Heterophile interference is not a factor in the day-to-day measurement of serum Tg levels, with fewer than 0.5% of blood samples encountered over an extended period in patients with thyroid cancer or controls

Verburg FA, Wäschle K, Reiners C, Giovanella L, Lentjes EG. Heterophile antibodies rarely influence the measurement of thyroglobulin and thyroglobulin antibodies in differentiated thyroid cancer patients. Horm Metab Res 2010. 10.1055/s-0030-1254132 [doi]

SUMMARY

BACKGROUND

Serum thyroglobulin (Tg) measurements play key roles in the follow-up management and treatment of patients with differentiated thyroid cancer (DTC). However, serum antithyroglobulin antibodies (TgAb) are present in approximately 25% of patients with DTC, which produces substantial alterations in the immunometric measurement of Tg, leaving Tg factitiously low, usually in the undetectable range. The other spurious alteration with Tg immunometric assays occurs with heterophile antibodies (HABs; antibodies that can bind to animal antigens such as antibodies [mouse, goat or rabbit] used in immunoassays) that are not as prevalent, but produce equally important alterations in serum Tg measurements, usually increasing the serum Tg level that is often in the range that suggests persistent disease; although Tg levels may be low in some cases. The aim of this study was to determine the impact of heterophile antibodies on the measurement of serum Tg recovery and TgAb levels in patients with DTC. HABs can interfere with immunometric assays by forming a bridge between capture and detection antibody, which may result in a falsely lower or even a false positive result in the case of an absent analyte. On the other hand, HABs may bind to the capture antibody in such a way that binding of the analyte is hindered, thus casing a false negative result. Most immunometric assays contain additives to reduce HAB interference; this has not been completely successful in blocking HAB interference.

Methods and Study Patients

This is a study of 201 patients that were undergoing follow-up for DTC in the authors' hospitals in Germany, Switzerland, and The Netherlands. Also, 52 control samples were studied. Half of the samples were treated by incubating for 1 hour in HAB-blocking tubes (HABBT). Multiple series of controls were anonimyzed before use: 10 serum samples from patients with Hashimoto's thyroiditis, 10 serum samples from patients with isolated elevation of TgAb levels; 10 serum samples from healthy subjects, and 19 serum samples from patients with a poor Tg recovery independent of TgAb status.

Procedures

Samples were obtained from patients for clinical follow-up and sent for regular Tg measurement. After Tg measurement, any remaining serum samples were frozen to –20°C until the experiment was performed. After thawing, the samples were centrifuged for 5 minutes at 3000 rpm, after which the samples were split: 5 ml was left standing untreated at room temperature for 1 hour and 0.5 ml was incubated for 1 hour at room temperature in HABBT. After incubation, the samples were again centrifuged for 5 minutes at 3000 rpm. Tg, Tg recovery, and TgAb levels were measured in each of the HABBT-treated and untreated samples. The blocked and untreated samples were measured together in the same run using the same kit; all samples in the experiment were measured using kits for the same lot. The Tg-plus method was standardized against the international standard CRM-457, which has a lower detection limit of 0.04 µg/L and a functional sensitivity of 0.2 µg/L.

Criteria for Sample Analyses

For each sample, the differences were calculated between the original Tg value and the Tg measurement obtained after HABBT treatment. The difference was considered significant if the HABBT-treated sample differed from the untreated sample by more than threefold the samples in the same run, as both samples were analyzed within the same run. The ±3 SD cutoff was used because statistically only 0.2% of the measurement would be expected to fall outside this range, which makes this a highly specific cutoff that is not likely to be influenced by random experimental error produced by sample manipulation. This was done because of the high coefficient of variation in the range of TgAb values that are considered negative.

RESULTS

Thyroglobulin Tests

Only 2 of 201 (1%) patient samples had a significant deviation between the native and HABBT-treated samples. In one case the Tg sample dropped from 11.2 µg/L to 8.0 µg/L after HABBT treatment; in another sample a native Tg of 6.3 µg/L was reduced to 4.1 µg/L after HABBT treatment. The Tg levels after HABBT treatment in neither of these patients was great enough to have affected the clinical management.

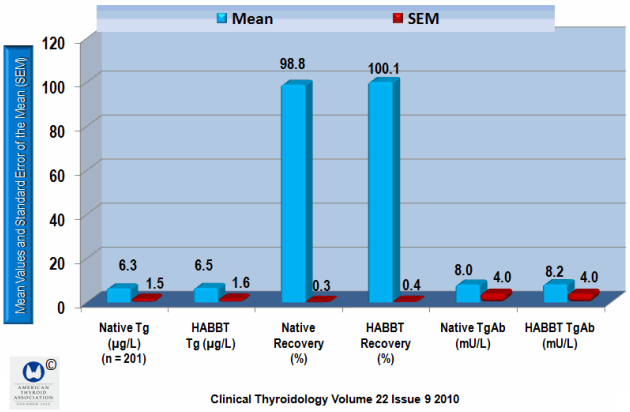

Figure 1. This figure shows the means (±SEM) before (native) and after heterophile antibody blocking tube (HABBT) treatment for thyroglobulin (Tg) levels, Tg recovery rates, and anti-Tg antibody (TgAb). Fewer than 0.5% of blood tests had HAB interference with Tg measurement.

Controls

In none of the 52 control samples was a significant difference in Tg levels found between the native and HABBT-treated samples.

Thyroglobulin Recovery Tests

A significant difference in TgAb levels could not be found between the native and HABBT-treated samples.

Controls

A significant decrease in Tg recovery was found in one patient between the native (129 mIU/L) and the HABBT-treated sample (39 mIU/L). All 51 other samples were free from apparent HAB interference.

CONCLUSION

Two patients had a moderate, but significant lowering of Tg levels after an HABBT treatment of serum Tg that was too little to affect clinical management. None of the 52 controls showed HAB interference in Tg measurement. Neither patients with DTC nor controls experienced HAB interference, and all patients with thyroid cancer and all but one control were found to have interference in TgAb measurement. In all, a possible HAB interference was encountered in 3 of 759 tests (0.4%). The authors concluded that one can assume that heterophile antibody interference is not a factor to be reckoned with in the daily practice of Tg measurement in the treatment and follow-up of patients with DTC.

COMMENTARY

The main observation in this study was that only 3 of 759 tests (0.4%) had possible HAB influence. Moreover, falsely elevated Tg values (n = 2) and TgAb (n = 1) were involved in all 3 cases.

There is no consensus regarding the frequency of HAB interference on different tumor marker immunoassays, with ranges reported from 1% to 80% in the literature (1). One of the earlier studies of HAB influence on serum Tg levels, by Preissner et al. (2) in 2003, suggested that unlike anti-Tg autoantibody interference, HAB immunoassay interferences were not well recognized as a Tg assay problem. They noted that when HAB interference does occur, it usually results in false positive Tg test results. They provided the caveat that some patients with thyroid cancer may be treated with ^{131}I on the basis of an elevated serum Tg result alone, which has the potential of administering unnecessary therapy. The study evaluated the prevalence of HAB interference in a commonly used automated immunoassay in 1106 consecutive specimens with Tg values >1 ng/ml. All Tg measurements were repeated after the samples were incubated in heterophile-blocking tubes, which showed a >3 SD percentage difference from the original result, which was considered to be HAB interference. Tg levels fell to <1 ng/ml in 32 specimens after HABBT treatment, 20 of which fell to <0.1 ng/ml. Of these 20 specimens, 17 were TgAb-negative. All 32 specimens had a fall of >3 SD percentage points (>56.91%) as compared with the original result. There also were two samples that showed a significant increase of greater than 56.91% after HABBT treatment. The authors concluded that HAB interference is relatively prevalent (1.5 to 3.0%) in a commonly used automated Tg assay and can lead to clinically significant artifacts. The authors suggested that unless a Tg assay is confirmed to be free of HAB interference or uses additional blocking steps, HAB interference should be suspected if Tg results do not fit the clinical picture. Verburg found that HAB interference results mostly in falsely elevated Tg or TgAb levels.

A study by Persoon et al. (3) identified HAB interference in Tg measurements in only 1 of 127 patients with the Nichols Advantage chemiluminometric assay, but the study did not use HABBT and identified HAB influence, instead identifying an HAB effect through an elevated Tg recovery rate.

Verburg et al. express concern about using HABBT in their study (and in others), as the exact formulation of these tubes in this study is considered a trade secret by the manufacturer, which leaves the possibility that the tubes contain substances that might interfere with the Tg measurement. The authors opine that the rare cases in which HAB interference can be seen are thus of no clinical consequence. They offer the caveat that this may not hold true for other assays and other populations.

Most authors who have studied this problem have concluded that this is a difficult situation that must be left to the discretion of the attending physician. This is true. In my view, one of the most important concepts in the treatment of patients with DTC is that serum Tg and TgAb concentrations are not static phenomena and that thyroglobulin and TgAb are best observed and evaluated over time, during which the direction of these markers must be carefully assessed. A serum Tg level clearly rising over time is usually the most likely signal of increasing tumor volume. Also, serious conclusions for repeated therapy also evolve around sensitive imaging studies such as ultrasonography, computed tomography–positron-emission tomography, or other imaging studies, depending on the potential location of the residual or recurrent tumor. Such fastidious evaluations almost always will avoid unnecessary ^{131}I therapy when HAB interference is a factor.

— Ernest L. Mazzaferri, MD, MACP

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