Clinical Biochemistry xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Clinical Biochemistry

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

Total and free thyroxine and triiodothyronine: Measurement discrepancies, particularly in inpatients

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ARTICLE INFO

Article history:
Received 27 April 2014
Received in revised form 4 June 2014
Accepted 6 June 2014
Available online xxxx

Keywords:
Free thyroxine
Thyroxine
Free triiodothyronine
Triiodothyronine
Immunoassays
Tandem mass spectrometry

ABSTRACT

Objective: We compared the performance of tandem mass spectrometry versus immunoassay for measuring thyroid hormones in a diverse group of inpatients and outpatients.

Methods: Thyroxine (T4), triiodothyronine (T3), free thyroxine (FT4), and free triiodothyronine (FT3) were measured by liquid chromatography tandem mass spectrometry and immunoassay in 100 patients and the two assays were compared.

Results: T4 and T3 values measured by the two different assays correlated well with each other (r =0.91–0.95). However, the correlation was less good at the extremes (r = 0.51–0.75). FT4 and FT3 concentrations measured by the two assays correlated less well with each other (r = 0.75 and 0.50 respectively). The studied analytes had poor inverse correlation with the log-transformed TSH values (r= -0.22– 0.51) in the population as a whole. The strongest correlations were seen in the groups of outpatients (r = −0.25−0.61). The weakest degree of correlation was noted in the inpatient group, with many correlations actually being positive.

Conclusion: The worst between-assay correlation was demonstrated at low and high hormone concentrations, in the very concentration ranges where accurate assay performance is typically most clinically important. Based on the lesser susceptibility of mass spectrometry to interferences from conditions such as binding protein abnormalities, we speculate that mass spectrometry better reflects the clinical situation. In this mixed population of inpatients and outpatients, we also note failure of assays to conform to the anticipated inverse linear relationship between thyroid hormones and log-transformed TSH.

Introduction

Thyroid hormone assays should ideally be accurate and truly reflect the concentration of analyte in the sample. Barriers to accurate measurement using immunoassays (IA) can include changes in binding proteins, presence of heterophilic antibodies, and concentration of non-esterified free fatty acids [1,2]. Such factors may account for the method-dependent variation documented in thyroid hormone measurements made during such physiologic and medical conditions as pregnancy, renal failure, non-thyroidal illness, and genetic abnormalities in binding proteins [3–7]. Discrepant values between thyroid hormone assays can be illustrated by examining the correlation between the results obtained when using different assays to assess the same sample [8]. Another means of judging the validity of thyroid hormone measurements is to examine the relationship between thyroid hormone concentration and the logarithmically transformed thyroid stimulating hormone (TSH) value, which is shown to follow a complex, but generally inverse linear relationship [8–13].

The performance of thyroid hormone assays is of importance across all range of values. However, assay performance may be particularly important at the low and high values for thyroid hormones, as it is at these two extremes that presence of thyroid disorders is more likely. Erroneous values for thyroid hormones may prevent the correct and timely diagnosis of hypothyroidism or hyperthyroidism, particularly in difficult or challenging clinical cases in which the diagnosis may be confounded by incongruent laboratory values.

Measurement by tandem mass spectrometry (MS) is accurate, precise, and more specific than immunoassays [14]. When coupled with physical separation methods it permits the reliable measurement of free thyroid hormone in any of the conditions that may result in changes in binding protein concentrations [7,14,15]. These situations include, for example, pregnancy, non-thyroidal illness, and renal disease.

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http://dx.doi.org/10.1016/j.clinbiochem.2014.06.007
0009-9120/

Table 1
Tandem mass spectrometry method for FT4 and FT3.

1a. Mass spectrometer gradient parameters

	Time, min	Solvent A, %	Solvent B, %
Cleaning	0.00	70	30
	1.00	70	30
	2.00	54	46
Elution	3.50	30	70
	4.00	30	70
	4.01	10	90
	5.00	10	90
	5.01	70	30
	7.00	70	30

1b. MRM conditions for FT3, FT4 and internal standards in negative ion mode

Compound	MRM transition	DP	EP	CE	CXP
FT3	649.7/126.8	−109	−10	−94	−15
T3-6C13	655.5/126.9	−110	−10	−99	−7
FT4	775.5/126.8	−90	−10	−110	−17
T4-d_5	780.7/126.8	−99	−10	−90	−17

1c. Tandem mass spectrometer working parameters

Parameter	Value
Collision gas (CAD)	High
Curtain gas (CUR)	30
Gas 1 (GS1)	35
Gas 2 (GS2)	65
Ionspray voltage, V	−4500
Probe temperature, °C	650
Dwell time, ms	250

Solvent A: 0.05% formic acid in 2% (v/v) methanol/water.
Solvent B: 50% (v/v) methanol/acetonitrile.

We were therefore interested in the performance of these two assay methodologies in samples from a diverse group of patients. The goal of this analysis was to examine in the same sample analyte values generated by IA compared with the values generated by MS with particular attention to thyroid hormone concentrations in the lower and higher concentration ranges.

Methods

Patient eligibility and recruitment

One hundred patients were recruited for this study. Patients were recruited from the outpatient clinics and inpatient services at Georgetown University and Medstar Washington Hospital Center. This protocol had been approved by the Institutional Review Board at both institutions. Individuals of any age with any medical diagnoses were included in order to capture conditions that could potentially affect assay performance. All patients signed a written informed consent form and donated a single blood sample. The status of each patient as an inpatient or outpatient was recorded.

Assays used in study

Blood was collected in red top tubes without serum separator, as the latter has been shown to inhibit signal by 80–90% in methods employing ESI ionization [16]. All sera were stored at −80 °C, at which temperature they had excellent stability, and analyzed in one batch for each assay at the completion of the study. The thyroid hormone and related analytes measured by MS were thyroxine (T4) and triiodothyronine (T3) in one mass spectrometry method, and free thyroxine (FT4), and free triiodothyronine (FT3) in another mass spectrometry method. These analytes were also measured by IA.

T4 and T3 by MS

This method, instrumentation, conditions, and working parameters have been previously described [17,18]. An API-5000 tandem mass spectrometer equipped with TurbolonSpray source and Shimadzu HPLC system was employed. 100 μL of patient serum was deproteinized by adding 150 microliters (μL) of acetonitrile containing labeled internal standards. The supernatant was diluted with 500 μL deionized water and a 300 μL aliquot was injected onto an Agilent ZORBAX SB-C18 column (2.1 × 30 mm 1.8 μm). After washing the column for 4.5 min with mobile phase A (0.01% formic acid in 98% water, 2% methanol) at a flow rate of 0.25 mL/min, the switching valve was activated and the analytes of interest were eluted into the mass spectrometer with a gradient of 35% mobile phase B (methanol with 0.01% formic acid) to 64% B in 2.5 min before equilibration for the next injection. Quantification by multiple reaction-monitoring analysis was performed in the positive mode. The ESI source was operated with ionspray voltage at 5500 V and heated temperature at 650 °C. Gas settings were as follows: curtain gas 35, collision gas 4, nebulizer and heated gas 50. Retention time and ion pair for each analyte, their internal standards and compound-dependent parameters are listed. T4 (9.4, m/z 777.9/634.3), T4$^{13}C_6$ (9.4, m/z 783.8/640.2) DP = 120, CE = 37, CXP = 21. T3 (8.83, 9.2, m/z 651.9/606.1), T3-$^{13}C_6$ (8.83, 9.2, m/z 657.9/612.1) DP = 120, CE = 29, CXP = 13.

The within-day coefficients of variation (CVs) were <8.9% and between day CVs were between 1.6% and 7.6%. Recovery ranged from 92.8% to 95.4%. The lower limits of detection were 1.93 nmol/L for T4 and 0.015 nmol/L for T3. Normal reference intervals for females and males (Gaussian method on 130 females and 130 males) for each of the analytes are: T4 54.00–140.00 and 60.40–131.00 nmol/L; T3 1.15–2.60 and 1.29–2.65 nmol/L respectively [19].

FT4 and FT3 by MS

Method. This method was performed in the Department of Laboratory Medicine at the National Institutes of Health, Bethesda, MD. The

Table 2
Within-analyte, between-assay correlations.

	Analyte 1	Analyte 2	Spearman correlation coefficient (r)	P	Slope and Y intercept
Across entire range	T3 IA	T3 MS	0.95	<0.01	y = 1.1249x + 34.13
	T4 IA	T4 MS	0.91	<0.01	y = 1.0806x + 1.23
	FT4 IA	FT4 MS	0.75	<0.01	y = 0.4891x + 0.46
	FT3 IA	FT3 MS	0.50	<0.01	y = 0.3180x + 194.59
Below reference interval	T3 IA	T3 MS	0.72	<0.01	y = 0.8612x + 45.08
	T4 IA	T4 MS	0.75	<0.01	y = 2.9194x − 3.27
	FT4 IA	FT4 MS	0.37	<0.01	y = 0.3701x + 0.56
	FT3 IA	FT3 MS	0.01	0.85	y = 0.0339x + 190.67
Above reference interval	T3 IA	T3 MS	–	–	–
	T4 IA	T4 MS	0.51	<0.01	y = 1.0424x + 1.91
	FT4 IA	FT4 MS	0.38	<0.01	y = 0.1552x + 1.41
	FT3 IA	FT3 MS	–	–	–

Please cite this article as: Jonklaas J, et al, Total and free thyroxine and triiodothyronine: Measurement discrepancies, particularly in inpatients, Clin Biochem (2014), http://dx.doi.org/10.1016/j.clinbiochem.2014.06.007