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Age-specific correlation between thyroid hormone concentrations and ultrasound thyroid volume for diagnosing thyroid dysfunction in preterm infants: a single-centre, prospective, observational study protocol

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ABSTRACT

Introduction Thyroid disorders are commonly concomitant with premature birth; however, indications to start therapy remain unclear due to lack of gestational age-specific reference ranges and thyroid ultrasound nomograms. We aim to evaluate the age-specific correlation between circulating free thyroxine (FT4) and thyrotropin stimulating hormone (TSH) levels and ultrasound thyroid volume to assist identify infants requiring thyroid hormone replacement therapy.

Methods and analysis This is an observational, prospective, single-centre study that will include 200 preterm infants born between 24 and 32 weeks of gestational age, without any congenital diseases or malformation that may affect thyroid function. Venous blood will be obtained in infants at 14–21 days of life, and at 32 and 36 weeks of postconceptional age (PCA) to measure FT4 and TSH concentrations. Thyroid ultrasound will be performed at 32 and 36 weeks of PCA. Relevant outcomes will include determination of FT4 and TSH values and ultrasound thyroid volume for preterm infants born at 24–28 weeks of gestation and 29–32 weeks of gestation. Correlations among circulating hormone concentrations and thyroid volumes with the head circumference and body mass will also be determined.

Ethics and dissemination The Ethics Committee of the Medical University of Warsaw has approved the study protocol prior to recruitment (KB44/2019). Informed consent will be obtained from caretakers of preterm infants at the time of enrolment. Consent for participation in the study can be withdrawn at any time, without consequences and without obligation to justify the decision. All data will be stored in a secure, password-protected Excel file that is only accessible to researchers involved in the study. Findings will be published in a peer-reviewed journal and disseminated at relevant national and international conferences.

Trial registration number NCT04208503.

Strengths and limitations of this study

- This study will evaluate the correlation between thyroid hormone levels and ultrasound thyroid volume in preterm infants via the prospective collection of data and the inclusion of an adequate number of patients to assure statistically powered data.
- The study is unique as it combines thyroid ultrasound with blood tests to assist neonatologists in the decision to initiate thyroid hormone replacement therapy in preterm infants.
- Our results should inform the use and interpretation of thyroid ultrasound in vulnerable preterm infants.
- A limitation of the study is the potential biases that could arise due to missing data, for example, due to medical treatment that contradicts blood collection and/or thyroid ultrasound.
- Another limitation is that the analysis will be limited to the variables collected in the observational data set, and therefore, there is potential bias due to important unmeasured variables.

INTRODUCTION

Congenital hypothyroidism (CH) is a serious endocrinological thyroid disorder and the most common preventable cause of mental retardation.[1] Early diagnosis and treatment of CH can help prevent neurodevelopment disability and improve long-term outcomes.[2]

Thyroid disorders are commonly concomitant with prematurity.[3 4] The increasing incidence of preterm deliveries (and increased survival of preterm neonates), along with improved screening for CH, likely accounts for the higher incidence of hypothyroidism detected in infants. Preterm infants may be more susceptible to thyroid disorders for several reasons, including immaturity of the hypothalamopituitary-thyroid axis, non-thyroidal illness, impaired synthesis and metabolism of thyroid hormones, and administration of medications (eg, dopamine or steroids).[2–5] Moreover, the incidence of

a temporary form of hypothyroidism, termed transient prematurity hypothyroxinemia, is higher among preterm neonates than in the general population. Transient prematurity hypothyroxinemia is defined as a temporary reduction in free thyroxine (FT4) values without an increase in thyrotropin stimulating hormone (TSH) values and might be associated with neurocognitive retardation of preterm infants.[4] Therefore, neonatologists are increasingly aware of the importance of diagnosing and treating thyroid dysfunction in preterm infants.

Unfortunately, thyroid disorders are often unrecognised in preterm infants, likely due to the delay in the elevation of TSH concentration after delivery.[2 5] Although TSH concentrations increase from 2 to 6 weeks of life, the time for TSH elevation can vary. Indeed, McGrath *et al*[2] reported that 50.9% of preterm infants born at <33 weeks of gestation who were diagnosed with hypothyroidism had a delayed TSH elevation. Kaluarachchi *et al*[5] found that delayed TSH elevation in preterm infants born at <30 weeks of gestation was more frequently associated with multiple gestations, small weight for gestation age and a lower 5 min Apgar score. To overcome this issue of delayed TSH elevation, both European and American guidelines recommend a repeated screening test for hypothyroidism in preterm neonates. European guidelines recommend that the test should be repeated 2 weeks after the first screening (or in the second week of life), while the American Academy of Paediatrics advocates its repetition between the second and the sixth weeks of life.[3] However, the optimal timing of blood sample collection for hypothyroidism assessment in preterm infants remains highly debated.

The indications to start therapy for hypothyroidism in neonates also remain unclear due to the lack of gestational age-specific reference ranges and nomograms of thyroid function.[6] Kilchemmann *et al*[7] reported significant differences between the average FT4 values according to gestational age ranges but found no differences in TSH levels. The observed lack of benefits of thyroid hormone replacement therapy in preterm infants may reflect the lack of appropriate reference ranges for thyroid hormone levels in this population. Indeed, no universal repeat screening protocol for assessing thyroid disorders is currently available,[3] and there are no clear guidelines for assessing thyroid volume in preterm infants. Therefore, novel methods of assessing thyroid function are being sought.

Ultrasound may help determine the anatomy and function of the thyroid gland in infants.[1] In particular, ultrasound assessment of thyroid volume in neonates could be used to detect thyroid dysfunction. Apart from thyroid volume, the examination also provides information on the location and structure of the gland. However, it is important to note that although ultrasonography is useful for determining the presence or absence of the thyroid gland in the normal location, the diagnosis of hypertrophy or hypoplasia requires further examination.[8] Nonetheless, as ultrasound is a simple, non-invasive and repeatable examination, we propose it should be included as a standard examination in the initial diagnosis in neonates with abnormal results of screening tests for hypothyroidism. Indeed, the guidelines on CH from the European Society for Paediatric Endocrinology recommend performing imaging studies in order to determine a specific aetiology.[9]

Although significant differences in mean thyroid volume have been detected on ultrasound according to gestational age,[10] thyroid ultrasound normative data for neonates are currently unavailable. Therefore, determining ultrasound thyroid volumes could allow us to objectively identify a gland as normal, small or enlarged. Such data, combined with age-specific thyroid hormone values, could be used to assist neonatologists in more accurately interpreting thyroid hormone and ultrasound results in neonates.

Objectives

This study aims to find additional methods to assess the development and dysfunction of the thyroid gland in preterm infants. The primary objective is to determine FT4 and TSH values in preterm infants born at 24–28 weeks of gestation and in those born at 29–32 weeks of gestation. We also plan to determine the thyroid volume via ultrasound for the two groups of preterm infants. Other objectives include assessing whether circulating FT4 and TSH concentrations correlate with thyroid volume and gestational age and determining the optimal age that correlates with the delayed elevation of TSH concentration.

METHODS AND ANALYSIS

Study design

This an observational, prospective, single-centre, cohort study that will include 200 preterm infants. Thyroid ultrasound and circulating FT4 and TSH measurements will be repeated at 32 and 36 weeks of postconceptional age (PCA). The schedule of assessment is shown in table 1. The study started in January 2020 and is due to be completed in December 2021. A schematic overview of the flow of participants through the study is presented in figure 1.

Study setting

Participants will be recruited among inpatients of the Neonatal and Intensive Care Department of the Medical University of Warsaw in Princess Anna Mazowiecka Hospital, Poland. In case of a low recruitment rate, other hospitals may be a source of patients.

Study population

The study will include 200 preterm infants born between 24 and 32 weeks of gestation (estimated by ultrasound) at the Anna Mazowiecka Hospital or who are admitted to the neonatal intensive care unit (NICU) within 7 days of birth. The eligibility of prospective patients will be

Table 1 Proposed schedule of enrolment, interventions and assessments

Study plan	Birth	0–14 days	14–21 days	32 weeks PCA	36 weeks PCA
Anthropometry	x			x	x
Medical history collection	x				
Mothers' thyroid history	x				
Eligibility assessment		x			
Written parental consent		x			
Medications (dopamine, steroids)		x			
Enrolment			x		
Blood sample for TSH and FT4 analysis			x*	x	x
Assessment of thyroid function			x	x	x
AEs (thyroid-related disorders)			x	x	x
SAEs (sepsis)			x	x	x
Ultrasound of thyroid volume				x	x

*Only in the group born at 24–28 weeks of gestational age.
AE, adverse event; FT4, free thyroxine; PCA, postconceptional age; SAE, serious adverse event; TSH, thyrotropin stimulating hormone.

determined by recruiting physicians who are familiar with the study protocol. Eligibility will initially be based on patients' medical records. Recruiting physicians will collate further information related to the inclusion and exclusion criteria in an interview with the patient's caregivers, explain the study procedures to them and provide them with an information leaflet describing the study. The caregivers will then be requested to provide two copies of written informed consent. The patient's case report form (CRF) will be prepared and filed, along with one copy of the informed consent. Information consent forms and information sheets will only be provided in Polish. The inclusion and exclusion criteria are listed in table 2.

Blood sample collection and laboratory analysis

Approximately 1 mL of blood will be collected and examined in the hospital laboratory to determine thyroid hormone concentrations during other routine blood tests. Serum concentrations of FT4 and TSH will be analysed by immunofluorescence assays using the Architect i1000SR (Abbott Diagnostics).

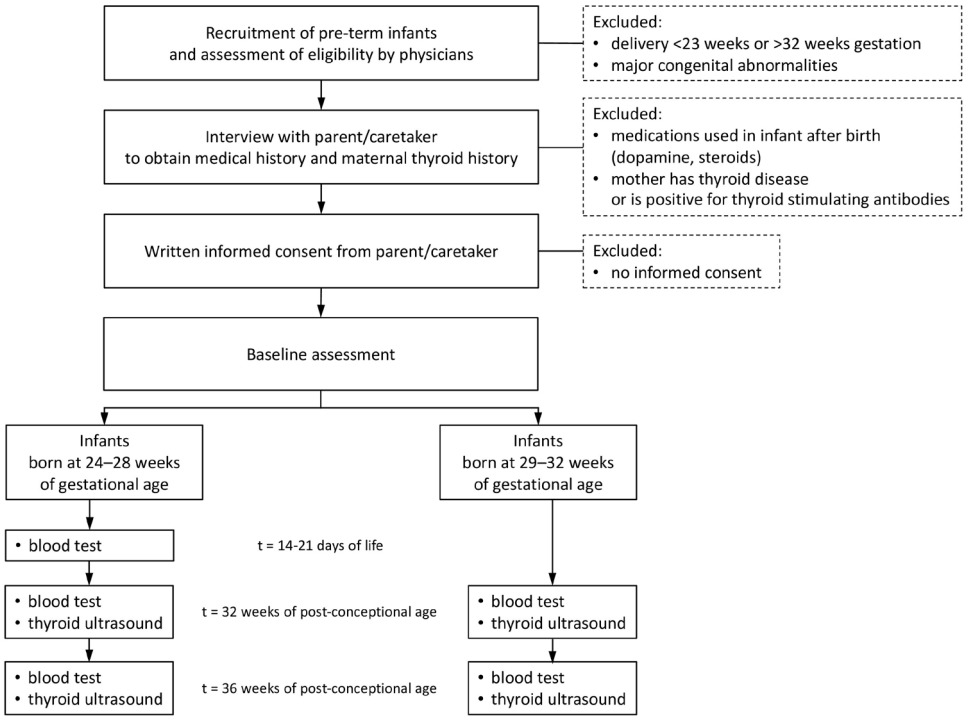

Figure 1 Flow of participants.

Table 2 Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
► Preterm infants born between 24 and 32 weeks of gestation (estimated by ultrasound) ► Born in or admitted to the Neonatal Intensive Care Unit within 7 days of birth ► Without congenital disease/malformation that may affect thyroid function ► Signed informed parental consent	► Preterm delivery <23 weeks of gestation or >32 weeks (estimated by ultrasound) ► Major congenital abnormalities ► Medications used after birth, including steroids or vasopressors (up to 12 hours after the end of treatment) ► Positive maternal thyrotropin antibodies ► Mother has thyroid disease treated with antithyroid drugs ► Mother treated with amiodarone ► Lack of parental consent

Preterm infants will be divided into two groups: those born at 24–28 weeks of gestational age and those born at 29–32 weeks of gestational age. In the group born at 24–28 weeks of gestational age, venous blood will be obtained at 14–21 days of life and 32 and 36 weeks of PCA. In the group born at 29–32 weeks of gestational age, the venous blood test will be done at 32 and 36 weeks of PCA.

Ultrasound examination

Thyroid ultrasound will be performed in preterm infants at 32 and 36 weeks of PCA. Ultrasound evaluation of the thyroid gland will be performed using the Philips HD XE system (Philips healthcare, Eidhoven, the Netherlands). The thyroid gland will be measured using a linear array transducer with the high-frequency probe L7-15 MHz. The volume of the thyroid gland is calculated by the formula for a prolate ellipsoid, where thyroid volume=length × breadth ×depth × $\pi/6$ (0.52).[11] The total volume is calculated as the sum of the volume of the individual lobes. The isthmus will not be included since it is low in normal neonates.

Treatment or intervention

No study-related interventions are planned. All preterm infants will be treated according to current guidelines.

Protocol deviations due to concomitant treatment or disease

In case steroids or vasopressors are found to have been administered within 12 hours prior to the scheduled examination time, the examination time will be changed to another time or skipped.

Primary and secondary outcomes

The primary outcomes will include:

1. Determination of FT4 and TSH values at 14–21 days of life and 32 and 36 weeks of PCA in preterm infants born at 24–28 weeks of gestation.
2. Determination of FT4 and TSH values at 14–21 days of life and 32 and 36 weeks of PCA in preterm infants born at 29–32 weeks of gestation.
3. Determination of ultrasound thyroid volume at 32 and 36 weeks of PCA in both groups of preterm infants (ie, those born at 24–28 weeks of gestation and those born at 29–32 weeks of gestation).
4. Evaluation of correlations between circulating thyroid hormone concentrations and thyroid volume.

Secondary outcomes will include:

1. Comparison of changes in FT4 evaluated at 32 and 36 weeks of PCA in each group of preterm infants.
2. Comparison of changes in TSH evaluated at 32 and 36 weeks of PCA in each group of preterm infants.
3. Analysis of TSH values over time (to determine the optimal time for TSH measurement).
4. Evaluation of changes in ultrasound thyroid volume examined at 32 and 36 weeks of PCA in each group of preterm infants.
5. Evaluation of the correlation between thyroid volume and circulating thyroid hormone concentrations with the head circumference and body mass at 32 and 36 weeks of PCA.

Follow-up

The primary and secondary outcomes will be assessed during the study period. There will be no follow-up period.

Sample size

The size of the sample has been calculated so that the scope of the CI will not exceed±0.25 (with a 95% confidence level and normal distribution assumption). With these assumptions and the variability specified in the preliminary study (SD of 0.115), the required number of patients is 85.

Data collection and management

The following baseline data will be collected in the hospital electronic database: gestational age of birth, sex, pregnancy status and thyroid treatment weight, Apgar score, concomitant treatment (steroids, dopamine), concomitant disease (sepsis). As sepsis can affect thyroid function, all infants will be monitored throughout this study for this condition, which will be reported as an adverse event. The influence of sepsis on the study outcomes will subsequently be analysed.

An electronic CRF will be used for this study. For each subject enrolled, a CRF must be completed by the investigator or a designated subinvestigator. This also applies to those subjects who fail to complete the study. If a subject withdraws from the study, the reason must be noted on the CRF. The CRF will be completed on an ongoing basis.

CRF entries and corrections will only be performed by study site staff, authorised by the investigator.

For the purpose of verifying the compliance of the data recorded in the electronic database with the original source data, access to source documentation (medical and ultrasound records) will be open to the ethics committee and regulatory authorities. Responsibility for compliance will rest on the principal investigator. The electronic database will be consistent with the original source data. Checks for possible errors and inconsistencies in the entered data will be carried out periodically.

Confidentiality

Complete patient and study information will be stored on a secure, password-protected Excel platform. Only researchers involved in the study will be provided with a personalised login and password to access the study information. The statistical team will not have access to sensitive data such as date of birth, address and contact details. All records relevant medical history, together with copies of ultrasound clips and radiology reports, will be stored separately in a locked file cabinet.

Statistical analysis

For establishing norms in the thyroid volume, the distribution of this variable will be estimated for two groups of preterm infants. Based on these distributions, we will estimate the percentiles of thyroid volume. Extreme values (eg, below the third percentile or above the 97th percentile) will be treated as outliers. Comparisons of means of thyroid volume as well as thyroid hormone concentrations will be conducted using Student's t-test if normally distributed, or the Mann–Whitney U test if the data do not follow a normal distribution.

Pearson's or Spearman's correlations will be applied for evaluating relationships between the thyroid volume and hormone concentrations. A generalised estimating equations will also be performed to examine these relationships.

Inter-rater agreement coefficients (eg, Cohen's Kappa) will be applied to evaluate agreement in the detection of hypothyroidism in infants of different age.

Evaluation of the influence of variables (eg, body weight, pregnancy age and pathological factors) on thyroid volume and hormone levels will be assessed using multiple regression analysis.

Statistical analysis will be carried out in R software, V.4.0.5 or later.

Monitoring and harms

This is a non-interventional, observational study with minimal study-related risk for the participant. The biological risk in this study is limited to repeated draws of small amounts of venous blood at 14–21 days of life and 32 and 36 weeks of PCA. To minimise local pain at the venepuncture site, the time of blood collection in the preterm infant will be correlated with the required TSH and FT4 control. Before the commencement of the study, the study team will be trained in good clinical practice.

There is no need for a data monitoring committee to be established as the trial complies with the standard care of very low birth weight infants. Any medical development, even of a potential causal character, will be treated as an adverse event (AE). We will record all AEs reported after the enrolment in the study until hospital discharge. The AEs to be recorded will be thyroid-related and include sepsis, treatment with steroids or dopamine. The electronic questionnaire will provide a list of anticipated thyroid-related AEs to be monitored.

Patient and public involvement

Neither parents of our patients nor the public were involved in the development of the research question, outcome measures or study design. We do not plan to include patients in the recruitment and conduct of the study. After completing the study, we will present our results to parents during our annual NICU Graduates Day.

Implications for practice

Our results should inform the use and interpretation of thyroid ultrasound in vulnerable preterm infants.

Ethics and dissemination policy

The Bioethical Committee of the Medical University of Warsaw has approved the study (KB44/2019). All significant modifications to the protocol will be reported to the committee. Written informed consent will be obtained from all parents/caretakers of infants who are willing to participate in the study. The consent for participation in the study can be withdrawn at any time, without consequences and without an obligation to justify the decision.

All deidentified data collected during the trial will be available. These documents will be accessible to anyone who provides a methodologically sound proposal immediately following publication with no end date.

We plan to submit our findings to international peer-reviewed journals and conferences (paediatric, endocrinological neonatal).

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Patient consent for publication Not required.

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