Best Practice & Research Clinical Endocrinology & Metabolism 27 (2013) 689–700

Contents lists available at SciVerse ScienceDirect

Best Practice & Research Clinical Endocrinology & Metabolism

journal homepage: www.elsevier.com/locate/beem

5

Determination of free thyroid hormones

Linda M. Thienpont, PhD, Specialist in Laboratory Medicine, Professor of Instrumental Analytical Chemistry, Statistics and Quality Control, Head of Mass Spectrometric Reference Laboratory, Chair of the IFCC Committee for Standardization of Thyroid Function Test (C-STFT) [a,*], Katleen Van Uytfanghe, PhD, Technical Supervisor of the Reference Laboratory, Scientific Secretary of the C-STFT [a,1], Kris Poppe, MD, PhD, Professor, Doctor [b,2], Brigitte Velkeniers, MD, PhD, Professor, Doctor [b,3]

[a] *Faculty of Pharmaceutical Sciences, Ghent University, Harelbekestraat 72, 9000 Gent, Belgium*
[b] *Departments of Endocrinology and General Internal Medicine, University Hospital Brussels, Free University Brussels (VUB), Laarbeeklaan 101, 1090 Brussels, Belgium*

Keywords:
thyrotropin
TSH
thyroxine
T4
tri-iodothyronine
T3
one-step labeled analog assay
one-step labeled antibody assay
two-step labeled analog assay
free T4 index
equilibrium dialysis
ultrafiltration
isotope dilution-liquid chromatography-tandem mass spectrometry

Timely diagnosis and treatment of thyroid dysfunction is compelling given the prevalence and severity of the disease. It requires reliance on adequate laboratory testing of serum TSH as a hallmark in combination with free thyroxine/triiodothyronine. Free hormone methods have to accommodate variations in the concentration and binding capacity of binding proteins. This is a challenge because none of the methodologies developed so far measures the actual unbound hormone in serum. The indirect methods provide an approximation while the direct ones estimate the free hormone concentration either in the presence of the protein-bound counterpart, or after physical separation of the free from bound fraction. The ongoing controversy on the validity and lack of comparability of methodologies points to their imperfectness to reflect real *in-vivo* free hormone concentrations. Therefore, laboratories and clinicians should know the window of

* Corresponding author. Tel.: +32 92648104; Fax: +32 92648195.
E-mail addresses: linda.thienpont@ugent.be (L.M. Thienpont), katleen.vanuytfanghe@ugent.be (K. Van Uytfanghe), kris.poppe@uzbrussel.be (K. Poppe), brigitte.velkeniers@uzbrussel.be (B. Velkeniers).
[1] Tel.: +32 92648105; Fax: +32 92648195.
[2] Tel.: +32 24763080; Fax: +32 24776820.
[3] Tel.: +32 24776418; Fax: +32 24776428.

1521-690X/$ – see front matter
http://dx.doi.org/10.1016/j.beem.2013.05.012

international conventional reference measurement procedure

validity and limitations of their methods. The recently developed reference measurement system is a key advance towards improved standardization and clinical validity of free thyroid hormone measurements.

Introduction

Thyroid dysfunction is a frequent clinical problem that affects millions of individuals. The prevalence of subclinical thyroid dysfunction is estimated to be in the order of 1–5% in individuals older than 60 years and, depending on the gender and age of the population studied it may be even higher [1]. Timely diagnosis and treatment of thyroid dysfunction is compelling given the severity of the disease. Whereas physicians can accurately establish the clinical diagnosis of overt hypo- or hyperthyroidism in patients presenting with obvious symptoms, identification of subclinical dysfunction relies on adequate laboratory tests. For the assessment and management of the thyroid function in general, in thyroid cancer, during pregnancy and postpartum in particular, integrated laboratory-clinical approaches and practice guidelines have been released [2–7]. The recommended laboratory assessment comprises testing of thyrotropin (more commonly referred to as thyroid stimulating hormone, TSH) with an assay of adequate sensitivity, but also requires consideration of the TSH-thyroxine (T4) relationship. For differential diagnosis, algorithms have been proposed that comprise testing of T4 and/or triiodothyronine (T3) and thyroid antibodies. Although preferably the free form of T4/T3 is assessed, total hormone testing finds its place to exclude clinically discordant free hormone results. For follow-up of a differentiated thyroid cancer after thyroidectomy and I-131 ablation, the diagnostic strategy includes measurement of stimulated thyroglobulin [5].

In 2008, about 59 million TSH and 18 million free T4 tests were performed in the USA, and about three times these numbers in the rest of the world. The testing volume may even increase given the fact that recent research links subclinical thyroid dysfunction to coronary heart disease and cardiovascular and all-cause mortality [8]. Also pregnancy outcome may drive the decision to screen or treat subclinical thyroid disease [9]. This underpins the indisputable value of robust laboratory tests for thyroid function. The tests must be clinically and analytically valid, so that they accurately reflect the activity of the thyroid gland and hormone concentrations, particularly the free fractions of T3 and T4. In addition, different assays should provide equivalent results to allow interpretation against common reference intervals or cut-off values. The latter requirement must, however, be put into perspective for some key thyroid hormones, for which the medical decision values necessarily depend on the population examined. For example, free T4 and TSH concentrations in young pregnant females differ substantially from those in older female patients [10–12]. Also the reference range of TSH has become a matter of non-resolved debate (e.g., [13–15]). Therefore, the establishment of appropriate reference ranges is compelling for proper interpretation of thyroid hormone results.

Scope

This review will cover approximately one decade of literature since a review by Midgley [16] and two leading monographs, i.e., from the National Academy of Clinical Biochemistry (NACB), and from the Clinical and Laboratory Standards Institute (CLSI) [17,18]. Note that the NACB meanwhile archived the aforementioned guidelines [17], but in Ref. 2 an update of some sections relevant to this review (e.g., Section III: Thyroid Tests for the Clinical Biochemist and Physician) can be found (more in particular, Chapter: Assay of thyroid hormones and related substances). Although written many years earlier, we consider that the Ekin's standard text on free thyroid hormone measurements still deserves active citation in this review [19]. We will only recapitulate the different methodologies for measurement of serum/plasma free thyroid hormone concentrations, including their potential deficiencies, because they are comprehensively described in Refs. [16–18]. In contrast, we will critically evaluate the recently proposed solutions to accommodate the known limitations. These range from the introduction of isotope dilution-liquid chromatography-tandem mass spectrometry (ID-LC/tandem MS) combined with equilibrium dialysis (ED) or ultrafiltration (UF), to the resurrection of indirect methods. We will