corticoids UFF + UFE as an outcome. In both models, the only significant associations observable for men were between GC3 and UFF (β, 3.7; P < 0.01) and GC3 and UFF + UFE (β, 10.3; P < 0.01). In women, in addition to a priori adjusted GC3, nitrogen also showed an association with UFF, resulting in a total explained variation of 47% (Table 1) for UFF alone. However, with UFF + UFE as the outcome, total $R^2$ in women increased to 0.72 and—in addition to urinary nitrogen—plasma leptin also explained a significant portion of variation of potential fGcA after adjustment for glucocorticoid secretion (GC3; Table 1). In line with the known stimulating effect of increased 5α-reductase activity on cortisol clearance, a trend ($P = 0.057$) for a negative association of this enzyme’s activity index (5α-THF/THF) with UFF + UFE was seen (Table 1). Accordingly, metabolic and nutritional influences on fGcA (assessed in 24-h urine samples) can be unraveled if the influence of the adrenocortical secretory activity is taken into account (e.g., as GC3).

With both UFF + UFE and UFF (the conventional measure for fGcA), increases in the protein intake–related postmeal plasma cortisol, as reported in the literature, are mirrored by the significant positive $β$ values for 24-h nitrogen excretion rates. However, the frequently reported interaction of leptin with the hypothalamus–pituitary–adrenal axis and the 5α-reductase influence on cortisol clearance remain masked if only UFF is analyzed. A major limitation of our study is that no individuals with abnormal glucocorticoid values were examined. Although recent findings (2, 4) and the present data suggest that UFE may be a useful complementary analyte to UFF for a more meaningful assessment of fGcA, further studies on healthy individuals and hypercortisolemic patients are required before manufacturers can be encouraged to develop adequate multiple glucocorticoid metabolite immunoassays for clinical use.

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References

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Undetectable Serum Thyroglobulin Due to Negative Interference of Heterophile Antibodies in Relapsing Thyroid Carcinoma

To the Editor: Undetectable serum thyroglobulin (Tg) after thyroid-stimulating hormone (TSH) stimulation is considered the most reliable marker of cure in patients with differentiated thyroid carcinoma (DTC). Interference by Tg antibodies (TgAb) and heterophile antibodies (HAb) may lead to false decreases and increases in Tg concentrations, respectively (1, 2).

A 32-year-old woman with enlarged neck lymph nodes was referred to our center. She had undergone total thyroidectomy and radioactive treatment 4.2 years before for pT1Nx papillary thyroid carcinoma (PTC). Six months later a neck ultrasound and rhTSH-stimulated Tg assay were negative (stimulated Tg <0.9 μg/L). Subsequently both clinical examination and Tg assay under thyroxine were performed every 12 months, with negative results.

We performed neck ultrasound, which revealed 2 round hypoechoic partially colliquated lymph nodes in the right neck (III level). A fine-needle aspiration biopsy (FNAB) was performed, and the needle-washing fluid was analyzed both by cytological examination and Tg assay (Tg-FNAB). Cytology specimens showed PTC recurrence, and FNAB-Tg was 950.70 μg/L. The serum Tg was undetectable (<0.9 μg/L) with negative

<table>
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* Stepwise multiple regression results (models a priori adjusted for GC3).

* Total $R^2$ for significant predictors ($P < 0.05$) without 5α-reductase index (5α-THF/THF).
TgAb (<60 kU/L), but recovery test results were abnormal (58%, range 80%–120%). The patient underwent modified right-neck dissection, which provided histological confirmation of PTC metastasis in 4 of 34 dissected lymph nodes. The Tg and TgAb measurement and recovery tests were performed on a fully-automated Immulite 2000 (DPC) system. In this case, because of the mismatch between serum and FNAB-Tg concentrations and the low Tg recovery, we further searched for interferences on the serum Tg assay. First, we retested Tg, Tg-recovery, FNAB-Tg, and TgAb using sensitive immunoassays (Tg-plus and Dyno-test Tg, BRAHMS). BRAHMS assay results indicated that Tg was pathologically increased and Tg-recovery was within the reference interval (Table 1). On the basis of these results we hypothesized that antibodies other than TgAb were causing interference. We then measured serum Tg on the Immulite platform after treating serum samples in a heterophilic blocking tube (Scantibodies Laboratory). After incubation in a heterophilic blocking tube, a Tg increase to 18.2 μg/L was found, confirming interference by HAb leading to a false-negative result.

TgAb interference is a major pitfall, leading to a falsely low or negative results in approximately 20% of DTC patient sera at the time of cancer diagnosis in the US. The frequency is lower in Europe and declines after thyroidectomy. Use of the TgAb immunometric assay is strongly recommended to screen for interferences in patients with DTC, but the usefulness of the recovery-test is under debate. However, because no consistent correlation pattern has been demonstrated between different Tg and TgAb immunoassays, undetectable TgAb in apparently TgAb-negative sera should be regarded with caution (2). In these cases a recovery test should be considered, especially if Tg testing did not fit the clinical picture (3). In our patient, the low recovery signaled interference on the Tg assay, with increased TgAb undetected by 2 different immunoassays. Generally the HAb binds to both the capture and detection antibody, simulating the presence of analyte in its absence and resulting in a false-positive result or a falsely increased measurement if the analyte is present. Preissner et al. (2) evaluated 1106 serum Tg samples and detected HAb interferences in approximately 3% of the specimens tested, without falsely low or negative results; however, samples with Tg concentrations <1 μg/L were excluded from the study. In some cases, HAb binds only to the capture (or detection) antibody, leading to falsely low or negative analyte measurement results (4). As shown in the present case (for the 1st time, to our knowledge) HAb may also interfere with testing by decreasing the measured Tg, leading to false-negative results. The interfering antibody cannot be differentiated with the blocking tube, but animal immunoglobulins added to serum should be useful. Interestingly, HAb did not significantly interfere with Tg measurement in FNAB washing fluid. Similarly, the Tg-FNAB did not appear to be substantially affected by TgAb, probably because of the very high Tg concentrations in the needle-washing fluids (5).

In conclusion, the Tg assay is a cornerstone in DTC follow-up and management. Tg measurement is significantly limited in TgAb-positive patients, however, and HAb may increase or, as shown here, even decrease the measured Tg. Ultrasound examination is of pivotal importance in the management of DTC neck recurrences, and in this case was supported by FNAB, allowing a definitive diagnosis. Thus effective management of DTC will continue to depend on multidisciplinary collaboration, especially for high-risk or relapsing patients.

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