies make you prone
To many injuries, some diagnosed as a swollen bone.
Although a pain, you are needed
To run and jump and play unimpeded.
Navicular, navicular,
This ode is for you,
You have come to be my favorite bone, tried and true.
These were my thoughts when I was diagnosed as
having a swollen navicular bone at the completion
of basketball season during my junior year in high school.

Erin E. Barney