Case report: When measured free T₄ and free T₃ may be misleading. Interference with free thyroid hormones measurements on Roche® and Siemens® platforms

Krzysztof C Lewandowski¹,², Katarzyna Dąbrowska¹ and Andrzej Lewiński¹,²*

Abstract

A 59-year old female patient presented with apathy and 6 kg weight gain. Investigations revealed severe primary hypothyroidism (TSH>100 μIU/ml). L-thyroxine (L-T₄) was started and titrated up to 75 μg, once daily, with clinical improvement. Other investigations revealed very high titres of anti-thyroid peroxidase (anti-TPO) and anti-thyroglobulin (anti-Tg) antibodies. After three months, there was a fall in TSH to 12.74 μIU/ml, however, with unexpectedly high free T₄ (FT₄) - 6.8 ng/ml and free T₃ (FT₃) - 6.7 pg/ml concentrations [reference range (rr): 0.8-1.9 ng/ml and 1.5-4.1 pg/ml (Siemens®), respectively]. At this stage L-T₄ was stopped, and this was followed by a rapid increase in TSH (to 77.76 μIU/ml) and some decrease in FT₄ and FT₃, however FT₄ concentration remained elevated (2.1 ng/ml). Following this, L-T₄ was restarted. On admission to our Department, she was clinically euthyroid on L-T₄, 88 μg, once daily. Investigations on Roche® platform confirmed mildly elevated TSH - 5.14 (rr: 0.27-4.2 μIU/ml) with high FT₄ [4.59 (rr: 0.93-1.7 ng/ml)] and FT₃ [4.98 (rr: 2.6-4.4 pg/ml)] concentrations. Other tests revealed hypoechogenic ultrasound pattern typical for Hashimoto thyroiditis. There was no discrepancy in calculated TSH value following TSH dilution (101% recovery). Concentrations of FT₄ and FT₃ were assessed on the day of discontinuation of L-T₄ and after four days by the means of Abbott® Architect I 1000SR platform. These revealed FT₄ and FT₃ concentrations within the reference range [e.g., FT₄ - 1.08 ng/ml (rr: 0.7-1.48)] vs 4.59 ng/ml (rr: 0.93-1.7, Roche®), FT₃ - 3.70 pg/ml (rr: 1.71-3.71) vs 4.98 (rr: 2.6-4.4, Roche®),] confirming assay interference. Concentrations of ferritin and SHBG were normal.

Conclusions: Clinicians must be aware of possible assay interference, including the measurements of FT₄ and FT₃ in the differential diagnosis of abnormal results of thyroid function tests that do not fit the patient clinical presentation.

Keywords: Free T₄, Free T₃, Assay interference

Case presentation

Written informed consent was obtained from the patient for publication of this case report and any accompanying images. The publication in question was approved by the ethical committee of the Polish Mother’s Memorial Hospital – Research Institute. A 59-year old female patient presented with a history of apathy and weight gain (about 5–6 kg). Initial investigations revealed severe primary hypothyroidism (TSH>100 μIU/ml), moderate hyperlipidaemia (total cholesterol 239 mg/dl, triglycerides 185 mg/dl) and normal fasting glucose (83 mg/dl). She was started on L-thyroxine (L-T₄), 25 μg, once daily, the dose of which was gradually titrated up to 75 μg, once daily, with clinical improvement. Other investigations revealed very high titre of anti-thyroid peroxidase antibodies (>4000 IU/ml (reference up to 115 IU/ml)). After about 3 months of treatment test revealed expected fall in TSH concentrations (to 12.74 μIU/ml), however, with unexpectedly high free thyroxine (FT₄)
and free triiodothyronine (FT₃) concentrations (Siemens® platform - see Table 1). At this stage L-T₄ was stopped, this was followed by a rapid increase in TSH (to 77.76 μIU/ml) and some decrease in FT₄ and FT₃, however, FT₄ concentration still remained elevated. Following this, L-T₄ was restarted. There was a gradual decrease in TSH, with FT₄ and FT₃ concentrations above the reference range. The patient was referred for the second opinion.

On admission to our Department, she was clinically eu-thyroid. Her medication included L-T₄, 88 μg, once daily, Rosuvastatin, 10 mg, once daily, Amlodipine, 5 mg x 1, once daily, Metoprolol slow release, 25 mg, once daily. There was no overt history of angina. She did not smoke and did not consume alcoholic beverages in excess.

Investigations performed in our Department (Roche® platform) confirmed mildly elevated TSH with high FT₄ and FT₃ concentrations (Table 2). The patient vehemently denied any compliance problems. Other tests confirmed very high titres of anti-thyroid peroxidase (anti-TPO) anti-thyroglobulin (anti-Tg) antibodies [anti-Tg > 4000.00 IU/ml (rr: up to 115 IU/ml); anti-TPO antibodies > 600.00 IU/ml (rr: up to 34 IU/ml)] and hypoechogenic ultrasound pattern typical for Hashimoto thyroiditis. Cortisol concentration was 10.52 μg/dl, ACTH 14.7 pg/ml (rr: 0–46 pg/ml). There was no discrepancy in calculated TSH value following TSH dilution (Table 2). Following this, L-T₄ was stopped with assessment of thyroid function (Table 3). Again there was a gradual increase of TSH with some decrease in FT₄ and FT₃, however, still above the reference ranges. We also assessed FT₄ and FT₃ on the day of discontinuation of L-T₄ and after 4 days in a different laboratory (Abbott® Architect I 1000SR platform – Table 3). This revealed FT₄ and FT₃ concentrations within reference ranges. Furthermore, the measured FT₄ concentration was about 4 times lower than on the Roche® platform (Table 3). Concentrations of ferritin and SHBG were normal (Table 3). Following administration of a single dose of 300 μg, once daily, there was a decrease in TSH with very little change of FT₄ and FT₃ (Roche® platform). Measurements of heterophilic antibodies were unfortunately not available in our Department. A diagnosis of hypothyroidism due to Hashimoto thyroiditis was made. The patient was discharged on L-T₄, 100 μg, once daily. The patient, as well as her GP were informed about problems with FT₄ and FT₃ measurements, and that further adjustments of L-T₄ dose should be based on clinical picture, as well as TSH measurements alone.

### Table 1 Initial results in the 59-year old female patient before and during treatment with L-T₄

| Date          | TSH   | FT₄ | FT₃ | L-T₄ dose | Reference Range                        |
|---------------|-------|-----|-----|-----------|----------------------------------------|
| Initial results | >100  | -   | -   | none      | TSH 0.27-4.0 μIU/ml (Siemens®)          |
|               |       |     |     |           | FT₄ 0.8-1.9 ng/ml (Siemens®)           |
|               |       |     |     |           | FT₃ 1.5-4.1 pg/ml (Siemens®)           |
| 13-14 weeks   | 12.74 | 6.8 | 6.7 | 75 μg     |                                        |
| 16 weeks      | 77.76 | 2.1 | 4.0 | None for two weeks |                                    |
| Seven months  | 49.23 | 3.1 | 4.5 | 50 μg     |                                        |
| Twelve months | 8.41  | 4.8 | 5.6 | 88 μg     |                                        |

### Discussion

Measurements of thyrotropin (TSH) and of total and FT₄ and FT₃ are widely used for thyroid function evaluation. However, some serum samples might demonstrate a nonspecific binding with assay reagents that can interfere with the measurement of these hormones. Several authors have reported the presence of such interferences, resulting in abnormal concentrations of thyroid hormones inconsistent with the patient’s clinical picture [1-3]. Interference in immunoassays is a widely recognized problem, which could potentially lead to unnecessary investigations and treatment. We describe a case, with interference in the FT₄ and FT₃ assay that led to falsely elevated serum FT₃ and FT₄ concentrations.

Clinical and biochemical investigations of our patient showed that biochemical thyroid status did not match her clinical presentation. Namely, the patient had high FT₄ and FT₃ levels with grossly elevated TSH, while simultaneously she did not have clinical features of hyperthyroidism. Further investigations revealed that she had very high titres for anti-TPO antibodies and anti-Tg antibodies, as well as thyroid ultrasound pattern typical for Hashimoto thyroiditis. Moreover, following treatment with L-T₄, we observed an expected fall in TSH concentrations, accompanied by clinical improvement, but also accompanied by further increase of FT₄ and

### Table 2 TSH dilution test in the 59-year old female patient on 88 μg of L-T₄ daily

| Dilution | TSH | FT₄ | FT₃ | Reference Range |
|----------|-----|-----|-----|-----------------|
| none     | 4.37| 6.61| 5.57|                 |
| 1:5      | 0.885 | -   | -   |                 |
| Calculated | 5 x 0.885 = 4.42 | -   | -   | (101.1% recovery) |

Furthermore, the measured FT₄ concentration was about 4 times lower than on the Roche® platform (Table 3). Concentrations of ferritin and SHBG were normal (Table 3). Following administration of a single dose of 300 μg, once daily, there was a decrease in TSH with very little change of FT₄ and FT₃ (Roche® platform). Measurements of heterophilic antibodies were unfortunately not available in our Department. A diagnosis of hypothyroidism due to Hashimoto thyroiditis was made. The patient was discharged on L-T₄, 100 μg, once daily. The patient, as well as her GP were informed about problems with FT₄ and FT₃ measurements, and that further adjustments of L-T₄ dose should be based on clinical picture, as well as TSH measurements alone.

### Discussion

Measurements of thyrotropin (TSH) and of total and FT₄ and FT₃ are widely used for thyroid function evaluation. However, some serum samples might demonstrate a nonspecific binding with assay reagents that can interfere with the measurement of these hormones. Several authors have reported the presence of such interferences, resulting in abnormal concentrations of thyroid hormones inconsistent with the patient’s clinical picture [1-3]. Interference in immunoassays is a widely recognized problem, which could potentially lead to unnecessary investigations and treatment. We describe a case, with interference in the FT₄ and FT₃ assay that led to falsely elevated serum FT₃ and FT₄ concentrations.

Clinical and biochemical investigations of our patient showed that biochemical thyroid status did not match her clinical presentation. Namely, the patient had high FT₄ and FT₃ levels with grossly elevated TSH, while simultaneously she did not have clinical features of hyperthyroidism. Further investigations revealed that she had very high titres for anti-TPO antibodies and anti-Tg antibodies, as well as thyroid ultrasound pattern typical for Hashimoto thyroiditis. Moreover, following treatment with L-T₄, we observed an expected fall in TSH concentrations, accompanied by clinical improvement, but also accompanied by further increase of FT₄ and FT₃ concentrations.
FT$_3$, far above the reference ranges. On the other hand, L-T$_4$ withdrawal resulted in fast increase in TSH, with only gradual decrease in FT$_4$ and FT$_3$, where FT$_4$ still remained above the reference range, despite very high TSH concentration (see Table 1). For these reasons, we considered assay interference. Therefore, we carried out dilution in TSH serum, which demonstrated linear correlation and almost 100% recovery. Manufacturer’s instructions precluded dilution tests with FT$_4$ and FT$_3$ assays. Such situation was different from recently described falsely elevated TSH level due to macro-TSH [4]. Furthermore, investigations in our Department confirmed an expected rise of TSH after L-T$_4$ withdrawal. In our patient, based on biochemical studies alone, the differential diagnosis also included thyroid hormone resistance syndrome or a TSH-oma. Both entities are characterized by clinical thyrotoxicosis, diffuse goiters, elevated circulating levels of FT$_4$ and FT$_3$, and non-suppressed serum TSH, though such very high initial TSH concentrations (i.e., above the upper limit of TSH assay), are not typical for these entities. However, given significant clinical improvement on L-T$_4$ treatment, high antibody titres, as well as thyroid ultrasound picture typical for Hashimoto thyroiditis, we had decided not to perform either a TRH test, or pituitary MR scanning. We are aware of the fact that in case of thyroid hormone resistance or a TSH-oma one might expect marked and progressive worsening of thyrotoxicosis following gradual increase in the dose of L-T$_4$. Moreover, we also investigated concentrations of SHBG and ferritin in serum. Thyroid hormone is one of several factors that modulate the level of sex hormone-binding globulin (SHBG) in serum. SHBG levels are usually elevated in thyrotoxicosis and have been reported to be normal in a few patients with generalized resistance to thyroid hormone (GRTH) [5,6]. Also, a significant body of evidence exists showing a positive correlation between the serum levels of T$_3$, T$_4$ and ferritin [7,8] in patients with abnormal thyroid status. Namely, all of these studies documented elevated serum ferritin levels in patients with hyperthyroidism which normalized when the T$_3$ and T$_4$ levels returned to normal. In our patient levels of SHBG and ferritin were normal.

For these reasons we concluded that assay interference was the only plausible explanation for the observed abnormalities. As direct test for heterophilic antibodies was not available in our institution, then we decided to repeat the blood tests using a different assay (i.e. two step assay – Abbott Architect®) in contrast to one step Siemens® and Roche® assays. This showed markedly lower concentration of FT$_4$ and also lower concentration of FT$_3$, consistent with the clinical picture. A possibility of assay interference is often under-recognised in clinical practice, while some patients’ sera contain autoantibodies to thyroid hormone that result in methodological artifacts in total or free hormone measurements [9,10].

Heterophilic antibodies are antibodies produced against poorly defined antigens. They are generally weak antibodies with multi-specific activities. Heterophilic

| Date       | TSH    | FT$_4$ | FT$_3$ | Ferritin | SHBG   | Reference Ranges                                      |
|------------|--------|--------|--------|----------|--------|-------------------------------------------------------|
| 14.06.2012# | 5.14   | 4.59   | 4.98   | 79.9     |        | TSH 0.27-4.2 μIU/ml (Roche®) FT$_3$ 0.93-1.7 ng/ml (Roche®) FT$_4$ 2.6-4.4 pg/ml (Roche®) |
| 14.06.2012  | not repeated: Roche® platform | 1.08* | 3.70* | 79.5     |        | *Abbott® Architect 11000SR FT$_3$: 0.7-1.48 ng/dl FT$_4$: 171.371 pg/ml |
| 15.06.2012  | 6.5    | 4.5    | 4.97   |          |        | Ferritin: 5–148 ng/ml                                  |
| 16.06.2012  | 9.49   | 3.48   | 4.82   |          |        | SHBG: 18.8-115.2 nmol/l                               |
| 18.06.2012  | 11.01  | 3.41   | 4.74   | 81.00    |        |                                                       |
| 18.06.2012## | not repeated: Roche® platform | 1.00* | 3.38* | 69.5     |        | *Abbott® Architect 11000SR FT$_3$: 0.7-1.48 ng/dl FT$_4$: 171.371 pg/ml |
| 19.06.2012  | 9.53   | 3.57   | 4.74   |          |        |                                                       |

# L-T$_4$ stopped on this day.
## 300 μg of L-T$_4$ administered after blood test on 18.06.2012.
antibodies are present in 30–40% of the population. However, they seldom cause interference with diagnostic immunoassays (0.05–0.5%), unless present in particularly high titres [12]. By definition, heterophilic antibodies are directed against specific animal immunoglobulins or against immunoglobulins of various animal species, depending on the recognized epitope and on the cross-reactivities between species immunoglobulins [10,13]. The recent development of two-site immunometric assays with specific antibodies, such as mouse monoclonal antibodies, has enabled higher specificities and sensitivities. Since the introduction of these assays, there have been several reports of abnormal concentrations of TSH resulting from heterophilic antibody interference [10]. To counteract this problem, all commercial assays now include blocking reagents, such as nonspecific and polymerized murine IgG. However, the presence of blocking reagents does not completely eliminate the problem of interference in some specimens and with some kits.

The FT4 and FT3 assays in our lab used a kit produced by Roche Diagnosis GmbH, Mannheim (sandwich Eleysys). Eleysys is an electrochemiluminescent immunoassay (ECLIA) involving ruthenium as the luminescent material. To exclude interference, the thyroid function tests were performed again, using the Architect system (Abbott). The Architect FT3 and FT4 assay is a two-step immunoassay determining the presence of free (unbound) T3 and T4 in human serum and plasma using chemiluminescent microparticle immunoassay (CMIA). Unfortunately, as we mentioned before, direct tests to detect particularly high titres of heterophilic antibodies were not available in our Department. However, given the patient’s clinical condition, results of other investigations, as well as significant improvement in comfort after treatment with L-T4, we concluded that the cause of disharmony between clinical picture and biochemical results was related to FT4 and FT3 assay interference.

Conclusion
Clinicians need to be aware of the potential for interference in immunoassays by heterophilic antibodies since such inaccurate results of thyroid function tests may lead to inappropriate treatment decisions.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
KCL was a senior physician responsible for patient’s treatment and he prepared the manuscript; KD was a medical resident responsible for patient’s care; AL was a senior author, he conceived the study and revised the text of manuscript. All authors have read and approved the final manuscript.

Acknowledgements
The study was supported by the grant of the Ministry of Science and Higher Education of Poland - No. NN402 476637, contract No. 4766/B/P01/2009/37 (grant No. 507-11-384 of the Medical University of Lodz).

Received: 20 September 2012 Accepted: 24 October 2012

References
1. Ismail AA, Walker PL, Barth JH, Lewandowski KC, Jones R, Burr WA: Wrong biochemistry results: two case reports and observational study in 5310 patients on potentially misleading thyroid-stimulating hormone and gonadotropin immunoassay results. Clin Chem 2002, 48:2023–2029.
2. Ghosh S, Howlett M, Boag D, Malik I, Collier A: Interference in free thyroxine immunoassay. Eur J Intern Med 2008, 19:221–222.
3. Obha K, Noh JH, Unno T, Satoh T, Ishara K, Matsuhashi A, Sasaki S, Oki Y, Nakamura H: Falsely elevated thyroid hormone levels caused by anti-ruthenium interference in the Eleyecs assay resembling the syndrome of inappropriate secretion of thyrotropin. Endocr J 2012, 59:663–711.
4. Loh TP, Kao SL, Halsall DJ, Toh SA, Chan E, Ho SC, Tai ES, Khoo CM: Macro-thyrotropin: a case report and review of literature. J Clin Endocrinol Metab 2012, 97:1823–1828.
5. Carani C, Isidori AM, Granata A, Carosa E, Maggi M, Lenz A, Iannini EA: Multicenter study on the prevalence of sexual symptoms in male hypo- and hyperthyroid patients. J Clin Endocrinol Metab 2005, 90:6472–6479.
6. Sarne DH, Refetoff S, Nelson JC, Linarelli LG: A new inherited abnormality of thyroid-binding globulin (TBG-San Diego) with decreased affinity for thyroxine and triiodothyronine. J Clin Endocrinol Metab 1989, 68:114–119.
7. Hashimoto T, Matsubara F: Changes in the tumor marker concentration in female patients with hyper-, eu-, and hypo-hyperthyroidism. Endocr Jpn 1989, 36:871–879.
8. Kubota K, Tamura J, Kurabayashi H, Shirakura T, Kobayashi I: Evaluation of increased serum ferritin levels in patients with hyperthyroidism. Clin Invest 1993, 72:26–29.
9. Ismail AA, Snowden N: Autoantibodies and specific serum proteins in the diagnosis of rheumatological disorders. Ann Clin Biochem 1999, 36:565–578.
10. Després N, Grant AM: Antibody interference in thyroid assays: a potential for clinical misinformation. Clin Chem 1998, 44:440–454.
11. Sakata S, Nakamura S, Miura K: Autoantibodies against thyroid hormones or iodothyronine. Autoimmunity 1985, 4:297–302.
12. Ismail AA, Barth JH: Wrong biochemistry results. Br Med J 2001, 323:705–706.
13. Knicka LJ: Human anti-animal antibody interferences in immunological assays. Clin Chem 1999, 45:942–956.

Cite this article as: Lewandowski et al: Case report: When measured free T4 and free T3 may be misleading. Interference with free thyroid hormones measurements on Roche® and Siemens® platforms. Thyroid Research 2012 5:11.