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The Thyroxine Screening Index in congenital hypothyroidism screening

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A modification of the RIA total thyroxine assay from a dry drop of blood has been applied in 5-day-old neonates for the screening of congenital hypothyroidism. For first classification, the thyroxine screening index was used in 13 500 newborns; it proved to be simple, rapid and economically acceptable. In borderline cases, more detailed examinations were carried out, viz. estimation by RIA of thyroid stimulating hormone, thyroxine and thyroxine-binding globulin from venous blood. The incidence of permanent and transient impairment of thyroid function is shown in a special graph.

Two methods are usually employed for mass screening of congenital hypothyroidism, radioimmunoassay (RIA) and enzyme immunoassay (ELISA) for determining thyroid hormones in the blood serum of neonates. It is possible to determine the levels of thyroxine (T_4) and of thyroid-stimulating hormone (TSH) or their combination; the latter may be regarded as the best solution [4, 6]. TSH has hitherto been considered a more reliable indicator of thyroid function than T_4, especially in the case of ectopic thyroid glands [6]. It has, however, been shown recently that primary T_4 screening may have a similar sensitivity as TSH for mass screening programs for congenital hypothyroidism [1].

To introduce the method in Czechoslovakia, we elaborated and tested clinically a modification of the total thyroxine assay from a dry drop of blood in neonates by the so-called single step T_4 RIA [5].

For first classification, we adopted a procedure which employed the thyroxine screening index (TSI). This was used for assessing the borderline and pathological values.

Material and Methods

For single step T_4 RIA, a drop of blood is withdrawn from the heel. On Schleicher and Schüll 2992 filter paper the spot measures 12 mm in diameter. Spots 3 mm in diameter containing about 3.0 μl of blood (1.5 μl of serum with an assumed haematocrit value of 50%) were cut out and analysed. The method is based on single step addition of the reagents. One ml of a stock solution containing specific serum, a buffer, labelled antigen, immunoglobulin G, polyethylene glycol and 8-amino-1--naphthalene sulphuric acid were pipetted into test-tubes, then the dried blood spot was added.

For determining TSI, the original determination of total T_4 was modified by eliminating the calibration curve and modifying the calculations. Instead of employing several samples for constructing

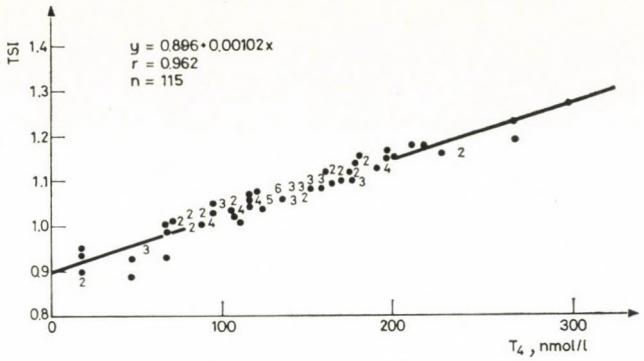

FIG. 1. Correlation between total T_4 and TSI in 115 blood samples collected on the 5th day of life. Numerals indicate the number of estimations for a given value

the calibration curve, we used a pooled sample from normal donors as the reference standard. Samples for estimation were processed in series. Each series contained estimated and reference sera for the calculation of TSI. The reference sample was represented by the pooled T_4 values of blood donors. For checking the error of analysis, we used serum from a patient with confirmed hypothyroidism. Results were evaluated by calculating TSI from the ratio of the reference and tested samples, viz.

$$\text{TSI} = \frac{\text{cpm reference sample}}{\text{cpm unknown sample}}.$$

In a normal newborn, the TSI value was 1.0 ± 0.1. Values between 0.90—0.99 indicated that the analysis must be repeated to eliminate some laboratory error. A recall was essential when TSI was below 0.90 (1.0 sigma). The validity of the analysis was controlled by the value of non-specific binding, the dispersion of values of the reference samples, and the value of the control sample. The long-term stability of the above-mentioned parameters was followed in profile diagrams.

The relation of TSI values and T_4 levels was studied in 115 neonatal blood samples collected on the 5th day after birth. Total T_4 concentrations were determined in the same samples by T_4 RIA kit (DRG) from a dry blood spot (Fig. 1). It is evident that there was a significant correlation between these values of $r = 0.962$ at the 0.0005 level of significance. So far we have examined 13 500 5-day newborns employing the TSI parameter.

RESULTS AND DISCUSSION

TSI was found to be a simple, rapid and economically acceptable test which proved useful for providing first information about the functional state of the neonatal thyroid gland. The results were classified into three groups (Fig. 2).

In Group A, pathological values are shown which require an immediate detailed assay from venous blood.

Group B includes borderline values which require repeated examination from original samples of dry blood on filter paper.

Entirely normal values were found in Group C.

From this scheme it is evident that, if necessary, this first examination can be specified in more detail by further tests such as RIA T_4, TSH and/or TBG. TSI is in accordance with the instructions of the European Thyroid Association for introducing SKH and the procedure which was recommended by Fischer et al [2] for use in the USA.

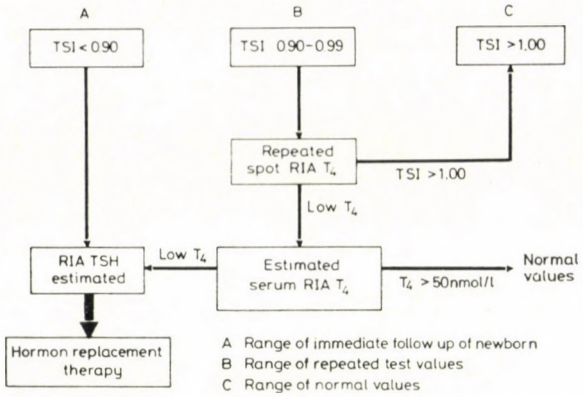

FIG. 2. Evaluation of TSI results of screening for congenital hypthyroidism. Group A: pathological values which require immediate detailed assay from venous blood. Group B: includes borderline values which require repeated examination of the original sample of dry blood. Values in Group C correspond to normal values

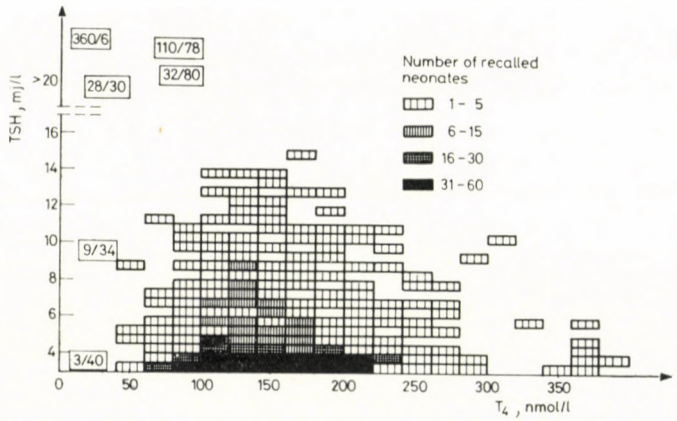

FIG. 3. Statistical distribution of total T_4 and TSH values in neonatal venous blood serum. The black area represents the highest frequency attained during a given period:
360/6: 360 I.U./l TSH, 6 nmol/l T_4 — primary hypothyroidism (athyreosis)
110/78:110 I.U./l TSH, 78 nmol/l T_4 — primary hypothyroidism (rudimentary thyroid gland)
32/80:32 I.U./l TSH, 80 nmol/l T_4 — transient hypothyroidism
28/30:28 I.U./l TSH, 30 nmol/l T_4 — transient hypothyroidism
9/34:9 I.U./l TSH, 34 nmol/l T_4 — defect in TBG production
3/40:3 I.U./l TSH, 40 nmol/l T_4 — defect in TBG production

In our material we encountered 3 cases of primary hypothyroidism, 2 cases of TBG defect and 3 cases of transient hypothyroxinaemia.

The distribution of the results of venous serum samples is shown in Fig. 3. The radioimmunological values of total T_4 obtained by the method of single step analysis are on the abscissa, while the TSH values on the ordinate. The TSH values were obtained by RIA using the Calbiochem

semikit. The black area represents the highest incidence of neonates recalled during the given period. In the upper part of the map are cases of primary hypothyroidism, such as athyreosis and ectopic thyroid gland, and below are the cases of transient hypothyroidism. In the bottom part are cases of secondary hypothyroidism and those with a defect in TBG production.

After having introduced a national screening program for congenital hypothyroidism, computer evaluation is being planned for processing the results of the above model.

Our investigations are part of a pilot study which is being employed in five regions of Czechoslovakia, where 60 252 newborns were examined on the fifth day after birth by the end of 1982 [3]. The incidence was so far 1:5 000 newborn infants. It is expected that in the near future this screening program for hypothyroidism will cover the whole of Czechoslovakia.

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