CONTROLLED ANTENATAL THYROID SCREENING STUDY

PROTOCOL

04/04/02 version including subsequent revisions

Co-Ordinating Centres:

University of Wales College of Medicine
Departments of Medicine, Medical Biochemistry and Child Health
University Hospital of Wales
Heath Park
Cardiff
CF14 4XN

Department of Environmental and Preventive Medicine
Wolfson Institute of Preventive Medicine
St. Bartholomew's and the Royal London School of Medicine and Dentistry
Queen Mary and Westfield College
Charterhouse Square
London
EC1M 6BQ

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Abbreviations

UHW – **U**niversity **H**ospital of **W**ales, Cardiff

WI – **W**olfson **I**nstitute, London

T4 – **T**hyroxine. The major hormone controlling basal metabolic rate. It is secreted by the thyroid gland. The 4 relates to the number of iodine atoms the compound contains.

FT4 – **F**ree **T**hyroxine. In the blood plasma, T4 is predominantly bound to proteins. The test measures the level of unbound T4.

TSH – **T**hyroid **S**timulating **H**ormone. TSH is a hormone produced by the pituitary gland. It stimulates secretion of T4 by the thyroid gland.

1. Aims

The study aims to determine whether screening pregnant women for hypothyroidism based on a high thyroid stimulating hormone or a low free thyroxine level in maternal serum, and offering serum screen positive women thyroxine, will decrease the incidence of childhood intellectual impairment.

2. Background

Thyroxine (T4) is a hormone secreted by the thyroid gland. T4 is predominantly bound to protein in the blood but a small percentage remains unbound. This is referred to as free thyroxine or FT4.

Thyroid stimulating hormone (TSH) is a hormone secreted by the pituitary gland. It stimulates the secretion of T4 by the thyroid gland. Increased T4 production inhibits TSH production, thereby creating a self-regulating feedback loop.

Disorders affecting the thyroid gland can result in decreased production of T4. This increases production of TSH by the pituitary gland. Elevated TSH levels are thus an indicator of hypothyroidism. This form of hypothyroidism is known as primary hypothyroidism. Secondary hypothyroidism occurs when the pituitary gland does not secrete adequate levels of TSH. The thyroid gland responds by underproducing T4.

Low levels of T4 in the developing foetus lead to impaired intellectual development in infants and in children.

Data published by Haddow and his colleagues (1999) showed that pregnant women with a high TSH level were three times more likely to have children with an IQ less than or equal to 85, than women with normal TSH during pregnancy.

The value of using serum TSH and T4 as screening tests in a large group of pregnant women and treating those with results that suggest hypothyroidism is unknown. This is being tested in this prevention trial by examining the intellectual function of the children at age three and comparing the results with a control group of women.

3. Design

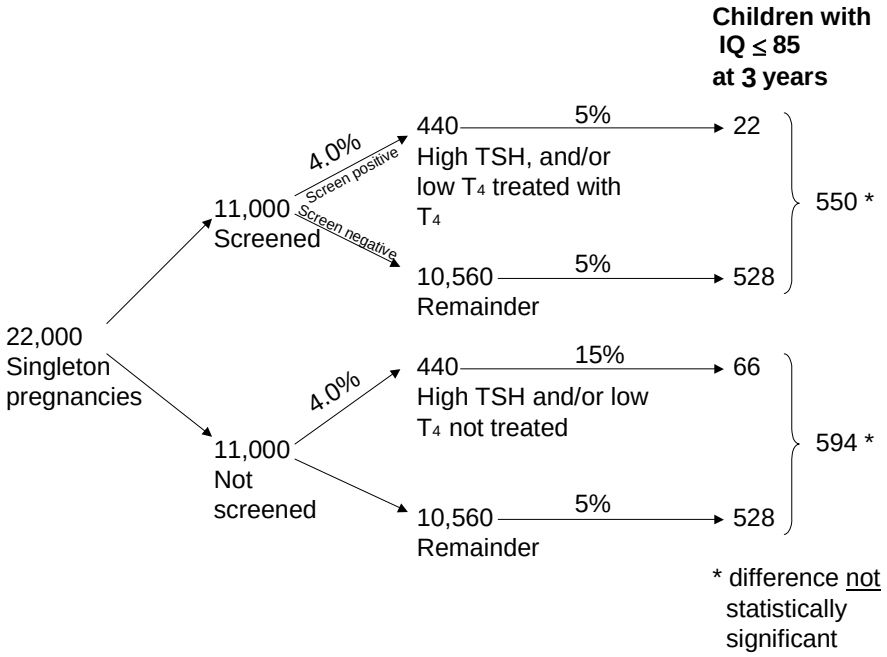
It is intended to recruit 22 000 pregnant mothers from the four designated centres in the South Wales area over the course of two years. 11 000 of these will be allocated to the screen group and 11 000 to the control group.

Of the 11 000 in the screen group, the top 2.5% with high TSH and the bottom 2.5% with low T4 will be treated with T4. This would give approximately 275 pregnant mothers in each of these categories. However, these two categories are not mutually exclusive and it is therefore expected that there will be an overlap. The extent of this overlap is not precisely known but if we assume that we end up treating 4% of the screen group rather than 5%, we have the following groupings:

Category	Number of Mothers	Treated with T4
High TSH only	165	Yes
Low FT4 only	165	Yes
High TSH and Low FT4	110	Yes
Remainder	10 560	No

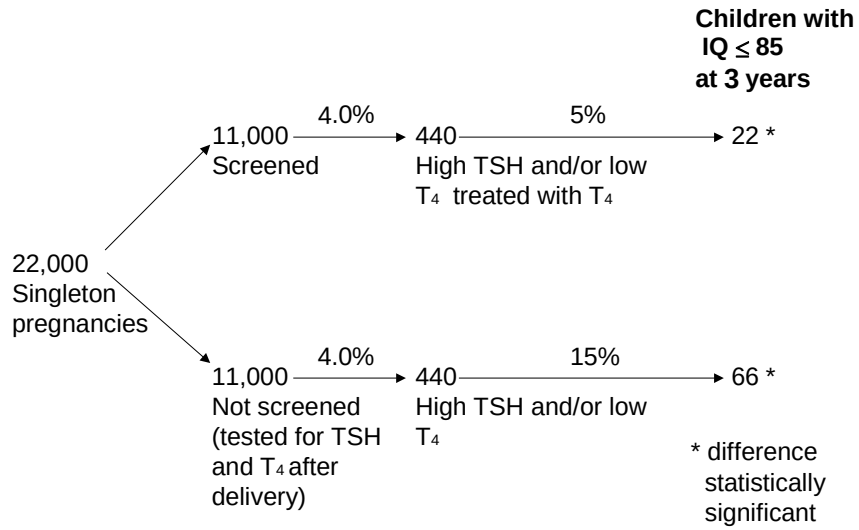
The following flow diagram (A) illustrates the numbers assuming that screening and treatments reduce the percentage of children with low IQ's from 15% to 5%. A direct comparison of IQ's in the screened and control groups would not be significant.

A



Storing serum from the controls and testing them after delivery will identify women with high TSH and/or low T4 levels. Only the IQ of the children from this group and the treatment group will be tested and compared. This enhances the statistical power of the study; the comparison is between 22 and 66 'cases' instead of 550 and 594 (see B). The 10 560 in each group without high TSH and/or low T4 can be ignored; they are of no interest as they do not have hypothyroidism by our definition, their presence serves only to dilute the results.

B



A random sample of women without hypothyroidism will be selected from the control group for comparison purposes. A sample of 5% i.e. 275 mothers should prove sufficient. This is depicted in diagram C below.

C

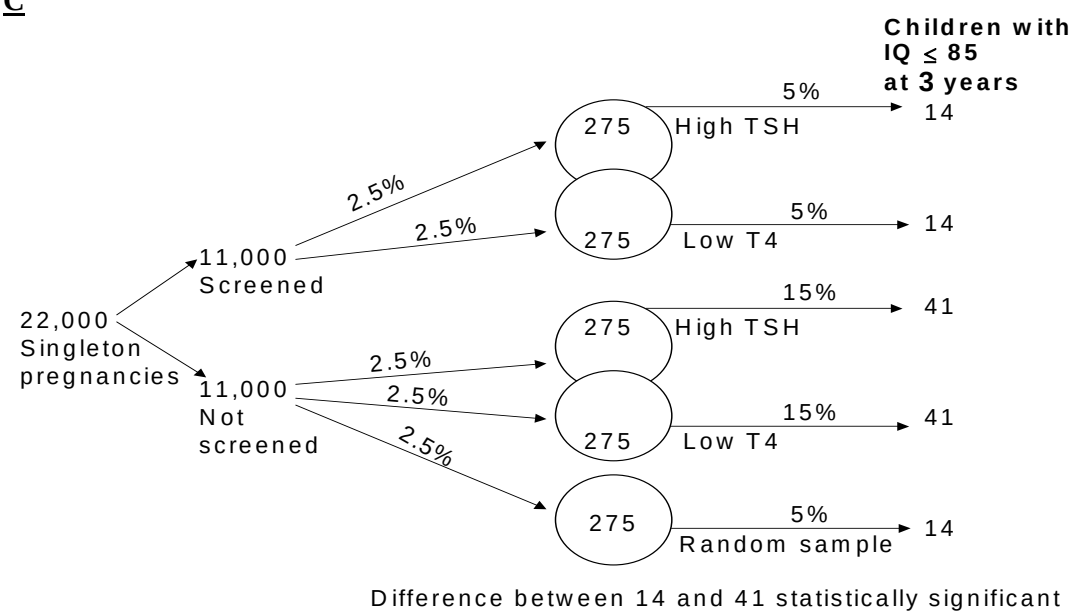
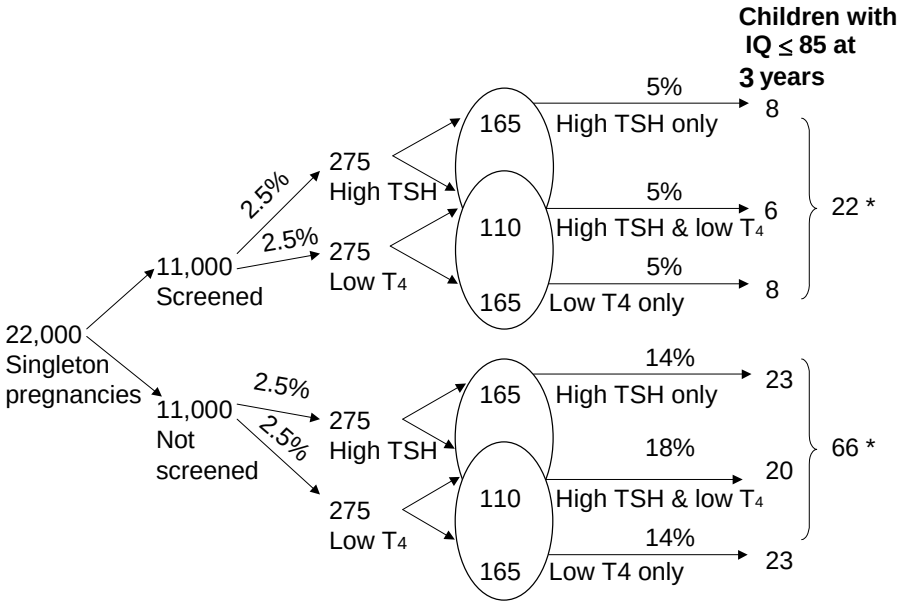


Diagram D gives a breakdown of how many cases would be expected in the high TSH, low T4 and combined categories in both the screen and control group. This has been adjusted numerically to avoid fractional numbers, but illustrates the point clearly.

D



4. Implementation

The study will be based on the recruitment of 22 000 pregnant mothers from four centres in the South Wales area and other centres willing and able to follow the protocol that would help achieve the target number of participants. They will be randomly allocated into two separate groups, a screen group and a control group. The groups will be evenly split with 11 000 mothers in each. The centres will be:

- University Hospital of Wales, Cardiff
- Llandough Hospital, Cardiff
- Royal Gwent Hospital, Newport
- Singleton Hospital, Swansea

When a woman suspects that she is pregnant, her G.P. will arrange for a pregnancy test. If it is positive the woman sees the community midwife, and she will give her a study leaflet to take away and read. At her routine week twelve antenatal booking appointment, the attending midwife will ask the mother if she has read the leaflet and wishes to participate, providing the woman is eligible for the study (see section 5 below). If she does she will be asked to sign the consent form and supply a blood and urine sample. The midwife will take the necessary booking details. The consent, blood sample and booking details are essential. The urine sample is desirable but not essential, this will be used to check for iodine deficiency at the end of the study.

A plain 7ml tube (without anticoagulants) will be used for the blood sample. A urine sample will be taken into a clean receptacle without additives.

The blood and urine samples will be refrigerated and transported to the University Hospital of Wales (UHW) with the booking form on the same day.

At UHW the blood sample will be centrifuged and separated in the Department of Medicine. Serum will be split into three serum tubes. One 0.5ml sample will be for analysis at UHW, and the other two 2ml samples for long-term storage at UHW and Wolfson Institute (WI). The storage at the WI is in case of freezer failure and for verification purposes.

A 2ml urine sample will be stored at UHW in an identifiable long-term freezer location.

The serum and urine samples will be labelled with identical UHW barcodes. Data from the booking form will be entered into the study computer, which will allocate the woman to the screen or control group. A box on the booking form will be ticked to indicate whether the woman is in the screen group or control group.

The UHW screen group serum samples will be tested for TSH and FT4 levels immediately and the results fed back to the study PC. High TSH and/or low T4 will be identified by the computer. The cut-offs for these values will be set from a pilot study and adjusted during the study if necessary.

The WI frozen serum samples will be sent periodically on dry ice to the WI. They will be accompanied by a floppy disk or CD generated by the PC system at UHW. This will contain an extract of the sample details (name, date of birth, sample date and UHW barcode number), which will be linked to a storage location code at the WI to enable relabelling and storage of the samples. These data will be fed into the computerized laboratory system for keeping track of samples and the samples stored appropriately.

Women with high TSH and/or low FT4 in the screen group will be given appointments for them to attend a clinic for treatment. The corresponding GPs for these women will be sent letters generated by the PC.

At the clinic the doctor will arrange for the woman to receive T4 (dose will be 0.15mg of T4 each day to be taken orally in tablet form). The woman will be asked to attend again 6 weeks following the initial clinic visit and again at 30 weeks gestation to check the dosage and progress. This will also happen six weeks after delivery, to see if the woman needs further treatment. Blood samples will be taken at these visits to monitor TSH and FT4 levels. Paper copies of their results will be entered into the PC. These will include details of the mother's compliance or otherwise with the protocol. PC listings will be produced on a regular basis to indicate when mothers are due for their four and six month checkups.

Birth notification will be made available to the study centre and details entered into the PC for women in the high TSH and/or low T4 screen group and all those in the control group (whose bloods require analysis post delivery).

The PC on a regular basis will produce a list of women in the screen group for whom a birth notification form has been received, or who are past their Estimated Delivery Date. This will be sent to the Cardiff Study Office to arrange appointments with the mothers for their week six postnatal check-up.

A regular list of women in the control group who are past their Estimated Delivery Date

will be produced by the PC so that the stored antenatal samples can be assayed for TSH and FT4. The results will be put into the PC. Women with high TSH and/or low FT4 levels will be identified, and a random sample of women with normal TSH and FT4 levels. The women in the control and treatment groups will be contacted for their 6 weeks postnatal appointment. The random sample group of control women will be told that they are in this group, and will be contacted in 3 years time for psychological testing.

In respect of the following women:

- High TSH and/or low FT4 in the screen group.
- High TSH and/or lowFT4 in the control group.
- Random controls in the control group.

The PC will generate a list of children who are about to attain three years of age. A psychologist will visit these children at their home and conduct an intelligence test using the British Ability Scales, this will take approximately 90 minutes. Again, the psychologist will be told to avoid finding out which group the mother falls into. The written results will be entered into the study PC.

The study will terminate when 22 000 mothers have been recruited. This is expected to take two years. The statisticians will analyze the data primarily on an intention-to-treat basis and secondarily on an on-treatment basis and the findings will be published and discussed at professional meetings.

If the parents wish to know the results of the IQ test, they may be informed after the test at three years of age.

5. Eligibility

In order for a pregnant mother to participate in the study she must satisfy the following criteria:

1. Not already receiving thyroxine or anti-thyroid drug therapy.

2. Not suffering from an existing medical condition that contraindicates the administration of thyroxine.

3. Consents to participation in the study.

4. Attends before sixteenth weeks of gestation.

5. A blood sample is successfully obtained at the booking clinic.

Multiple births will be excluded from the analysis. This cannot be applied as an exclusion criterion as it will not be known whether there are multiple foetuses at the week twelve booking (not all centres perform an ultrasound scan). Women with multiple pregnancies may therefore receive T4, but the children will not undergo psychological testing.

6. Consent

Consent will be sort at the booking antenatal visit and recorded on the booking form. This will be kept in the Cardiff study office.

7. Recording Data

All data will be collected on forms and input to the study PC at UHW by the study secretary. There will only be one PC.

8. Serum TSH and T4 Measurements

These measurements will be performed at the UHW laboratory.

A woman will be defined as positive for the purposes of the study if she has a high TSH level and/or a low FT4 level. A high TSH is defined as the top 2.5%, and low FT4 as the bottom 2.5%. These values will be refined as the study progresses.

9. Urine Measurements

The urine samples from the positive women will be tested for iodine to determine the distribution of urinary iodine in this population, and identify the prevalence of iodine deficiency.

10. Assessment of Compliance

Treatment compliance will be monitored at the clinic.

11. Follow-up

Follow-up will continue until the children have reached their 5^{th} birthdays and the psychological tests are complete.

12. Ethical Approval

Ethical approval has been obtained from the Local Research Ethics Committee (Panel D) of the Bro Taf Health Authority, NHS Cymru, Wales and from the relevant Ethics Committees in Newport and Swansea and Multiple Research Ethics Committee.

13. Data Collection

The data will be collected on paper forms and input to the study PC at the Cardiff Study Office. The paper forms will be stored.

14. Data Analysis

The data will be analyzed primarily on an "intention-to–treat" analysis, i.e. all cases will be included whether they observe the protocol or not. This is considered to be the most impartial analysis. There will also be a secondary "on-treatment" analysis where cases that violate the protocol are excluded.

Any data that is put into the public domain will be anonymised to protect patient confidentiality.

15. Data Monitoring

The data will be periodically transferred from the study PC at UHW to the WI where it will be checked for validity and interim analysis performed where possible to ascertain the probable direction of the study.

16. References

Haddow JE, Palomaki GE, ALLan WC et al. Maternal thyroid deficiency during pregnancy and subsequent neuropsychological development of the child. N Eng J Med 1999;341:549-555

Protocol Amendment

In August 2004 a decision was made by all the investigators to perform IQ and other psychological assessment of children once at age 3 instead of twice at age 2 and age 5. Mothers were informed, by letter, of the protocol change in October 2004.

Controlled Antenatal Thyroid Screening Study (CATS)

Statistical Analysis Plan

1) Aim

Randomised trial to investigate whether treatment of pregnant women with sub-clinical hypothyroidism (judged as having thyroid stimulating hormone [TSH] > 97.5th centile and/or thyroxine [FT4] < 2.5th centile, measured during the late first or early second trimester) prevents impaired cognitive function in the children at age 3.

2) Randomisation

Eligible women who agree to participate at their first antenatal visit will provide a blood sample for TSH and FT4 measurement. Upon laboratory receipt of the sample women will be randomised by computer generated block design to either a screen or control group.

3) Blinding

Women randomised to the screen group will have their blood sample assayed for TSH and FT4 immediately. Doctors of screen positive women will be asked to prescribe 150mcg of levo-thyroxine. Women randomised to the control group will have their blood sample assayed for TSH and FT4 shortly after delivery. IQ will be assessed in the children of screen and control group women with positive TSH and/or FT4 results at age 3. Psychologists performing IQ assessments will be blinded as to whether the mother of the children being assessed received treatment.

4) Statistical analysis

(i) Baseline characteristics
In screen positive women, measurements of TSH and FT4, as well as other characteristics such as maternal age, will be summarised using the mean and standard deviation or median and inter-quartile range if the data are non-Gaussian. Comparisons between screen and control group women will be made using t-tests or Wilcoxon rank sum tests as appropriate. Categorical variables will be summarised using percentages and screen and control groups will be compared using the Chi-squared test.

(ii) IQ standarisation
Due to the study taking place in Britain and Italy and there being more than one psychologist assessing IQ of the children, to allow for differences in language and psychologists' test interpretation, IQ scores will be standardised by adding or subtracting the difference in mean control group IQ from 100 to all IQ scores tested by that psychologist.

(iii) Primary outcome

The mean standardised IQ scores in screen and control groups will be summarised using the mean and standard deviation and compared using a t-test. The proportion with IQ<85 in screen and control groups will be compared using a chi-squared test. At different IQ cut-offs (for example 80, 90, 95) the proportions with IQ scores less than the specified cut-offs will be compared using relative risks with 95% confidence intervals. This will be based on an intention-to-treat analysis. A similar on-treatment analysis will also be performed assuming at least a 10% decrease in TSH level, and at least a 10% increase in FT4 level measured at a check-up 6-weeks after initial blood sampling indicating compliance.

P-values will be two-tailed and differences in means and proportions between screen and control groups will be presented with 95% confidence intervals.