

Yield of Recommended Blood Tests for Neonates Requiring Phototherapy for Hyperbilirubinemia

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ABSTRACT: **Background:** Hyperbilirubinemia of the newborn is common. Rarely is an underlying disease other than physiologic hyperbilirubinemia considered the cause of high bilirubin levels. Some of the laboratory tests recommended by the American Academy of Pediatrics are expensive and do not always lead to diagnosis.

Objective: To evaluate the efficacy of standard laboratory tests performed on newborn infants requiring phototherapy for hyperbilirubinemia.

Methods: We conducted a retrospective chart review that included neonates born during a 6 month period with birth weight > 2500 g treated with phototherapy for hyperbilirubinemia (n=282) according to published guidelines. The main outcome measures were primary and maximal bilirubin values (mg/dl), time to jaundice (in days), the number of bilirubin tests undertaken and whether the patient showed abnormal functioning, and the number of days in follow-up.

Results: Thirty-three neonates (11.7%) were positive in at least one laboratory test (defined as "Abnormal" in our study), 45.5% of whom met the criteria for phototherapy during the first 48 hours of life. Among the newborns who were negative for all laboratory tests (defined as "Normal"), only 6.8% met phototherapy criteria within their first 48 hours of life ($P < 0.001$). In the Normal group there was a consistent decrease in total serum bilirubin values shortly after phototherapy was begun, while the Abnormal group presented an increase in serum bilirubin values during the first 12 hours of phototherapy. None of the infants had conjugated (direct) hyperbilirubinemia during the study period.

Conclusions: Most neonates presenting with a laboratory identifiable etiology for hyperbilirubinemia (i.e., hemolysis) can be distinguished from those who test negative, mainly based on the timing of presentation and response to phototherapy. A more meticulous selection of patients and reduction in the magnitude of routine laboratory testing can safely reduce discomfort to infants with hyperbilirubinemia as well as costs.

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Neonatal hyperbilirubinemia is diagnosed when the total serum bilirubin is higher than accepted with respect to weight and age of the newborn. According to the American Academy of Pediatrics guidelines for the management of hyperbilirubinemia in infants of ≥ 35 weeks gestation, if a newborn requires phototherapy the following laboratory evaluation is recommended to detect the hyperbilirubinemia etiology [1,2]: TSB or transcutaneous bilirubin, neonate's blood type and direct Coombs test (if not already performed on cord blood), complete blood count and smear, direct/conjugated bilirubin level, glucose 6-phosphate dehydrogenase activity or end-tidal CO₂ measurement as an option, and measurement of TSB level every 4–24 hours depending on the neonate's age and TSB level. When TSB level approaches the need for exchange transfusion or when the newborn is unresponsive to phototherapy, the following tests are recommended: reticulocyte count, G6PD activity profile, albumin level, and end-tidal CO₂ if available.

Newman et al. [3] found that most of the laboratory workup for determining the etiology for hyperbilirubinemia is futile. Most laboratory tests have poor sensitivity and specificity, and rarely is an underlying disease other than physiologic hyperbilirubinemia considered the cause of high levels of bilirubin severe enough to meet criteria of phototherapy. According to their study, only blood type (of mother and neonate) and direct Coombs test are obligatory. They concluded that there is no value in measuring direct bilirubin, unless jaundice persists for 2–4 weeks.

Bilirubin toxicity is rare in the absence of hemolysis; therefore, if there is no hemolysis, the risk, cost and discomfort of further evaluation for hyperbilirubinemia may outweigh the benefit. It has been demonstrated that most of the laboratory tests recommended by the AAP are expensive and rarely lead to a diagnosis other than ABO incompatibility or Rh isoimmunization. Moreover, if hemolysis did occur, it may coexist with other etiologies such as *Streptococcus* group B bacteremia [4].

In 1994 Newman and Easterling [4] investigated reticulocyte count and blood smear in jaundiced neonates. They

TSB = total serum bilirubin

G6PD = glucose 6-phosphate dehydrogenase

AAP = American Academy of Pediatrics

found that when those tests are done as part of hemolysis screening in hyperbilirubinemic neonates, the diagnosis of hemolysis is rarely made in conditions other than isoimmunization. That study also demonstrated that if a direct Coombs test is positive there is no need to conduct other laboratory tests, and if the test is negative a full laboratory workup is required only for neonates in whom other reasons for hemolysis are suspected, such as a family history of red blood cell dysfunction [5].

During the period of our research, when a newborn met the criteria for phototherapy, a routine battery of laboratory tests was performed in some cases, including G6PD activity qualitative screen, blood type, direct Coombs test, complete blood count, reticulocyte count, total and direct bilirubin, and liver function tests. The tests were performed to establish the cause of severe hyperbilirubinemia. Our main objective was to assess the efficacy of the screening tests in evaluating significant neonatal hyperbilirubinemia requiring phototherapy in our institute.

PATIENTS AND METHODS

We conducted a retrospective study, using data from the medical charts of neonates who were born and hospitalized in Soroka Medical Center in Beer Sheva, Israel, for 6 months and treated with phototherapy due to jaundice during their first month of life. The overall number of births in our tertiary medical center during the research year reached 13,000, of which 7474 occurred within the study period. A total of 282 neonates required phototherapy, an incidence of 3.8%. Newborn medical charts were selected according to our inclusion criteria (i.e., birth weight \geq 2500 g and phototherapy).

The recorded data included details such as gender and ethnicity, gestational age, birth weight, blood type, age upon discharge, mode of delivery, relevant medical history, bilirubin values (first, maximal, and upon-discharge), number of capillary bilirubin tests, and time under phototherapy. Laboratory evaluation comprised complete blood count, blood cultures and electrolytes and liver functions including total and direct bilirubin, direct Coombs test and qualitative G6PD activity test. Other data included number of readmissions 30 days after discharge and number of follow-up days.

Our study design was approved by the Helsinki Subcommittee for trials in human subjects at the Soroka Medical Center (research # 4078).

STATISTICAL ANALYSIS

Statistical analysis was performed using SPSS (Statistical Package for Social Sciences) for Windows 14.0 (Chicago, IL, USA). Average and standard deviations were used for comparisons of data, and parametric tests (paired *t*-test, independent *t*-test) and non-parametric tests (Mann-Whitney and chi-

square tests) when applicable. *P* values < 0.05 with confidence interval of 95% were considered statistically significant.

RESULTS

Altogether, 282 newborn infants were found suitable for phototherapy during the study period; of these, 181 (64.2%) were male, 186 (66%) were Jewish and 96 (34%) were Muslim Arabs. Regarding mode of delivery, 215 (76.2%) were delivered vaginally, 59 (20.9%) by cesarean section and 8 (2.8%) by vacuum extraction. The average length of pregnancy was 38.25 weeks (\pm 1.74), with an average birth weight of 3179.2 g (\pm 460.3); 51 (18%) were born < 37 weeks.

With regard to blood type, 48.6% of the mothers (137 mothers) were O+, while 30.5% (85) of the newborns were A+ blood type; 156 mothers (55.3%) were either blood type O or Rh-

The average postnatal age of babies beginning phototherapy was 4.7 \pm 2.53 days. Average postnatal age upon discharge was 7 days; mean duration of admission was 6.7 \pm 3.19 days. Mean bilirubin value at the start of phototherapy reached 16.6 mg/dl, and the mean maximal recorded value was 18.4 \pm 2.63 mg/dl; the mean value upon discharge was 12.6 \pm 2.18 mg/dl.

LABORATORY TEST DATA

The mean number of specific bilirubin tests was 9.7 \pm 5.5 per neonate with a maximum of 34 tests; the average time under phototherapy was 38.15 \pm 21.94 hours with a maximum of 132 hours. All other laboratory tests are described in Table 1a.

Neonates positive for at least one of the laboratory tests (direct Coombs test, reticulocyte count, and G6PD deficiency)

Table 1a. Laboratory evaluation performed at start of phototherapy

| Test | No. of tests | Mean result*** | No. of newborns |
|--------------------------------|--------------|-----------------------|--|
| Liver functions tests (mg/dl)* | 271 | | 100% of tests showed direct/ total ratio < 0.2 |
| Total bilirubin | | 16.8 (\pm 3.8) | |
| Direct bilirubin | | 0.68 (\pm 0.2) | |
| Blood type | 240 | | |
| No incompatibility | | | 136 (56.7%) |
| Incompatibility | | | 104 (43.3%) |
| Reticulocyte count | 236 | 4.05% (\pm 2.8) | Reticulocytosis was found in 9 newborns (3.8%)** |
| Direct Coombs test | 282 | | 20 (7.1%) positive |
| G6PD activity | 282 | | 7 (2.5%) showed zero activity |
| Liver function test | 42 | | |
| AST | | 59 U/L (\pm 33.16) | |
| ALT | | 34 U/L (\pm 28.75) | |
| Blood culture | 48 | | 3 (6.4%) were positive |

* Liver functions tests includes direct and total bilirubin

** > 10% reticulocytes defined as reticulocytosis

*** Values in brackets represent standard deviation

AST = aspartate aminotransferase, ALT = alanine aminotransferase

Of all 282 neonates, 33 (11.7%) were found positive for at least one of the tests and were defined as "Abnormal" in our study. The remaining 249 (88.3%) were found negative in all laboratory tests and as such were defined as "Normal." There were no significant statistical differences between the two groups regarding demographics (gender and ethnicity), birth weight, gestational age, and blood type of mother and newborn.

Direct and total bilirubin values were not used as distinguishing factors between the two groups since 100% of liver function tests yielded direct/total bilirubin ratios less than 20%, suggesting there was no evidence for direct hyperbilirubinemia during the study period. Reticulocyte count above 10% was defined as a positive test.

Significant differences ($P < 0.05$) were detected for starting phototherapy: a) hyperbilirubinemia was prevalent earlier in the Abnormal group and the first bilirubin levels were measured earlier in this group, b) the total number of hours under phototherapy was greater in the Abnormal group, and c) hemoglobin level (both initial and second) was lower in the Abnormal group because more neonates in this group developed hemolysis [Table 1b]. We found that 45.5% in the Abnormal group compared to only 6.8% in the Normal group required phototherapy during the first 48 hours of life, and the difference between the two groups was statistically significant using the chi-square test ($P < 0.001$) [Figure 1].

Table 1b. Differences between the two groups during hospital stay

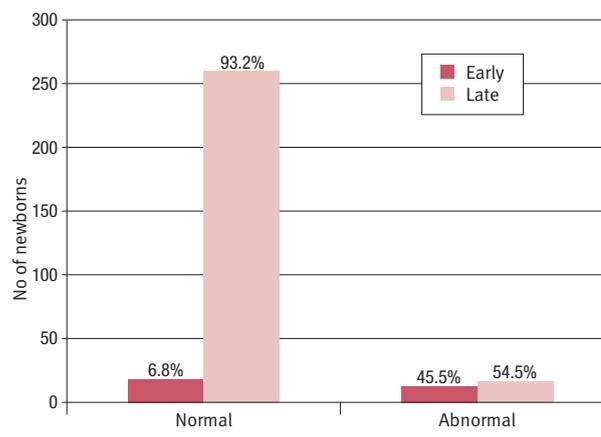
| | Normal | Abnormal | P value |
|-------------------------------|--------------------|--------------------|-------------------|
| Length of stay (days)* | 6.5 (\pm 2.62) | 7.6 (\pm 6.02) | 0.339 |
| Age at discharge (days)* | 6.6 (\pm 2.618) | 7.7 (\pm 5.998) | 0.323 |
| Onset of phototherapy (days)* | 5 (\pm 2.51) | 2.5 (\pm 1.35) | < 0.001 |
| Bilirubin value (mg/dl)* | | | |
| Initial | 17.7 (\pm 2.73) | 14 (\pm 3.8) | < 0.001 |
| Maximal | 18.3 (\pm 2.59) | 17.5 (\pm 2.5) | 0.129 |
| Number of bilirubin tests* | 10 (\pm 5.57) | 12 (\pm 4.77) | 0.063 |
| Hours under phototherapy* | 38.8 (\pm 22.5) | 49.9 (\pm 30.6) | 0.001 |
| Direct/total bilirubin ratio* | < 0.2 in all tests | < 0.2 in all tests | – |
| Hemoglobin level (mg/dl)* | | | |
| Initial | 18.5 (\pm 2.28) | 15.8 (\pm 3.1) | < 0.001 |
| Second | 18.9 (\pm 2.54) | 14.9 (\pm 3.76) | 0.02 |
| Blood culture results** | 8.6% positive | 0% positive | 0.338 |
| Liver function tests (U/L) | | | |
| AST | 60 (\pm 35.5) | 45 (\pm 28.24) | 0.211 |
| ALT | 30 (\pm 28.13) | 37 (\pm 35.3) | 0.653 |
| Discharge with follow-up** | 38% | 69% | 0.001 |

* Statistical analysis using Independent samples *t*-test

** Statistical analysis using chi-square test

AST = aspartate aminotransferase, ALT = alanine aminotransferase

Figure 1. Timing of phototherapy initiation. In the Abnormal group 45.5% of infants met phototherapy criteria during the first 48 hours of life, versus only 6.8% of infants in the Normal group ($P < 0.001$)



DISCUSSION

Hyperbilirubinemia of the newborn is defined as TSB levels \geq the 95th percentile according to the Bhutani nomogram [6]. Although jaundice is common and is usually physiologic in nature, it can represent a pathologic process or a complication. Clinically apparent jaundice can be seen in as many as 60% of all term newborns during the first week of life. However, it was previously observed that most of the laboratory workup conducted while searching for the cause of hyperbilirubinemia does not help establish a diagnosis. The sensitivity and specificity of these tests are poor, and rarely is an underlying disease (other than physiologic hyperbilirubinemia) considered the cause of a bilirubin level high enough to require phototherapy [7-9].

Neonates are discharged from Israeli hospitals 36–48 hours after delivery, limiting our ability to screen for and diagnose hyperbilirubinemia in the newborn. Therefore, the need for phototherapy has become a leading cause for readmission.

It has been established that if a direct Coombs test is positive there is no need for other laboratory tests, and even if the result is negative a full laboratory workup is indicated only for neonates in whom there is a reason to suspect other causes for hemolysis such as anemia, early jaundice, or familial history of red blood cell dysmorphism.

A comparative analysis of three strategies for screening and prevention of kernicterus in the newborn did not demonstrate any benefit of conducting a full laboratory workup to detect and prevent one incident of kernicterus [10-12].

Regarding phototherapy, the AAP guidelines state that if TSB level does not decrease, or continues to rise despite proper phototherapy, there is a reasonable basis to suspect hemolysis. It can be concluded that if bilirubin level decreases

under phototherapy, there is no reason to suspect hemolysis as the cause of the hyperbilirubinemia.

To date, the question of whether to perform a full laboratory evaluation immediately after starting phototherapy or to wait a few hours, observe the pattern of bilirubin levels under phototherapy, and then conduct the recommended laboratory tests if necessary has not been sufficiently studied. One of our main objectives was to consider the necessity of performing all the recommended tests at the beginning of phototherapy, searching for direct hyperbilirubinemia or hemolysis, or wait for a few hours and widen our investigation depending on the response of bilirubin values to phototherapy. The incidence of phototherapy among newborns weighing > 2500 g in our study was 4%; 11.7% of them were found positive for at least one of the tests, defined as Abnormal, while those who were found negative to all tests were referred to as Normal.

All 271 liver function tests showed a direct/total bilirubin ratio of less than 0.2, meaning all newborns who required phototherapy and underwent liver function tests presented with indirect hyperbilirubinemia.

In the Abnormal group 45.5% began phototherapy during the first 48 hours of life compared to only 6.8% in the Normal group ($P < 0.001$). We can therefore conclude that if early jaundice is present (< 48 hours from delivery) and the newborn requires phototherapy, further laboratory workup is recommended since the chance of encountering hemolysis is high. That notion fits the definition of a screening test, known to be more efficient in populations at risk than in the general population. When we compared the pattern of bilirubin values during the first 24 hours under phototherapy, we found that although the Abnormal group started phototherapy with lower bilirubin values (because jaundice is apparent earlier in life among hemolytic newborns), the pattern of bilirubin values also differed from that of Normal neonates. While the Abnormal group presented with an increase in bilirubin values during the first 12 hours of phototherapy, followed by a plateau between 12 and 18 hours and a decrease after 18 hours, the Normal group showed a consistent decrease immediately after starting phototherapy [Figure 2].

Therefore, it can be concluded that if jaundice is not evident early in the neonate's life and the newborn requires phototherapy, establishing the etiology of hyperbilirubinemia during the first few hours of phototherapy can be avoided. If bilirubin levels continue to decline there is no reason to try to establish the cause (since in this case jaundice is probably physiologic in nature). But if values do increase (or stay the same) it is feasible to expand the laboratory evaluation [Figure 3].

Our results also showed that only 65% of all blood type tests were justifiable because only 156 of the mothers had either O blood type or Rh-negative factor. To date, every jaundiced neonate who starts phototherapy is screened for

Figure 2. Total serum bilirubin values during the first 24 hours of phototherapy. Total serum bilirubin level was stable and began to decline 6–12 hours from the start of phototherapy in the Normal group (17.7, 17.6, 17.2, 16.4, and 15.8 mg/dl at 0, 6, 12, 18, and 24 hours, respectively). In the Abnormal group, there was an increment during the first 12 hours of treatment, followed by a plateau and a decrease 18 hours from phototherapy initiation (14, 14.5, 16.2, 16.19, and 15.7 mg/dl at 0, 6, 12, 18, and 24 hours, respectively).

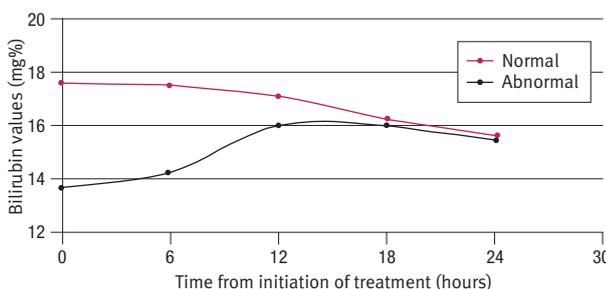
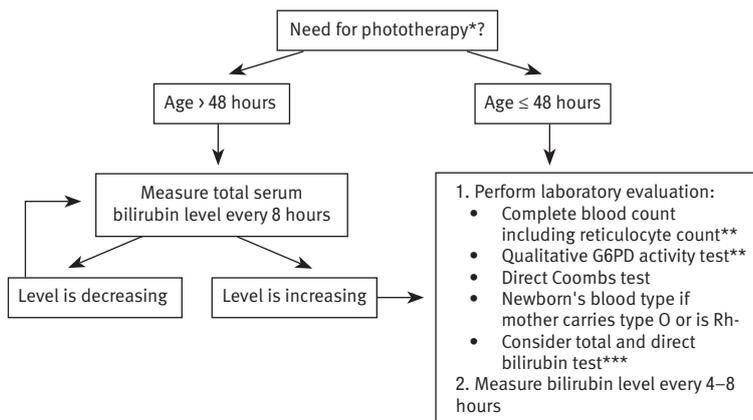


Figure 3. A suggested protocol for the evaluation of neonatal hyperbilirubinemia Table 1a. Laboratory evaluation performed upon starting phototherapy



* According to AAP guidelines.

** Reticulocyte count and qualitative G6PD activity test are performed if the newborn is not responsive to phototherapy or when TSB level approaches the need for exchange transfusion.

*** Study results showed the yield of total and direct bilirubin to be extremely low (if any) for detecting direct hyperbilirubinemia.

blood type, regardless of the mother's blood type. Forty-two liver function tests were performed with no indication according to medical charts; all were found normal.

The study has several limitations. The study period was 7 months, which means that the yearly incidence of hyperbilirubinemic neonates requiring phototherapy could only be extrapolated from our results. Among the 33 newborns who were defined as Abnormal, only 20 capillary bilirubin tests were documented at 24 hours; if more tests had been documented at 24 hours, the curve for the abnormal group in Figure 2 might have shown continuance of a plateau or even an increase in bilirubin levels instead of a decline (as presented).

CONCLUSIONS

According to our findings there is no financial or clinical benefit in conducting a full laboratory evaluation (according to AAP guidelines) to identify possible causes of severe hyperbilirubinemia in neonates found suitable for phototherapy, unless the jaundice was early (< 48 hours from birth). Neonates presenting with non-physiologic jaundice (i.e., hemolysis) based on laboratory results, can be distinguished from those who develop physiologic jaundice, according to the pattern of bilirubin values during the first 18 hours under phototherapy.

If early jaundice is diagnosed (< 48 hours from birth), we recommend a full laboratory evaluation at the start of phototherapy (by indication) because the chance of detecting hemolysis at this age is high. Given that the yield of direct and total bilirubin in detecting direct hyperbilirubinemia is very low, one should reconsider its relevance in the laboratory workup while searching for causes of hyperbilirubinemia in neonates.

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