Clinical Biochemistry xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Clinical Biochemistry

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

Assessment of thyroid function in intensive care unit patients by liquid chromatography tandem mass spectrometry methods☆,☆☆

Kerry J. Welsh, Brian R. Stolze, Xiaolin Yu, Trisha R. Podsiadlo, Lisa S. Kim, Steven J. Soldin *

Department of Laboratory Medicine, National Institutes of Health, Bethesda, MD 20892, United States
(SJS) Departments of Endocrinology and Metabolism, Georgetown University, Washington, DC, United States

ARTICLE INFO

Article history:
Received 21 October 2016
Received in revised form 17 November 2016
Accepted 19 November 2016
Available online xxxx

Keywords:
Free thyroid hormone
Total thyroid hormone
LC-MSMS
Immunoassay

ABSTRACT

Objectives: Patients with non-thyroidal illness syndrome have many abnormalities in thyroid hormone tests. Such patients have medical comorbidities associated with low serum proteins and are on multiple medications that interfere with thyroid hormone measurement by immunoassay platforms. It is unknown if these thyroid hormone measurements reflect physiologic conditions or if they are artifacts of testing methodology.

Methods: Fifty patients were selected from the intensive care unit (ICU) from our institution. Total and free thyroid hormones in plasma were measured by gold standard liquid chromatography-tandem mass spectrometry (LC-MSMS). The results were compared to the Roche Cobas 6000. Patient medical comorbidities and binding protein levels were assessed.

Results: Concentrations of total 3,5,5′-triidothyronine (TT3) and total thyroxine (TT4) were significantly more likely to be low by LC-MSMS compared to immunoassay. Free 3,5,5′-triidothyronine (FT3) levels were similar by immunoassay and LC-MSMS. However, FT4 concentrations were mildly elevated for many patients when measured by ultrafiltration LC-MSMS (19/50, 38%) compared to 1/50 (2%) when measured by immunoassay ($p = 0.0001$). Decreased albumin and thyroxine binding globulin were common and patients were on an average of 11.7 ± 5.0 medications, all factors known to interfere with results found on immunoassays.

Conclusions: Marked discrepancies in thyroid hormone measurement were noted between reference LC-MSMS and a common immunoassay platform. It is hypothesized that T4 binding to low affinity albumin is displaced by several drugs, raising concentrations of FT4 by LC-MSMS compared to immunoassay, and that the immunoassay values are falsely decreased due to low binding proteins in our patient population.

1. Introduction

Hospitalized patients suffer from a number of medical conditions and receive many medications that cause typical changes in thyroid function tests. These changes are referred to as “non-thyroidal illness” or the “euthyroid sick syndrome.” Patients with non-thyroidal illness are reported to have decreased total 3,5,5′-triidothyronine (TT3) and free 3,5,5′-triidothyronine (FT3), increased reverse 3,5,5′-triidothyronine (rT3), and may have normal or decreased total thyroxine (TT4) and free thyroxine (FT4) [1] when measured by immunoassay. Thyroid stimulating hormone (TSH) is usually normal, and thus these patients are considered “euthyroid.” These changes in thyroid status are reported to occur in over 70% of hospitalized patients [1]. The marked abnormalities in thyroid hormone tests that occur in non-thyroidal illness are generally considered to reflect a response to systemic illness rather than clinical thyroid dysfunction, and treatment with thyroid hormone is currently not recommended by most Endocrinology organizations including the American Thyroid Association [2,3]. Furthermore, routine testing for thyroid function in hospitalized patient in the absence of suspected thyroid disease is not recommended by most guidelines.

Thyroid hormones are measured by automated immunoassay platforms in the majority of clinical laboratories. However, a number of studies have shown discrepancies in free and total thyroid hormone measurement by immunoassay when compared to a reference method such as liquid chromatography-tandem mass spectrometry (LC-MSMS) in several populations (reviewed in [4,5]). Multiple studies have shown falsely normal values for TT3, FT3, and FT4 by immunoassays that are below the reference interval when measured by reference LC-MSMS in a number of patient populations [3,6–8]. Furthermore, several disease and physiologic conditions are known to cause interference in thyroid hormone measurement by immunoassay. Specifically, changes in the thyroid hormone binding proteins thyroxine-binding globulin (TBG)

☆ Declaration of interests: The authors have no conflicts of interest that could be perceived as prejudicing the impartiality of the research reported.

☆☆ The authors are supported by the National Institutes of Health Intramural Research Award.

* Corresponding author at: Department of Laboratory Medicine, NIH Clinical Center, Building 10, Room 2C-249, Bethesda, MD 20892, United States.
E-mail address: steven.soldin@nih.gov (S.J. Soldin).

http://dx.doi.org/10.1016/j.clinbiochem.2016.11.022
0009-9120/

and albumin that occur in a number of conditions cause unreliable immunoassay free thyroid hormone measurements [9,10]. Many commonly used drugs such as heparin, furosemide, anti-epileptics, and salicylates displace thyroid hormone binding from serum proteins [11–13]; immunoassays that use a dilution step cause a reduction in competing drugs, resulting in increased *in vitro* free thyroid hormone binding to proteins and a falsely decreased measure of free thyroid hormone [4]. Hospitalized patients, especially those in intensive care units (ICU), have a number of conditions that cause low protein states and are frequently on a large number of medications known to cause artifactual values in thyroid hormone assays. Thus, it is not clear if the abnormalities seen in non-thyroidal illness reflect physiologic conditions or if they are at least partially artifact due to analytical issues.

To our knowledge, no study has extensively studied thyroid hormone measurement by gold standard, validated LC-MSMS methods in a population of ICU patients. This study evaluates plasma FT3, FT4, TT3, TT4, and rT3 determined by reference method LC-MSMS in a cohort entirely of ICU patients. The results are further compared to a common automated immunoassay platform. Marked discrepancies were found by the immunoassay method in this population with multiple medical comorbidities, low binding proteins, and on multiple medications. These results have important implications for clinical studies evaluating patients with non-thyroidal illness syndrome.

2. Methods

2.1. Patient population

The Clinical Center at the National Institutes of Health is a 200 bed research facility. Fifty patients were selected from the ICU who were hospitalized for at least seven days between May 2016–July 2016. Patients were excluded if they had known thyroid disease, were receiving thyroid hormone replacement therapy, or if they were positive for antithyroperoxidase antibodies. Clinical characteristics including demographics, length of hospitalization, mortality, and number of medications were abstracted from the electronic medical record. This study was approved by the Institutional Review Board (Clinical protocol number 93-CC-0094).

2.2. Laboratory methods

Plasma samples were collected in lithium heparin tubes. Samples were stored at −80 degrees Celsius until analysis by LC-MSMS and immunoassay. The Roche Cobas 6000 (Indianapolis, IN) analyzer was used to measure TSH, FT3, FT4, TT3, TT4, albumin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, bilirubin, and creatinine. Hemoglobin was measured on a Sysmex XN-3000 (Lincolnshire, IL). Thyroid hormone reference intervals for immunoassays are 0.27–4.20 μIU/L for TSH, 2.0–4.4 pg/mL for FT3, 0.9–1.7 ng/dL for FT4, 80–200 ng/dL for TT3, and 4.5–11.7 μg/dL for TT4 as suggested by the manufacturer and verified by the laboratory. TBG and anti-thyroperoxidase antibodies were measured on the Siemens Immulite 2000 XPI analyzer (Malvern, PA).

FT3 and FT4 by LC-MSMS was performed by ultrafiltration isotope dilution LC-MSMS using a SCIEX Triple-Quad-6500 System (Framingham, MA) as previously described [7]. Briefly, 400 μL of plasma was filtered through a Centrifree YM-30 ultrafiltration device by centrifugation at 37 °C. Two-hundred fifty microliters of internal standard (T4-^{13}C6) in methanol was then added to 150 μL of ultrafiltrate for deproteinization. After vortexing and centrifugation, 325 μL of supernatant was diluted into 675 μL of deionized water and a 400 μL aliquot was injected onto a Poroshell 120 EC-C18 column. After washing, the switching valve was activated and the analytes were eluted from the column with a water/methanol gradient into the MS/MS system. Quantification by multiple reaction monitoring analysis was performed in the negative mode. Two levels of internal quality control were analyzed at the beginning and end of each run. Complete method validation details have been previously published [7,14]; recovery for FT3 and FT4 are between 95 and 105% and the intra- and inter-assay coefficients of variation are <9% for FT3 and <7% for FT4. The reference interval for FT3 is 2.0–6.0 pg/mL and 1.3–2.4 ng/dL for FT4 [14].

TT3, TT4, and rT3 were assayed by LC-MSMS using an Agilent 6460-Triple-Quad System as previously published [15]. Briefly, 100 μL of sample was added to 150 μL of 13C labeled internal standard for deproteinization. After vortexing and centrifugation, 200 μL of supernatant was diluted into 500 μL of 0.1 M ammonium acetate in deionized water. Two-hundred microliters was injected onto an Agilent Eclipse XBD-C8 cartridge column. After washing, the switch valve was activated and the analyte was eluted with a water/methanol gradient containing 0.01% formic acid into the MS/MS system. Quantification by multiple reaction mode monitoring was performed in the positive mode. Method validation details have been previously described [15,16]; recovery ranged from 92.8% to 95.4% and the intra-assay coefficient of variation is between 1.6% to 7.6%. The reference interval for TT3 by LC-MSMS is 80–177 ng/dL, 5.0–10.9 μg/dL for TT4, and 9–24 ng/dL for rT3 [17].

2.3. Statistical analysis

Analyses were performed using GraphPad Prism version 6 (GraphPad Software, La Jolla, CA).

3. Results

Characteristics of the patient population are shown in Table 1. The patient age range was 21–74 years, and 18.0% of patients died in the hospital. Length of hospitalization ranged from 7 to 252 days. Medical comorbidities were frequent, and the majority of patients were on substantial numbers of medications, ranging from 3 to 27. Decreases in binding proteins were found in most patients; 34/50 patients (68%) had albumin levels below the reference interval (3.5–5.0 g/dL) and

Table 1
Clinical characteristics of 50 intensive care unit patients evaluated for non-thyroidal illness syndrome.

Characteristics	
Age	47.3 ± 15.4
Gender	
Male	29 (58.0)
Female	21 (42.0)
Days in hospital	45.8 ± 49.4
Death in hospital	9 (18.0)
Number of medications	11.7 ± 5.0
Comorbidities	
Diabetes	11 (22.0)
Dialysis	1 (2.0)
Bone marrow transplant	20 (40.0)
Heart disease	10 (20.0)
Hematologic malignancy	25 (50.0)
Kidney disease	8 (16.0)
Mechanical ventilation	8 (16.0)
Solid malignancy	12 (24.0)
Transfusion	38 (76.0)
Laboratory values	
Albumin, g/dL	3.2 ± 0.8
Alanine aminotransferase, U/L	54.2 ± 92.5
Alkaline phosphatase, U/L	110.5 ± 102.3
Aspartate aminotransferase, U/L	43.2 ± 60.1
Bilirubin, total, mg/dL	1.6 ± 3.7
Bilirubin, direct, mg/dL	0.7 ± 1.9
Creatinine, mg/dL	1.2 ± 1.8
Hemoglobin, g/dL	9.5 ± 2.0
Thyroxine binding globulin, μg/mL	15.4 ± 4.6
reverse T3, ng/dL	42.4 ± 39.3

Categorical variables are shown as the number (percentage) and continuous variables as the mean ± standard deviation.

Please cite this article as: K.J. Welsh, et al., Assessment of thyroid function in intensive care unit patients by liquid chromatography tandem mass spectrometry methods, Clin Biochem (2016), http://dx.doi.org/10.1016/j.clinbiochem.2016.11.022