Subclinical hypothyroidism: a 'laboratory-induced' condition?

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Abstract

Objective: In current literature and guidelines, there is a tendency to define absolute TSH concentrations at which patient follow-up or even pharmaceutical intervention should be initiated. As TSH concentrations depend on the analytical method/platform used for TSH quantification, absolute cut-off values may pose threats for uniform clinical decision-making. In this study we therefore set out to clarify to what extent the method/platform and the reference values applied for TSH influence the clinical interpretation of thyroid parameters.

Design and methods: We retrospectively analyzed anonymous TSH results from the Dutch external quality assessment program (EQAS) in relation to reference values advised by different manufacturers. We also examined TSH/free thyroxine (fT₄) reference ranges and prevalence of thyroid pathology among different Dutch laboratories, including four cases in which a switch in the measuring platform was made.

Results: Our data show that interpretation of thyroid parameters is not only influenced by between-method/platform variation, but is also substantially affected by the variation in TSH/fT₄ reference intervals applied in individual laboratories. Additionally, we show that the transition to a novel analytical method/platform can result in a shift in the prevalence of thyroid pathology, especially for subclinical hypothyroidism.

Conclusions: Subclinical hypothyroidism can be a 'laboratory-induced' condition. This is an undesirable situation in regard to the clinical implications such a diagnosis can have for patients.

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Introduction

Biochemical evaluation of thyroid function involves the determination of plasma thyroid stimulating hormone (TSH) and free thyroxine (fT₄) concentrations. Subclinical thyroid disease is generally defined as the combination of an fT₄ concentration within the normal range with a TSH concentration outside the TSH reference interval. The clinical significance of these subclinical conditions is not clearly defined, which has led to discussion on whether asymptomatic patients suffering from these conditions should be monitored over time or even be treated (1). Subclinical hypothyroidism, for example, is a common biochemical finding with an estimated prevalence of 5–10%

in the general population (1). There is currently debate on whether fT₄ supplementation would be appropriate in this condition, as this therapy is suggested to have a positive effect on quality of life and cognitive function, and also on improving cardiovascular and lipid profile outcomes (1, 2). However, the randomized control trials that rendered these findings covered small, inhomogeneous study populations, indicating that these results should be interpreted with caution (3). Currently, an adequately powered randomized control trial is ongoing, which will hopefully resolve the uncertainties regarding risks and benefits of treatment in the elderly population with subclinical hypothyroidism

(the European multi-center TRUST study (2)). Also, for pregnant women, active management of subclinical hypothyroidism is advised in several guidelines based on observational studies showing adverse fetal and obstetric outcomes, even though a clear understanding of fetal response to fT_4 supplementation is absent (4).

Despite the ongoing debate regarding the optimal treatment strategy for subclinical hypothyroidism, there is a tendency in current literature and guidelines to define universal TSH 'decision points' at which fT_4 therapy should be initiated (generally at a TSH concentration ≥ 10 mU/l), or at which one should consider a confirmatory TSH measurement at a second time point to substantiate the diagnosis of subclinical hypothyroidism (1, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13). Definition of these universal TSH decision points is aimed to standardize the management of thyroid disease with the ultimate goal of improving patient care. However, thyroid biology itself partly argues against the delineation of universal TSH cut-off points, since each individual appears to have a specific, partially genetically determined set-point for the hypothalamic–pituitary–thyroid axis (14, 15). Also, a literature review by Kaptein *et al.* (16) pointed out that in chronically ill patients, isolated TSH elevations do not seem to indicate mild thyroid failure but are secondary to the underlying condition. From a laboratory point of view, the definition of universally applicable TSH-thresholds seems questionable as well, considering the inter-platform variation between TSH assays. As each manufacturer produces distinct TSH-recognizing antibodies, variation in TSH-epitope recognition and binding-affinity is to be expected, which is also influenced by post-translational modifications of TSH (17). Apart from this between-method variation, the TSH and fT_4 reference intervals applied by an individual laboratory are of crucial influence in the correct clinical classification of thyroid conditions. Taking this into account, the diagnosis of subclinical thyroid disease bears the hypothetical risk of being highly laboratory-dependent, possibly translating into a divergence in follow-up strategy or, when TSH exceeds the level of 10 mU/l, even in a treatment regime for patients with subclinical hypothyroidism. In this study, we have therefore aimed to shed light on the actual situation in the Dutch laboratory landscape regarding the clinical interpretation of thyroid laboratory parameters.

Subjects and methods

Data collection

Anonymous TSH data from the 2013 annual review of the Dutch national EQAS were provided by the Dutch

Foundation for Quality Assessment in Medical Laboratories (SKML). All 128 participating laboratories measured a set of 12 quality controls. These are human-based serum samples, six of which are spiked with TSH (from human pituitary glands; ICN Biomedicals, Aurora, Ohio, USA). These samples are stored at -80°C and transported in a frozen state to the participants. For each laboratory, the means of the lowest six and the highest six results were calculated and plotted in a graph together with the method specific reference values as provided by the manufacturer (Fig. 1). Actual applied TSH and fT_4 reference intervals from Dutch laboratories were obtained via personal communication and an online survey. In total, 27 laboratories provided the reference values for TSH and fT_4 used in their institution.

To estimate the prevalence of subclinical thyroid states, nine laboratories provided an actual anonymized set of laboratory results measured over 1 year. Four laboratories

Figure 1

EQAS annual TSH-performance data from 128 Dutch laboratories, in relation to the TSH reference ranges advised by the manufacturers. (A) URL and (B) LRL are shown for different measuring platforms as alternating grey and white bars; black dots represent cumulative performance of a single laboratory on a mean TSH LRL/URL level. Platforms are coded as follows: 1, Abbott Architect ($n=15$); 2, Siemens Advia Centaur ($n=7$); 3, Siemens Immulite ($n=7$); 4, Perkin Elmer ($n=1$); 5, Roche Cobas ($n=68$); 6, Beckman Coulter Access ($n=22$); 7, VIDAS ($n=2$); 8, Siemens Dimension Vista ($n=6$).

also provided an historical dataset measured with their previous measuring platform. All orders containing either a TSH result or a TSH result in combination with an fT_4 result were provided. Data were not corrected for age, sex, previous history of thyroid or other disease or concurrent use of medication. Datasets contained between 24000 and 41000 patient results each, as measured over a period of 1 year.

Reflex-testing bias correction

For specific patient categories, some laboratories use a diagnostic approach in which the fT_4 concentration is only measured if the TSH concentration falls outside the reference interval (also referred to as 'reflex-testing'). With using such an approach, the prevalence of thyroid conditions is biased towards non-euthyroid states. We could correct for reflex-testing by hypothesizing that in the dataset containing all TSH concentrations, a similar incidence of high, normal or low TSH concentrations should be present as in the dataset containing orders in which both TSH and fT_4 were known. This approach assumes that a TSH concentration within the reference limits without a measured fT_4 concentration reflects an euthyroid state. In practice, this correction led to an increase in the percentage of normal results (both TSH and fT_4 within reference ranges) and a leveled down-correction of the prevalence of (sub)clinical thyroid disease.

Results

TSH-performance in the Dutch EQAS in relation to clinical interpretation

In Fig. 1, the results of the Dutch EQAS are depicted in relation to the manufacturer-provided reference ranges. As shown in Fig. 1B, in the lower TSH concentration range, a substantial method-specific bias was observed, indicating lack of harmonization between different measuring platforms. However, the TSH lower reference limits (LRL) differed accordingly, largely compensating for the method-dependent differences in TSH concentrations. Therefore, the interpretation of TSH levels around the LRL is less influenced by the method used than the absolute differences in TSH concentrations might suggest. In the higher TSH concentration range, on the other hand, the opposite is seen. As shown in Fig. 1A, all methods measure approximately the same TSH concentration, but the manufacturer-provided upper reference limits (URLs) show large differences between methods. The application of different TSH URLs for similar TSH results will lead to a

divergence in the clinical interpretation of thyroid status. For example, a TSH concentration of 4.5 mU/l indicates euthyroidism for Abbott Architect and Beckman Coulter Access, while based on Siemens Immulite or Roche Cobas (subclinical), hypothyroidism would be diagnosed.

Inventory of Dutch platform/reference interval combinations for TSH and fT_4

Although reference values are provided by the manufacturer, as shown in Fig. 1, this does not necessarily implicate that laboratories use these values in daily practice. Therefore, we wanted to correlate the EQAS data with the reference values actually applied by different laboratories. As the EQAS data were anonymized, we could not trace TSH results from individual laboratories back to the actual reference limits in use. To assess the variation in TSH reference limits within and between platforms, 27 Dutch laboratories voluntarily provided their TSH/ fT_4 reference ranges. As expected, a large variation in reference ranges was observed between the five different methods used by these laboratories. However, large differences (up to 37%) within the same method group were also seen, for example for the TSH URL (Fig. 2). Combined with the between-method differences shown in Fig. 1,

Figure 2

Variation in TSH URLs for different measuring platforms. Boxplots show quartiles, medians (fat black lines) and extreme values (circles) for Roche Cobas ($n=15$), Siemens Immulite ($n=4$), Abbott Architect ($n=3$) or Beckman Coulter Access ($n=5$) users. Dashed black lines indicate the TSH URL advised by the manufacturer.

these findings imply that the clinical assessment of thyroid function is largely dependent on the laboratory performing the TSH measurements, as TSH concentrations and the reference intervals applied show substantial variation.

Comparison of the prevalence of (sub)clinical thyroid disease in nine Dutch laboratories

In order to analyze the estimated prevalence of (sub)-clinical thyroid conditions in Dutch laboratories, we investigated all TSH/ fT_4 concentrations measured over the course of 1 year by nine different laboratories. All TSH and fT_4 results were included in this analysis, regardless of patient age, sex, medication or previous history of thyroid or other disease. As shown in Fig. 3A, the variation in TSH/ fT_4 reference limits indeed translates into a high inter-laboratory variation in the prevalence of (sub)clinical thyroid pathology, both between as well as within methods. For example, Beckman Coulter Access or Siemens Immulite users reported a lower prevalence of subclinical hypothyroidism than Roche Cobas users (4.6, 7.6 and 14.3% respectively). The prevalence of subclinical hyperthyroidism, on the other hand, was highest for the single Abbott Architect user included (15.5% vs an overall average of 6.6%). Apart from these inter-platform differences, striking differences were also found between individual users of the same platform. For example, individual Roche Cobas users found a prevalence of subclinical hypothyroidism cases ranging from 11.8 to 17.8%; for a diagnosis of subclinical hyperthyroidism, the range was 3.4% to 11.2%.

Implications of a platform-switch on the incidence of (sub)clinical thyroid disease

The inter-platform differences that came up in our analysis outlined above suggest that the assessment of thyroid function is largely dependent on the laboratory performing the TSH and fT_4 measurements. However, individual laboratories accommodate different patient populations that harbor different distributions of thyroid pathology. To evaluate this, we compared the prevalence of thyroid conditions before and after a change in TSH/ fT_4 measuring platform for four different laboratories. Assuming that the diversity in patient population, and therefore the prevalence of thyroid disease, is identical before and after the platform change, potential shifts would have to be attributed to the differences in method and reference intervals applied.

Figure 3

Prevalence of (sub)clinical thyroid dysfunction in nine Dutch laboratories. Percentages were calculated based on the TSH and fT_4 concentrations measured over 1 year and were corrected for reflex-testing associated bias (see 'Materials and methods' section). Black represents the percentage of subclinical hypothyroidism, light grey the percentage of hypothyroidism, white the percentage of subclinical hyperthyroidism and dark grey the percentage of hyperthyroidism. The remainder of patients was euthyroid (not shown as bars). The prevalence of thyroid pathology is (A) categorized according to the platform used (upper numbers represent specific laboratory/analyser combination, lower numbers represent measuring platform used: 1, Roche Cobas; 2, Siemens Immulite; 3, Abbott Architect; 4, Beckman Coulter Access; 5, Advia Centaur) (B) shown for four actual cases involving a switch in measuring platform (coding similar as in Fig. 3A).

As shown in Fig. 3B, considerable changes in the prevalence of different thyroid conditions indeed were detected after a platform switch. For example, in the laboratory that changed from Siemens Immulite to

Figure 4

Illustration of TSH/ fT_4 year-results before and after a platform switch. Patient TSH/ fT_4 results (within the 70th percentile) measured with the Siemens Advia Centaur are outlined with a black continuous line and corresponding reference ranges are shown as black lined square. Patient TSH/ fT_4 results measured with Roche Cobas are outlined with a dashed line and corresponding reference ranges are shown as dashed lined square. Arrows indicate shifts following the platform switch.

Beckman Coulter Access, the prevalence of subclinical hyperthyroidism decreased with 3.3%, while the percentage of subclinical hypothyroidism cases did not significantly change. However, in all three laboratories that changed their method to the Roche Cobas platform, the prevalence of subclinical hyperthyroidism remained equal while a substantial increase in subclinical hypothyroidism (6.2% on average) was found. As these percentages were based on a mean number of 26 000 patient TSH results per laboratory, this would mean that approximately up to 5000 additional patients per year received a diagnosis of subclinical hypothyroidism over the three laboratories concerned. As illustrated in Fig. 4, the Roche method produced higher TSH concentrations, while the TSH URL applied shifted downwards compared to the situation for the Siemens Advia Centaur. For fT_4 , no relevant differences were observed. These findings point out that the increase in subclinical hypothyroidism diagnoses following a platform switch can be laboratory-induced by the change in method and the applied reference values, especially for TSH.

Relation between the estimated prevalence of subclinical hypothyroidism and the TSH URL applied

As we found that the measuring platform and TSH URL largely influenced the prevalence of subclinical hypothyroidism, we modeled the relationship between TSH URL and the estimated prevalence of subclinical hypothyroidism for the same laboratories as shown in Fig. 3A. From this Fig. 5, two important observations can be made. First, it is clear that a hyperbolic relation exists between the TSH URL and the percentage of subclinical hypothyroidism. This means that a small change in TSH concentration near the URL can result in a substantial change in the prevalence of subclinical hypothyroidism. Second, major differences exist between measuring platforms. For example, all Roche Cobas users report a higher prevalence of subclinical hypothyroidism than Beckman Coulter Access or Siemens Immulite users. This difference does not only exist near the TSH URL but also at a level of 10 mU/l, which is frequently considered as level for pharmaceutical intervention. At this TSH level, Roche Cobas users report on average 2.5% subclinical hypothyroidism cases, while for Beckman Coulter and Siemens Immulite users, this was 1.3%. This would translate to ~300 extra patients per Roche laboratory that receive fT_4 substitution based on TSH/ fT_4 results. From Fig. 5, it can also be concluded that the three laboratories that changed to the Roche measuring platform would have to shift their TSH URL substantially to achieve a similar prevalence of subclinical hypothyroidism as before the platform switch;

Figure 5

Correlation of the estimated prevalence of subclinical hypothyroidism to the TSH URL applied. The coding of the measuring platforms is as follows: thin black lines, Roche Cobas; dashed black line, Abbott Architect; white line, Siemens Advia Centaur; fat black lines, Siemens Immulite; fat grey lines, Beckman Coulter Access.

Clinical Study	K L M Coene and others	'Laboratory-induced' subclinical hypothyroidism	173:4	504
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e.g., for laboratory 1 (coding as in Fig. 3A), this would mean a change in TSH URL from 4.0 to 6.3 mU/l, for laboratory 2 from 4.2 to 5.2 mU/l and for laboratory 3 from 4.0 to 5.1 mU/l.

Discussion

In this study, we have aimed to shed light on the current situation among different laboratories in The Netherlands regarding the clinical interpretation of TSH/ft₄ results. Our data now point out that the prevalence of thyroid pathology shows large variation between laboratories, especially for the diagnosis of subclinical hypothyroidism. Several factors attribute to this variation. First, though overall TSH is a robust measure to screen for thyroid pathology, small differences between laboratories arise in absolute TSH concentrations because different measuring platforms are used. All TSH assays are calibrated to the same international standard (NIBSC 2nd IRP 80/558); however, no perfect commutability is reached, meaning that different methods are not optimally harmonized to produce equal results. Second, different laboratories, even those using the same method, apply different reference intervals for TSH and ft₄ to clinically define thyroid status. Third, variability in the patient population analyzed could give rise to the between-laboratory differences described in our study. As an example, in Fig. 5, one Roche Cobas user stands out with a prevalence of 4.5% of subclinical hypothyroidism cases when a TSH of 10 mU/l is applied as URL. This example involves a university laboratory, in contrast to the other laboratories that were connected to a general hospital. One can imagine that a university hospital in general serves a more chronically or critically ill patient population. In these patients, an altered hypothalamic–pituitary–thyroid set point can lead to the laboratory marks of subclinical hyper- or hypothyroidism, while no actual thyroid dysfunction is present, also referred to as 'euthyroid sick syndrome' (16). The estimated prevalences of thyroid disease reported in this study were based on datasets containing TSH/ft₄ results produced over a period of 1 year, that were uncorrected for underlying disease, therefore, the influence of distinct patient populations cannot be ruled out. Nevertheless, our analysis of four laboratories that made a platform switch for TSH/ft₄ measurement shows that, apart from the composition of patient population, variation in laboratory methods and reference intervals substantially influences the prevalence of thyroid conditions. As we did not exclude known thyroid pathology, use of

thyroid-affecting medication nor presence of anti-thyroid peroxidase (TPO) antibodies from our patient datasets, we cannot draw conclusions as to which measuring platform offers the best reflection of reality. However, a laboratory-induced change in subclinical hypothyroidism diagnoses following a platform switch is undesirable in any case.

In the light of ongoing efforts to standardize the clinical management of subclinical hypothyroidism (4, 5, 6, 7, 8, 9, 10, 11, 12, 13), our study highlights the importance of including the laboratory in this process. We show that the clinical interpretation of thyroid parameters highly depends on the specific analytical method and reference interval applied. This implicates that the validation of a novel measuring platform, apart from agreement between methods, should also include verification of the compatibility of novel reference ranges to the patient population analyzed. Our findings imply that subclinical hypothyroidism can be a laboratory-induced condition. Regarding the tendency to follow up or, when TSH exceeds 10 mU/l, even offer ft₄ replacement to patients diagnosed with subclinical hypothyroidism, this is an unwanted situation. Laboratories should therefore be aware of the implications a platform switch can have on the clinical interpretation of thyroid parameters. Visualization of patient data as we show in Fig. 4 can be helpful in estimating the effects of a change in measuring platform.

In conclusion, this study emphasizes the need for (inter)national harmonization/standardization programs for correct clinical interpretation of the thyroid biomarkers TSH and ft₄. Before the standardization of clinical management of subclinical thyroid disorders can be successful, harmonization on a laboratory level is required first. The International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) has recognized this necessity and has recently initiated a project to harmonize results from different TSH and ft₄ assays (18). The inclusion of reference values and TSH-decision points in this harmonization process will allow for laboratory-independent clinical decision-making regarding thyroid disorders.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

K L M Coene wrote the manuscript and collected and analyzed the TSH/ft₄ datasets, A-K Boer designed the study and edited the manuscript to its final version, A Y Demir, M A C Broeren and P Verschuure provided TSH/ft₄ datasets before and after a platform change and edited the manuscript to its final version and E G W M Lentjes provided the anonymized EQAS data and edited the manuscript to its final version.

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