

## RESEARCH ARTICLE

# Protocol modification proposed for congenital hypothyroidism screening programme in Romania

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**Objective:** In Romania, congenital hypothyroidism screening is performed by measuring thyroid-stimulating-hormone levels from dried blood samples. If the initial value is above the recommended cut-off value (10 mUI/L), the newborn is recalled for a second blood collection. The aim of this study was to investigate and report potential improvements on the screening protocol that is currently applied in our country in order to reduce the time between birth and treatment initiation in newborns positive to congenital hypothyroidism screening. **Methods:** Blood samples were collected from 41 full-term newborns between February and March 2019 at the maternity ward from Targu Mures Emergency County Hospital. Thyroid-stimulating-hormone values were measured with a chemiluminescent microparticle immunoassay in serum samples from cord blood collected at birth, and with a fluorometric enzyme-linked immunoassay in dried blood spots collected at day 3-5 after birth. To obtain whole blood values, serum values were transformed using a formula supplied by the kit manufacturer. Calculated cord blood values were compared with dried blood spots values using the Wilcoxon test. **Results:** After serum-to-whole-blood conversion, cord blood values ranged from 2.58 to 3.66 mUI/L (95% CI). Dried blood spot values ranged from 6.70 to 7.50 mUI/L (95% CI). The Wilcoxon test p value between cord blood and dried blood spots thyroid-stimulating-hormone levels was statistically significant ( $p < 0.01$ ). **Conclusions:** Thyroid-stimulating-hormone levels above the cut-off value were flagged by both techniques. An improvement to the existing protocol is proposed that may reduce time from positive screening results to confirmation of congenital hypothyroidism and treatment initiation.

**Keywords:** newborn screening, congenital hypothyroidism, TSH

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## Introduction

Congenital Hypothyroidism (CH) screening programs apply different strategies worldwide. These strategies are adapted to local conditions regarding primary and secondary CH incidence and earlier experience in screening programs [1]. Most programs use Thyroid-Stimulating Hormone (TSH) measurement as a primary strategy while others use T<sub>4</sub>-reflex TSH testing (TSH is measured only if T<sub>4</sub> is outside the reference values). Only one screening program, in Japan, uses TSH and freeT<sub>4</sub> measurement [1,2]. It is largely accepted that TSH-based screening programs using dried blood spots (DBS) have lower recall rates than those where TSH is measured from cord blood [3]. However, cord blood is preferred in some countries due to the early discharge of newborns and difficulties in recalling if the TSH level is above the cutoff value [4,5]. The American Association of Pediatrics recommends that treatment be started as soon as possible, especially for newborns that have initial TSH values above 40 mUI/L, even before venous blood sample results are available. Early treatment is recommended to minimize the negative effects of CH on brain development [2,6]. In Romania, DBS are collected at day 3-5 after birth and TSH is measured using a fluorometric enzyme-linked immunoassay (FEIA) technique. If the initial value is above the recommended cutoff value (20 mUI/L), the newborn is recalled for a second DBS collection [7]. The aim of this study was to investigate and

report potential improvements on the screening protocol that is currently applied in our country in order to reduce the time between birth and treatment initiation in newborns positive to CH screening.

## Methods

Written informed consent was obtained from the mothers prior to sample collection. Cord blood and DBS samples were collected between February and March 2019 from 41 full-term newborns at the maternity ward of the Emergency County Hospital Targu Mures, Romania. Cord blood samples were collected at birth for Rapid Plasma Reagin (RPR) screening, which is a routine procedure in our hospital for congenital syphilis detection, as a separate program than CH screening. After RPR testing, the remaining serum was recovered and stored at -20°C for up to 7 days. Serum TSH analysis was performed in a single run with a chemiluminescent microparticle immunoassay (CMIA) method (Architect i1000, Abbott, USA). From the same newborns, a DBS was collected within 3-5 days after birth as part of the Newborn Screening (NBS) program. DBS TSH levels were measured with the FEIA technique (Lab-systems Diagnostic, Oy, Finland). To obtain whole blood values, cord blood serum values were transformed using the formula supplied by the FEIA kit manufacturer as follows: TSH serum value  $\times 0.45 =$  TSH whole blood value. The same formula is used by the laboratory in reporting Proficiency Testing results. Normal distribution was verified with Kolmogorov-Smirnoff test and was rejected ( $p < 0.05$ ). Transformed serum values were compared with DBS val-

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ues, using the Wilcoxon statistical test. A diagnostic test was also performed, where we compared results on FEIA against CMIA (gold standard method). Statistical analysis was performed using MedCalc Statistical Software version 14.8.1 (MedCalc Software bvba, Ostend, Belgium; <http://www.medcalc.org>; 2014).

## Results

Cord blood serum TSH values measured with CMIA ranged between 5.84-8.25 mUI/L (95% CI). After applying the kit's manufacturer formula for serum-to-whole-blood conversion, calculated cord blood TSH values ranged between 2.58-3.66 mUI/L (95% CI). TSH values measured with FEIA from DBS ranged between 6.70-7.50 mUI/L (95% CI). The Wilcoxon test p-value between calculated cord blood TSH values and DBS TSH values was statistically significant ( $p < 0.01$ ).

Sensitivity obtained on the diagnostic test was 100%, meaning that positive results on CMIA are also positive on FEIA.

In one newborn, the DBS TSH value as measured by FEIA was 37.54 mUI/L, while the calculated cord blood TSH value was 8.14 mUI/L. After duplicate retesting on FEIA, the mean value (5.73 mUI/L) was below the cutoff value (10 mUI/L).

One newborn that had an above the cutoff TSH level of 12.12 mUI/L on CMIA was also flagged on FEIA (10.64 mUI/L). After duplicate retesting on FEIA, the mean value was 7.28 mUI/L. The results are shown in Figure 1.

## Discussions

TSH results obtained by FEIA were similar to those reported by the manufacturer. Results obtained by the manufacturer using the same test kit in 10.780 newborns in Shanghai showed that the 99th percentile was 7.5

mUI/L [8]. In a study from our screening center, using the same test kit and samples collected from 122 newborns, TSH ranged from 1.24 to 9.01 mUI/L [9]. A 20 mUI/L cutoff value is recommended by the Romanian Ministry of Health's guidelines for DBS samples [7]. A 17 mUI/L cutoff value is used by the largest screening center in our country [10] and a 10 mUI/L cutoff value is recommended by the kit manufacturer [8]. In CH programs, a high percentage (more than 40% in some areas) of newborns with TSH levels  $> 5$  mUI/L is considered indicative for iodine-deficient populations [11].

TSH findings on serum samples using CMIA (5.84-8.25 mUI/L, 95% CI) were similar to those reported by other studies employing similar techniques: 3.48-27.56 mUI/L from cord blood using CLIA (Chemiluminescent immunoassay) in Ethiopia [5], 4.56 mUI/L mean value for full-term newborns using DELFIA (Dissociation-Enhanced Lanthanide Fluorescent Immunoassay) in Columbia [12], and 3.36-22.09 mUI/L (90% CI) using CLIA in India [4]. In a previous study in our screening center, TSH was measured by another CLIA test (Immulite 2000, Siemens) and TSH results ranged between 0.20-11.43 mUI/L [9]. TSH values from cord blood measured with CMIA are lower than those obtained with FEIA from DBS. These results are similar to those reported by another study that compared TSH results from serum and DBS obtained in 118 adults from nursing homes where the correlation coefficient was  $r = 0.99$  and the slope was 1.64 with an intercept of -1.4. The diagnostic sensitivity, in this case, was 0.96 for DBS, showing that DBS results may also be used in screening other age groups [13]. It is well known that a TSH surge is expected in full-term newborns in the first 48 hours after birth, but programs that use cord blood for TSH measurement have a higher cutoff value (30 mUI/L) than programs that use DBS measurement (cutoff from 6-20 mUI/L). [1,4,5].

TSH values obtained from cord blood and DBS may not be comparable in absolute values for several reasons: TSH surge in the first 48 hours after birth, the difference between matrices [13], the inability to determine the true value of hematocrit at the time of TSH measurement [14], etc. Another reason may be the difference between the two techniques: functional and analytical sensitivity, different calibrators [8,15], lack of complete harmonization in TSH measurements [16].

The functional sensitivity of the CMIA test is  $< 0.01$  mUI/L and the analytical sensitivity of the test is  $< 0.0025$  mUI/L [5]. The functional sensitivity of the FEIA test was not declared by the manufacturer, while its analytical sensitivity is 0.9 mUI/L [8]. CMIA test is calibrated against WHO 85/558 reference material [15] and FEIA is calibrated against WHO and ISNS Reference Preparation for Neonatal Screening [8].

However, TSH values  $> 40$  mUI/L are above the cutoff value, regardless of the technique used, time of sampling, or the matrix used. In these cases, time is critical as these

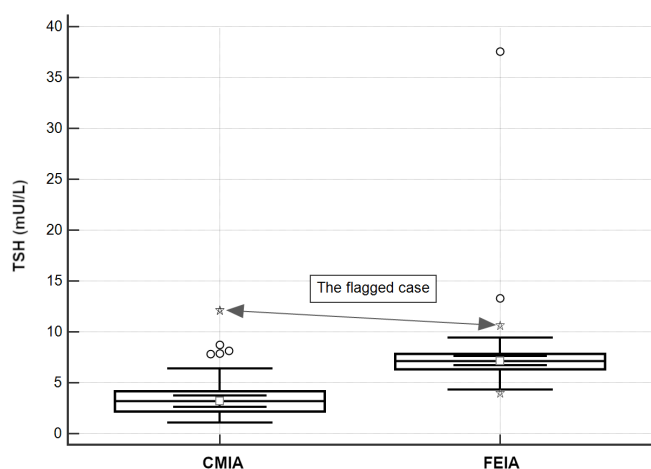


Fig. 1. Thyroid-Stimulating Hormone (TSH) results for the 41 matching samples. The chemiluminescent microparticle immunoassay (CMIA) results were obtained from cord blood (at birth), values ranged between 2.58-3.66 mUI/L. Fluorometric enzyme-linked immunoassay (FEIA) results from DBS collected within 3-5 days after birth ranged between 6.70-7.50 mUI/L. The marked case was flagged by both techniques.

newborns benefit the most from the early start of treatment [2,6].

In a study comparing recall rates for TSH from cord blood and DBS, all CH cases were identified by both techniques, but DBS testing had a higher recall rate than cord blood testing, for the studied group [3]. In a program study from Saudi Arabia, the recall rate for samples collected on DBS was 1.68%, while the recall rate for cord blood samples was 0.04% [17].

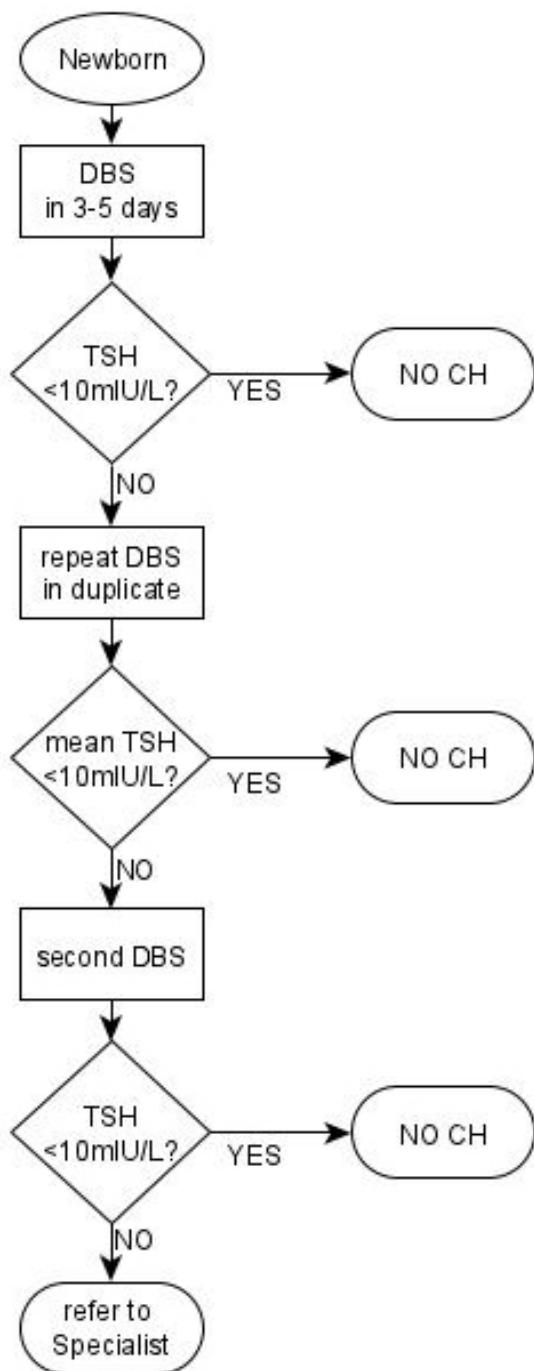


Fig. 2. The screening protocol currently applied in our center. A dried blood spot (DBS) is collected within 3-5 days after birth. If Thyroid-Stimulating Hormone (TSH) is lower than 10 mUI/L, then Congenital Hypothyroidism (CH) is ruled-out. If the TSH value is above 10 mUI/L, a duplicate measurement is performed. If mean TSH is above 10 mUI/L, a second DBS is collected and if TSH is above 10 mUI/L, the newborn is referred to a specialist.

In a study published in 2017, cord blood specificity for CH screening was 94.6% with a Positive Predictive Value of 7.25%. The recall rate in that study was 6.25%. In that program, values were measured with a CLIA method and a cutoff value of 20 mUI/L was used [18].

The median time until treatment initiation in our country is 19 days, as reported in the Medilog database (the database is used for surveillance of the CH screening program in Romania). This period is longer than the recommended 14 days period[10]. In Germany, the median time from birth to treatment is 11 days with the first results available after 7.3 days. In our country, the first results are obtained after 15 days due to a lengthy transportation phase (9 days until the samples arrive at the laboratory). In Scotland, therapy is started at 10.5 days after birth [10]. No data are available in the cited paper regarding the time span needed to recall the newborns, but at best this time is equal to the one necessary from birth to the first result.

The screening protocol currently applied in our center is described in Figure 2. In Figure 3, we propose a modified screening protocol that may help reduce the time between positive screening results and confirmation of CH.

Our study has some limitations due to the small sample size for comparison and that the cord blood samples are not always available in the screening laboratories.

In our modified protocol a DBS is collected within 3-5 days after birth and TSH is measured by FEIA. If the TSH is >10 mUI/L, a chemiluminescent immunoassay is performed from the stored serum that was collected for RPR

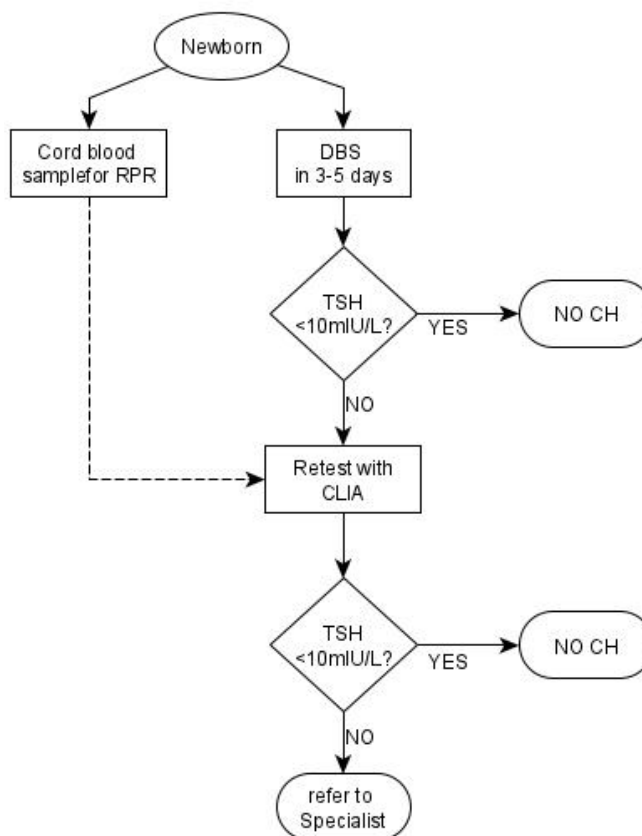


Fig. 3. The proposed improved protocol.

screening at birth. If the chemiluminescent value is >10 mUI/L, then the newborn is referred directly to an endocrinologist, without requesting a second DBS.

The modified protocol was successfully applied in our screening center in two cases that had DBS TSH levels above the cutoff value. The cord blood serum recovered from RPR testing was tested by CMIA. In both cases, TSH levels were >100 mUI/L for both DBS (FEIA) and cord blood (CMIA).

### Conclusions

Our proposed addition to the existing protocol may help reduce the time from birth to treatment initiation in newborns with congenital hypothyroidism. However, this modified protocol may not be applicable in all cases as cord blood samples are not available in all laboratories.

### Authors Contributions

IG- acquisition of data, drafting the article.

SB-acquisition of data, analysis and interpretation of data

OO- drafting the article, conception and design

MD- critical revising of the article, final approval of the version to be published

### Conflict of interest

None to declare.

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