standard method shows a correlation that is not fully satisfactory, but the fact that the two methods are based on completely different principles must be taken into account.

Given the specificity of the immunological method, its more widespread use would seem desirable. The method presented here is simpler and more rapid than traditional methods; perhaps further studies should lead to improvements.

Additional Keyphrase: age, weight, and time before analysis as sources of variability

Since we reported our method for measuring thyroxine in the eluate of blood spotted on filter paper (1–3), the screening program for neonatal hypothyroidism is now a service offered by the Quebec Network for Genetic Medicine to the Quebec population. We have already reported our preliminary results elsewhere (2–4). Here, we analyze several factors influencing these results, in an attempt further to decrease the proportion of falsely positive results.

Materials and Methods

A sample of 7358 results for thyroxine were taken from the period covering the month of January 1975. These results come from 24 different assays. This sample gives the possibility of studying more closely the influence of the interval between the sampling and the thyroxine determination because of the unusual delays after the Christmas holidays.

Factors Influencing Results for Thyroxine Concentration in Blood, as Measured in Paper Filter Spots in a Screening Program for Neonatal Hypothyroidism

J. H. Dussault, J. Morissette, P. Fiset, E. Laberge, and C. Laberge

To reduce the number of false-positives in our screening program for neonatal hypothyroidism, we investigated the effect of age, body weight, and interval between sampling and analysis on results for filter paper spot thyroxine determination. The statistical analysis included an analysis of covariance. The age of the infant did not influence the results, the interval influenced them minimally, but weight did require a correction factor of about 0.16 ng/kg below the mean weight. This correction factor should reduce our false-positives from 0.75% to 0.60%. Furthermore, because all hypothyroid infants had results that fell more than 2.8 SD below the geometric mean, we intend to measure thyrotropin concentrations in the blood eluate of all the spots with a thyroxine concentration below that cut-off point.

Statistical analysis of the results included an analysis of covariance and application of the following linear model.

\[ T_{ij} = M + \alpha_i + \beta_1 (P_{ij} - P_i) + \beta_2 (E_{ij} - E_i) + \beta_3 (A_{ij} - A_i) \]

where

- \( i = 1 \) @ 24 number of groups
- \( j = 1 \) @ M number of samples per group
- \( P_{ij} \) = weight of individual \( j \) in assay \( i \)
- \( P_i = \sum_{j=1}^{M} P_{ij} / M_i \)
- \( E_{ij} \) = difference between the sampling date of individual \( j \) and analysis assay \( (d_{ij}) \) date
- \( E_i = \sum_{j=1}^{M_i} E_{ij} / M_i \)
- \( A_{ij} \) = age in days of individual \( j \) in assay \( i \)
- \( A_i = \sum_{j=1}^{M_i} A_{ij} / M_i \)

This model has permitted us to determine:

- if there was a significant difference between the means of the different assays or groups, by testing if the different \( \alpha_i \) were all equal;
- if the different factors studied (weight of the infant, age, and time interval between sampling and analysis) influenced results for thyroxine, by calculating the \( B_i \) to see if they differed from zero.

Thyroxine concentration was determined as described previously (1–3).

Results

Table 1 shows the mean of the different variables analyzed, and the correlation between them and thyroxine concentration in five different groups of assays.

From Table 1 it can be seen that the means of the different assays are different with a \( F_{23} 7331 = 252 \). As seen in Table 2,
body weight is the principal factor influencing the results, with an increase or decrease of 0.16 ng of thyroxine per 40 µl of eluted blood for each kilogram below or above the mean weight. This influence is further illustrated in Figure 1. On the other hand, there is no significant difference in results for samples taken when the infant was three or five days old. Finally, even though the coefficient for interval between sampling and analysis is significantly different from zero, it means a correction of only .01 per day in from the mean, a negligible quantity. The mean thyroxine values in different assays vary from 1.2 to 3.0 ng/40 µl of eluted blood; therefore .01 represents a correction factor of <1%.

We conclude that only 3% of the variations in thyroxine are secondary to the three factors studied, weight being by far the most important.

Discussion

In our preliminary report on mass screening for neonatal hypothyroidism (2, 3) by use of our filter paper method we reported a prevalence of 0.75% false-positive results, which, although very acceptable for a screening program, in our case totaled in theory 514 recalled patients per year.

As shown in Table 2, age had no effect on the results for thyroxine concentration, the regression coefficient not differing significantly from zero. Since the blood is sampled between days 3 and 5 of age, we were not expecting this factor to influence greatly our T4 concentration.

Table 1. Mean of the Different Variables Analyzed, and Correlation between Them and Thyroxine (T4) Concentration

<table>
<thead>
<tr>
<th>n</th>
<th>T4, ng</th>
<th>Body wt, kg</th>
<th>Interval, days</th>
<th>Age, days</th>
<th>Weight</th>
<th>Interval</th>
<th>Age</th>
</tr>
</thead>
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<tr>
<td>367</td>
<td>1.93</td>
<td>3.25</td>
<td>16.2</td>
<td>3.9</td>
<td>.17</td>
<td>-.13</td>
<td>-.05</td>
</tr>
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<td>489</td>
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<td>3.27</td>
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<td>-.05</td>
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<td>3.20</td>
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<td>3.4</td>
<td>.12</td>
<td>-.14</td>
<td>-.18</td>
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<td>3.29</td>
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<td>.03</td>
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<td>2.80</td>
<td>3.27</td>
<td>9.4</td>
<td>3.9</td>
<td>.23</td>
<td>-.02</td>
<td>.05</td>
</tr>
</tbody>
</table>

The estimation of the weight coefficient gives the value of .16 ± .01, involving a correction for this factor. With use of this correction we are decreasing our false-positive results by about 15%. This influence of weight in lowering the thyroxine concentration for smaller babies was expected, because this relation is also present for serum thyroxine at birth (5).

Finally, the coefficient for the interval between sampling and analysis, which is -.012 ± .002 per day, implies a negligible correction except for samples for which analysis is delayed for more than 20 days. This occurs rarely and it is not of any consequence, because these samples have a high probability of being recalled.

Thus in our screening program we are now correcting our values for the weight of the newborn, hoping to decrease our false-positive proportion from 0.75% to 0.60%. On the other hand we are now experimenting with a filter paper spot method for thyrotropin measurement by immunoassay of the eluate with a highly sensitive antibody. This approach will permit us to decrease further our recalled population by measuring the thyrotropin in all samples with a thyroxine value that is smaller than 2.8 standard deviations below the geometric mean of the assay, since all hypothyroid infants detected to date were below that cut-off point.

References