Effect of Neonatal Phototherapy on Melanocytic Nevus Count in Children

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Background: Melanocytic nevus is the strongest risk factor for the development of cutaneous melanoma. Fair skin and exposure to UV light, especially in childhood, are correlated with the development of childhood nevi.

Objective: To assess the role of blue light neonatal phototherapy used to treat hyperbilirubinemia in nevus acquisition in childhood.

Design: Case-control prospective study.

Setting: University hospital.

Participants: Fifty-eight children were included in this study. Selection criteria included the following: age, 8 to 9 years; and skin type, less than IV by Fitzpatrick classification (ie, brown, always tans, rarely burns). The case group consisted of 18 children exposed to neonatal phototherapy (mostly intensive phototherapy) retrospectively found by review of consecutive neonatal medical records at Saint-Antoine Hospital, Paris, France. The control group was composed of 40 nonexposed children consecutively recruited from a public school in the same geographic area.

Main Outcome Measures: Total body nevus count in children, phenotypic characteristics, solar exposure, and demographic data were assessed by the same dermatologist.

Results: A comparison of both groups showed that the number of nevi larger than 2 mm was significantly higher in the exposed group. The mean (SD) nevus count was 3.5 (3.05; median, 3.0) per child in the exposed group, compared with 1.45 (1.99; median, 1.0) per child in the nonexposed group ($P_{\text{mean}}=.02$ and $P_{\text{median}}=.01$). Multivariate analysis confirmed these results, with a statistically significant correlation with nevus count, especially with nevi 2 to 5 mm in greatest diameter. The association between neonatal phototherapy and nevus count was not significant for nevi smaller than 2 mm or larger than 5 mm. Solar exposure, especially during vacations, was strongly associated with total nevus count and all nevus sizes (2-5 mm, <2 mm, and >5 mm). At univariate analysis, hair color was significantly associated with nevus size smaller than 2 mm ($P_{\text{mean}}=.03$).

Conclusions: Intensive neonatal phototherapy is a strong risk factor for nevus development in childhood. While childhood development of nevi is correlated with fair skin and solar light exposure, and having many nevi is a recognized risk factor in persons with melanoma, we must be careful not to equate childhood nevi development in response to neonatal phototherapy with an individual's risk of developing melanoma. The treatment of hyperbilirubinemia remains neonatal phototherapy. Exposed children should undergo dermatologic preventive measures and surveillance for the development of melanoma.

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Visible light phototherapy has been used since 1970 to treat neonatal hyperbilirubinemia (jaundice) that develops in a small percentage of healthy neonates (43%-60%) and a larger percentage of premature infants (80%).

Visible light phototherapy has a peak wavelength in the blue range of 450 nm. Blue light converts bilirubin in the skin of neonates with jaundice into metastable geometric isomers that are transported in blood and excreted in bile. Intensive phototherapy permitting high irradiance (3-4 mW/cm²) is required and creates high unconjugated bilirubin levels. Many studies about the safety of this therapy have focused on noncutaneous adverse effects. Numerous in vitro studies have shown that irradiation induces oxidative stress, with the formation of reactive oxygen species, leading to DNA damage and promoting tumorigenesis. To our knowledge, no study has evaluated the effects of blue light irradiation on melanocytes in neonates. It is not known whether neonatal phototherapy (in particular, intensive phototherapy) causes an increase in melanocytic nevus count in childhood.

We studied this association in a case-control study by comparing the nevus count in 2 groups of 8- to 9-year-old French children, one group composed of children who received intensive phototherapy at birth and the other comprising children who did not receive this treatment. The nevus count in both groups was compared after adjustment for classic clinical risk factors for nevus (skin types I [very white or freckled, always burns] and II [white, always burns, never tans], Fitzpatrick classification; blue eyes; fair hair and skin; lentigines; and UV light exposure).

**METHODS**

**PATIENTS**

Fifty-eight children born in 1994 and 1995 (8-9 years old at the time of the study) participated in this study. Written consent was obtained from the parents to enroll and to examine the children. Group 1 was composed of 18 children who received blue light phototherapy because of neonatal jaundice during the first few days of life. Interviews and physical examinations occurred at Necker Hospital, Paris, France, during July 1, 2003, through September 26, 2003. Group 2 (control group) was matched for age with the treatment group and included 40 children who had not been exposed to neonatal phototherapy. These children were recruited from a public school in Malakoff, France, during January 5, 2004, through March 31, 2004. Administrative authorization (school principal and physician) was obtained to conduct the study and to examine the children. Interviews and physical examinations occurred at the school. Parents were interviewed before the clinical examination and confirmed that the children had not received neonatal phototherapy. Neither group included children with skin types IV (brown, always tans, rarely burns) through VI (black, never burns) (Fitzpatrick classification).

**DATA COLLECTION**

All children and their parents underwent a standardized interview. The same dermatologist (E.M.) performed a complete clinical examination of every child. Data were entered on a preprinted examination sheet. Information obtained included the following: skin color, recorded as dark, medium, or light; eye color, classified as dark (brown) or light (blue, green, or gray); and hair color, classified into 5 categories including red, blond, light brown, dark brown, and black. Skin type was assessed according to the classification of Fitzpatrick as follows: very white or freckled, always burns, very white or freckled, sometimes burns, white to olive, white or freckled, always tans, brown, never tans, brown, always tans, rarely burns, brown, brown, always burns, rarely burns, brown, never tans, brown, always tans, never tans, and brown, always burns, never tans (Fitzpatrick classification).

![Table 1. Clinical and Epidemiologic Characteristics of the Total Population*](image)
ways burns, never tans (type I); white, usually burns, then tans (type II); white to olive, sometimes burns, always tans (type III); and brown, always tans, rarely burns (type IV).

Nevi were classified as follows: a melanocytic nevus was defined as a brown to black macule or papule; freckles and café au lait macules were excluded. Nevii were counted, measured, and classified into 3 size categories (<2 mm, or lentigo simplex; 2-5 mm; and >5 mm) to enable comparisons with previous studies.

The purpose of the questionnaire was to determine the history of severe sunburn, defined as erythema for longer than 48 hours or blistering (scored yes or no). Exposure to UV rays during vacations was defined as intermittent sun exposure, and beachside or sunny locations (scored as strong exposure). Sunscreen use was recorded (scored as never, sometimes, or always).

### STATISTICAL ANALYSIS

The following descriptive statistics were used to characterize the data. χ² analysis, or the Fisher exact test, when necessary, was used to compare pigmented traits and UV exposure between children exposed or not exposed to phototherapy. The statistical significance of the association between the prevalence of nevi and the risk factors (skin, hair, and eye color, and skin types I and II) was assessed using the Pearson correlation coefficient and the Wilcoxon, and Kruskal-Wallis tests. Furthermore, the total and size-dependent nevus count was studied and compared across the 2 groups. First, univariate analysis was used to compare nevus mean (t test) and median (Wilcoxon test) values between the groups. Second, multivariate analysis using a variance model was performed to consider potential confounders among the clinical risk factors, such as age, skin type, eye color, hair color, and UV exposure. All reported statistical significance levels were set at P<.05. Third, the relationship between irradiation dosage at birth and nevus count was evaluated (Pearson correlation coefficient).

### RESULTS

#### COMPOSITION OF THE SAMPLE

The analysis comprised 58 subjects: 18 children treated with phototherapy in the first days of life because of jaundice and 40 children who had not received phototherapy (control group). Both groups had similar demographic backgrounds (ie, age and ethnicity). Clinical characteristics and UV exposure history in all children are given in Table 1. In these respects, both groups were comparable (P<=.05, χ² test).

### NEVUS COUNT

Every child had at least 1 melanocytic nevus. The mean (SD) pigmented lesion count was 28.5 (15.33) per child, and the median count was 25.50 per child (interquartile range, 22 per child). When lentigo simplex was excluded from the analysis, only 37 (63%) of the children had nevi and the mean (SD) nevus count decreased to 2.09 (2.53) per child, with a median count of 1 per child (interquartile range, 3 per child).

### LINK BETWEEN PHOTOTHERAPY AND NEVUS COUNT

At univariate analysis, comparison of the 2 groups showed that the number of nevi that were 2 mm or larger was significantly higher in the exposed group (Table 2). For these nevi, the mean (SD) count was 3.5 (3.05) per child and the median was 3 per child (interquartile range, 5 per child), compared with 1.45 (±1.99) per child and 1 per child (interquartile range, 2), respectively, in the non-exposed group (Pmean=.02, and Pmedian=.01) (Table 2). When the analysis was limited to nevus size 2 to 5 mm, the difference was more significant (Pmean=.008, and Pmedian=.006). Conversely, the association between neonatal intensive phototherapy and nevus was not significant for the total nevus count for nevi smaller than 2 mm or larger than 5 mm. Clinical and epidemiologic data were used to further investigate the association between phototherapy and nevus count. Multivariate analysis using variance analysis models (Table 3) confirmed the results of the univariate analysis. After stratification for the classic clinical risk factors for the frequency of nevi (age, skin types I and II, medium-colored or light skin, fair hair, and light eye color), the link between phototherapy and nevi 2 to 5 mm and 2 mm or larger remained significant (respectively, P=.001 and P=.003).

### Table 2. Relation Between Phototherapy and Nevus Count: Univariate Analysis

<table>
<thead>
<tr>
<th>Nevi</th>
<th>Phototherapy</th>
<th>Size, mm</th>
<th>Mean</th>
<th>SD</th>
<th>P Value, t Test</th>
<th>Median (First to Third IQR)</th>
<th>P Value, Wilcoxon Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>Yes</td>
<td>Any</td>
<td>28.5</td>
<td>15.77</td>
<td>.88</td>
<td>22 (15-39)</td>
<td>.65</td>
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<tr>
<td>40</td>
<td>No</td>
<td>Any</td>
<td>26.7</td>
<td>15.33</td>
<td>.52</td>
<td>26 (17.5-36.5)</td>
<td>.41</td>
</tr>
<tr>
<td>18</td>
<td>Yes</td>
<td>&lt;2</td>
<td>25.56</td>
<td>14.06</td>
<td>.52</td>
<td>21 (12-36)</td>
<td>.41</td>
</tr>
<tr>
<td>40</td>
<td>No</td>
<td>&lt;2</td>
<td>27.25</td>
<td>14.87</td>
<td>.52</td>
<td>24 (17.0-39.5)</td>
<td>.41</td>
</tr>
<tr>
<td>18</td>
<td>Yes</td>
<td>2-5</td>
<td>3.17</td>
<td>2.67</td>
<td>.008</td>
<td>3 (1-5)</td>
<td>.006</td>
</tr>
<tr>
<td>40</td>
<td>No</td>
<td>2-5</td>
<td>1.23</td>
<td>1.49</td>
<td>.008</td>
<td>3 (1-6)</td>
<td>.006</td>
</tr>
<tr>
<td>18</td>
<td>Yes</td>
<td>&gt;5</td>
<td>3.5</td>
<td>3.05</td>
<td>.02</td>
<td>3 (1-6)</td>
<td>.01</td>
</tr>
<tr>
<td>40</td>
<td>No</td>
<td>&gt;5</td>
<td>1.45</td>
<td>1.99</td>
<td>.02</td>
<td>1 (0-2)</td>
<td>.01</td>
</tr>
<tr>
<td>18</td>
<td>Yes</td>
<td>&gt;5</td>
<td>0.33</td>
<td>0.59</td>
<td>.54</td>
<td>0 (0-1)</td>
<td>.29</td>
</tr>
<tr>
<td>40</td>
<td>No</td>
<td>&gt;5</td>
<td>0.23</td>
<td>0.62</td>
<td>.54</td>
<td>0 (0-1)</td>
<td>.29</td>
</tr>
</tbody>
</table>

Abbreviation: IQR, interquartile range.
The Pearson correlation coefficient was used to investigate the relation between light dosage at birth and nevus count, but did not demonstrate any link between these variables (results not shown).

ASSOCIATION BETWEEN PIGMENTARY CHARACTERISTICS AND NEVUS COUNT

The relationship between nevus count and classic phenotypic characteristics was examined in the total population and separately in the exposed and nonexposed groups. At univariate analysis in the nonexposed group, skin color was significantly related with total nevus count ($P_{\text{mean}}=.04$; data not shown), but this was not found for the total population. Light hair color was correlated with nevi smaller than 2 mm ($P_{\text{median}}=.03$). At multivariate analysis, a significant relation was found after stratification between the presence of freckles and nevi 2 to 5 mm ($P=.03$). This study does not show the role of other constitutional risk factors such as eye color and skin types I and II.

CONGENITAL NEVI

All parents were asked to identify nevi present at birth. Eleven percent of the children had nevi since early childhood, but univariate analysis did not suggest any relationship between congenital nevi and total nevus count ($P>.05$; data not shown).

ASSOCIATION BETWEEN SUN EXPOSURE AND NEVUS COUNT

Table 4 gives the results of univariate analysis for the total population. They confirm previous results that solar exposure, especially during vacations, is strongly associated with total nevus count. Separate analysis of nevi smaller than 2 mm, 2 mm or larger, 2 to 5 mm, and larger than 5 mm also confirm these results. The most significant results were obtained for nevi 2 to 5 mm and 2 mm or larger ($P_{\text{mean}}=.005$ and $P=.001$, respectively). In this study, severe sunburn was not recognized as a risk factor for nevus development. Multivariate analysis (Table 3), after controlling for other variables, confirmed the association of solar exposure to total nevus count ($P=.02$), but did not find any significant relationship for the different sizes of nevi except for lentigo simplex.

Table 3. Nevus Risk Factors: Multivariate Analysis

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>$P$ Value, Variance Analysis Model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Any Size</td>
</tr>
<tr>
<td>Intensive phototherapy</td>
<td>.50</td>
</tr>
<tr>
<td>Age</td>
<td>.36</td>
</tr>
<tr>
<td>Skin type I or II, Fitzpatrick classification*</td>
<td>.65</td>
</tr>
<tr>
<td>Skin color</td>
<td>.35</td>
</tr>
<tr>
<td>Eye color</td>
<td>.92</td>
</tr>
<tr>
<td>Hair color</td>
<td>.63</td>
</tr>
<tr>
<td>Freckles</td>
<td>.31</td>
</tr>
<tr>
<td>Solar exposure during vacations</td>
<td>.02</td>
</tr>
<tr>
<td>Severe sunburn</td>
<td>.94</td>
</tr>
</tbody>
</table>

*For a description of these classifications, see Table 1.

Table 4. Relation Between Solar Exposure and Nevus Count: Total Population, Univariate Analysis

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Size, mm</th>
<th>No.</th>
<th>Mean</th>
<th>SD</th>
<th>$P$ Value, $t$ Test</th>
<th>Median (First to Third IQR)</th>
<th>$P$ Value, Wilcoxon Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solar Exposure During Vacations</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Light</td>
<td>Any</td>
<td>4</td>
<td>11.25</td>
<td>7.04</td>
<td>.02</td>
<td>13.5 (7.0-15.5)</td>
<td>.01</td>
</tr>
<tr>
<td>Strong</td>
<td>54</td>
<td>29.78</td>
<td>15.03</td>
<td>.03</td>
<td>26 (18-39)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Light</td>
<td>$&lt;2$</td>
<td>4</td>
<td>11.00</td>
<td>6.98</td>
<td>.02</td>
<td>13 (6.5-15.5)</td>
<td>.02</td>
</tr>
<tr>
<td>Strong</td>
<td>54</td>
<td>27.56</td>
<td>14.35</td>
<td>.005</td>
<td>25 (17-36)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Light</td>
<td>2-5</td>
<td>4</td>
<td>0.25</td>
<td>0.50</td>
<td>.001</td>
<td>0 (0-0.5)</td>
<td>.06</td>
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<tr>
<td>Strong</td>
<td>54</td>
<td>1.94</td>
<td>2.14</td>
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<td></td>
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<tr>
<td>Light</td>
<td>$&gt;2$</td>
<td>4</td>
<td>0.25</td>
<td>0.50</td>
<td>.002</td>
<td>0 (0-0.5)</td>
<td>.34</td>
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<tr>
<td>Strong</td>
<td>54</td>
<td>0.28</td>
<td>0.63</td>
<td></td>
<td></td>
<td>0</td>
<td></td>
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<tr>
<td>Severe Sunburn</td>
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<td></td>
<td></td>
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<tr>
<td>Yes</td>
<td>Any</td>
<td>24</td>
<td>29.58</td>
<td>15.71</td>
<td>.66</td>
<td>24 (16.5-38.5)</td>
<td>.84</td>
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<tr>
<td>No</td>
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<td>27.74</td>
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<td></td>
<td></td>
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<tr>
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<td>14.90</td>
<td>.69</td>
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<td>2.40</td>
<td>.61</td>
<td>1 (0-2.5)</td>
<td>.71</td>
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<td>34</td>
<td>1.71</td>
<td>1.92</td>
<td></td>
<td></td>
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<td></td>
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<tr>
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<td>$&gt;2$</td>
<td>24</td>
<td>2.25</td>
<td>2.69</td>
<td>.69</td>
<td>2 (0-2.5)</td>
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<td></td>
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<td>0.67</td>
<td></td>
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<td></td>
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</table>

Abbreviation: IQR, interquartile range.
Phototherapy in neonates is common. It is not known whether neonatal phototherapy may cause an increase in melanocytic nevus count. We demonstrate in this study that neonatal phototherapy is associated with a higher count for nevi 2 mm or larger. Variance analysis models, considering clinical nevus risk factors and permitting multivariate analysis, confirm these results, with a high correlation of phototherapy to nevus count, especially nevi 2 to 5 mm, compared with other variables and known clinical risk factors. The frequency of nevi throughout childhood is now well documented. In our entire sample, after excluding nevi smaller than 2 mm, the mean (SD) number of nevi was 2.09 (2.53) per child, which is in accord with the results found by Mackie et al.

The results of this study are consistent with pigmen-
tary and environmental risk factors for the development of nevus that were reported previously. Univariate and multivariate analyses confirmed that sunlight exposure during vacations is responsible for the development of nevus, which is consistent with other reports that argue that intermittent solar exposure increases the development of nevus and melanoma and that prolonged exposure reduces the risk. Sunburn history was not identified as a risk factor for nevus, but parental interviews of our subjects were particularly difficult and subjective. Among pigmentedary characteristics, the statistical results were not significant for 2 phenotypic risk factors usually associated with nevus: skin type and eye color. This may be explained by the pigmentedary characteristics of the French population. In this study, only 1 child had skin type I. Skin types II and III were prevalent in the children in this sample and are representative of the French population, with different skin pigmentedary characteristics from those in the Northern European population. It would be necessary to use larger samples to confirm known risk factors.

Whereas numerous studies have previously identified clinical risk factors for nevus development, to our knowledge, this study is only the second, after that of Wiecker et al. to evaluate the relationship between pho-
therapy and nevus acquisition. Studies of the safety of this therapy have focused on noncutaneous adverse ef-
facts. Wiecker and colleagues concluded that pho-
therapy was not associated with nevus count. In their cross-
sectional retrospective study, phototherapy type and irradiation dosages were not precisely specified. Data were based only on interviews. It is difficult to ascertain retrospectively that a child received neonatal phototherapy. Only total body nevus count was taken into account, whatever the size of the nevi. The method of our study is quite dif-
ferent. Children in the treatment group were selected for intensive neonatal irradiation dosages according to high bil-
irubin rates. Precise data about equipment type and treat-
ment duration were found in the neonatal medical re-
cords at Saint-Antoine Hospital.

These results should be interpreted with caution. The relatively small group sizes limit the power of the re-
sults. Furthermore, we should have calculated the mean number of nevi per square meter of body surface, taking into account nevus density for comparison, to obtain more comparable data. However, before puberty, the body sur-
face of the examined children was not much different. Even though the interviews and clinical examinations were made by a single investigator, a standardized chest photo-
graph showing nevus distribution would have enabled more objective data collection, but it was difficult to obtain parental agreement to obtain such photographs. Finally, for lentigo simplex (nevi < 2 mm) and nevi larger than 5 mm, no statistical difference was observed between the 2 groups. Because lentigo simplex is common in white persons, our sample may be too small to draw definitive conclusions. Lentigo simplex may represent more recent nevi, whereas those nevi due to early events should be larger. Nevi larger than 5 mm probably are congenital nevi and are most probably associated with genetic predisposition.

The exact mechanisms underlying the increased risk in children exposed to phototherapy at birth to develop nevi are not known. Studies by Pope et al. identified that UV-A and visible spectral regions induce melanomas when the irradiation occurs very early in life. These different wavelengths may have similar effects on melanogenesis and cell proliferation. For blue irradiation with a wave-
length focused at 460 nm, reflection part, dermoeider-
al absorption, and subcutaneous tissue penetration are estimated to be 30%, 60% and 10% of the rays, respec-
tively. Melanocytic cells localized in the epidermis are clearly affected by irradiation of this type. It has been shown that cellular pigments of the retinal pigment epi-
ethelium, especially when illuminated with visible light, catalyze free radical activity sufficient to cause photo-
oxidation of several major cellular components, such as polyunsaturated fatty acids of the membranes. In the same way, melanin in skin cells must be responsible for the same biologic effects. If bilirubin has a role as photosensitizer, we can as-
sume that the visible light toxicity depends on the bili-
rubin concentration, with stronger biologic effects in the case of marked hyperbilirubinemia. That could explain why neonatal intensive phototherapy seems to be asso-
ciated with nevus count in this study and why this risk factor has not been identified until now. Furthermore, neonatal skin with reduced enzymatic activity, low meta-
bolic detoxification, greater penetrability, and incom-
pletely activated immunologic defense could explain the neonatal sensitivity and vulnerability to phototherapy-
induced biologic events.

Higher numbers of acquired benign nevi are associ-
ated with increased risk of melanoma. A detailed eval-
uation of the factors responsible for the development of nevi in children would be useful to identify high-risk groups to be targeted for prevention. The link between melanoma and phototherapy should be the focus of such a study. A recent retrospective case-control study of mela-
noma in children included 30 childhood melanoma cases in patients younger than 20 years born in Sweden and 120 control subjects. The authors did not find any sig-
nificant risk of developing childhood melanoma after neo-
natal phototherapy. We wonder whether neonatal pho-
totherapy could be a risk factor for adult melanoma.
Childhood melanoma is probably more strongly associated with genetic predisposition than environmental factors. Data for melanoma in adults are unavailable, and our results should not lead to an alarmist interpretation. The small difference in number of melanocytic nevi in the 2 groups may not indicate a difference in the risk between the subjects in the 2 groups. Neonatal phototherapy remains the treatment of choice for neonatal hyperbilirubinemia.

CONCLUSIONS

Our results suggest that neonatal intensive phototherapy increases nevus acquisition. Children who receive phototherapy must be targeted for prevention and surveillance. These first results must be interpreted with caution because of the small sizes of the exposed and non-exposed groups. To confirm our results, complementary studies are needed to explore the relationship between phototherapy and melanoma and to find a more precise link between blue light activity and melanocytes, in vitro and in vivo.

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Study supervision: Matichard and Descamps.

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REFERENCES