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Mutual interference between serum thyroglobulin and antithyroglobulin antibody in an automated chemiluminescent immunoassay

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Abstract

Objectives: To investigate the analytical interference between serum Tg and TgAb.

Design and methods: Tg and TgAb were measured on an automated chemiluminescent immunoassay system in mixed sera from DTC patients and individual samples spiked with exogenous Tg.

Results: Tg and TgAb recoveries in mixed patient samples were inversely correlated with expected TgAb or Tg concentrations, respectively. Impaired TgAb recoveries were also found in 50% (10/20) samples with high Tg in the exogenous recovery tests.

Conclusions: Mutual but not equal analytical interference between Tg and TgAb is present and concentration-dependent with interpatient variability.

Keywords: Thyroglobulin; Antithyroglobulin antibody; Chemiluminescent immunoassay; Analytical interference

Introduction

Serum thyroglobulin (Tg) is used as a sensitive and specific tumour marker in the postoperative follow-up of differentiated thyroid carcinoma (DTC) [1], 10–30% of whom are reported to be antithyroglobulin antibody (TgAb) positive [2–7]. In 187 DTC patients treated last year in our department, 15% had TgAb well above the upper normal reference limit (100 kIU/L) and 7.5% over 10-fold this limit.

Accumulating evidences have confirmed that interference from serum TgAb can cause inappropriately low Tg values in immunometric assays (IMA) [3–5,7].

Exogenous Tg recovery test is frequently performed for the detection of TgAb interference. In TgAb positive sera Tg recoveries lower than 70–80% are attributable to the interference [5,7], which, however, are not significantly correlated with the TgAb levels with normal recoveries found in sera with high TgAb and impaired recoveries in sera with low TgAb. So, the exogenous recovery test has been questioned [3,6–10].

Serum Tg interference with TgAb measurement was demonstrated in 3 samples with Tg over 40 000 μg/L [10], which needs to be further defined in sera with Tg at usual concentrations, that is, less than 1000 μg/L for DTC patients.

The present study carried out what we call endogenous recovery test in serials of mixed samples from DTC patients to study the concentration effect of the mutual interference between serum Tg and TgAb in a homogenous setting irrespective of interpatient variability. We also performed exogenous recovery tests in individual patient samples spiked with exogenous Tg to confirm Tg interference with TgAb measurement.

Materials and methods

Serum Tg and TgAb were measured in duplicate using an automated immunochemiluminometric analyzer and integrated reagents (LIAISON® Tg and anti-Tg, DiaSorin Inc., Italy). Human Tg in lyophilized human serum matrix (R_3 Tg, Roche Diagnostics, USA) was freshly reconstituted with distilled water and measured at Tg 861.1 μg/L and TgAb 83.3 kIU/L.

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Tg assay

Serum Tg is measured with polyclonal antibodies coated on magnetic particles as a capturer and monoclonal antibodies labelled with isoluminol as a tracer. The assay has an analytical sensitivity of 0.2 μg/L with a measuring range of 0.2–1000 μg/L and a normal range of 0.2–70 μg/L.

TgAb assay

Serum TgAb is measured with coated Tg as a capturer and polyclonal anti-human IgG antibodies conjugated with isoluminol as a tracer. The TgAb assay has an analytical sensitivity of 5 kIU/L, with a working range of 5–5000 kIU/L and a normal range of 5–100 kIU/L.

Serum samples

Frozen sera not visibly haemolytic and lipaemic with large volume were selected from postoperative DTC patients prior to radioiodine therapy. Special attention was paid to the concentrations of Tg and TgAb, irrespective of the respective disease status.

For the endogenous recovery test 3 pooled sera from 20 patients were prepared with Tg and TgAb measured at 11.1 μg/L and 9.0 kIU/L for Sample 1, 946.0 μg/L and 9.5 kIU/L for Sample 2, 1.1 μg/L and 1565.0 kIU/L for Sample 3, from 2 of which 3 serials of mixed samples were made. For example, to make the serial from Samples 1 and 2, transferring 10, 50, 100, 200, 300, 400, 500, 550, and 590 μL serum from Sample 1 into 9 tubes, respectively, adding Sample 2 into every tube to make up for 600 μL. The volume ratios of Samples 1 and 2 in this 9-tube serial were 1:59, 1:11, 1:5, 1:2, 1:1, 2:1, 5:1, 11:1 and 59:1. The mixing protocols for the other two serials from Samples 1 and 3, Samples 2 and 3 were all the same.

For exogenous recovery tests another 20 samples with low Tg (median, range: 1.5, 0.1–106.2 μg/L) and high TgAb (594.6, 134.1–1197.0 kIU/L) were mixed at 5:1 and 1:10 with the exogenous Tg reagent (Tg 861.1 μg/L, TgAb 83.3 kIU/L).

All mixed samples were capped and left to stand still overnight at ambient temperature prior to measurement for Tg to equilibrate with TgAb [6]. All the values were averages of duplicates directly read off the standard curves.

An expected value in a mixed sample was calculated according to the measurements of the constituent samples and their contributions. Recovery was defined as an observed value divided by the expected one, expressed as percentage.

Statistical analyses

We compared the observed and expected values of Tg and TgAb using Wilcoxon Signed Ranks Test. Differences in recoveries between two serials of samples were tested with Mann–Whitney test or Wilcoxon Signed Ranks Test. Associations of Tg or TgAb recoveries with expected TgAb or Tg concentrations were determined by using Spearman's coefficient of correlation. A two-side $P<0.05$ was considered to be significant.

Results

Analytical stability

Exogenous and endogenous recovery tests were done separately with averaged intra-assay CV of 5.6% (0.5%–10.2%) for Tg and 6.1% (0.9%–11.8%) for TgAb measurements.

Control sera supplied with the reagent sets were run concurrently and all the results were within the reference ranges. That is, 14.0 and 15.2 μg/L for the Tg-Low (11.2–16.6 μg/L), 220.1 and 242.5 μg/L for the Tg-High (196.0–264.0 μg/L), 91.8 and 110.8 kIU/L for the TgAb-Low (73.7–120.3 kIU/L) and 834.1 and 944.1 kIU/L for the TgAb-High (632.0–1118.0 kIU/L) in the endogenous and exogenous recovery tests, respectively.

TgAb interference with Tg measurements in the endogenous recovery tests

In mixed samples from Samples 1 and 2 with expected TgAb 9.0–9.5 kIU/L, observed Tg values were not significantly different from the expected ones ($P=0.214$) with a median recovery of 111%. In mixed samples from Samples 2 and 3 with expected TgAb 35.4–1539.1 kIU/L, observed Tg was significantly lower than the expected Tg ($P=0.008$) with a median recovery of 15%. The Tg recovery was inversely correlated with the expected TgAb concentrations ($r_s=-0.783$, $P=0.013$), and also markedly lower than that in the serial with low TgAb from Samples 1 and 2 ($P<0.001$) (Table 1).

Tg interference with TgAb measurements in the endogenous recovery tests

In mixed samples from Samples 1 and 3 with expected Tg 1.3–10.9 μg/L, observed TgAb values were not significantly different from the expected ones ($P=0.086$) with a median recovery of 97%. In mixed samples from Samples 2 and 3 with expected Tg 16.9–930.3 μg/L, observed TgAb was significantly lower than the expected TgAb ($P=0.021$) with a median recovery of 91%. Though the recovery was not significantly different from that in the serial with low Tg from Samples 1 and 3 ($P=0.136$), it was inversely correlated with the expected Tg ($r_s=-0.917$, $P=0.001$), and specifically, 89–101% at Tg 16.9–631.1 μg/L, and 54–80% at Tg 788.5–930.3 μg/L (Table 1).

Tg interference with TgAb measurements in the exogenous recovery tests

Individual samples mixed with the exogenous Tg reagent at 5:1 had expected Tg 145.4 μg/L, in which observed and expected TgAb were not significantly different ($P=0.064$), with a median recovery of 110% and impaired recovery (<80%) in 5% (1/20) samples. While in samples mixed with the exogenous