were balanced by increased serum cystatin C, which in addition to kininogens and α2-macroglobulin is the most important inhibitor for controlling the proteolytic activity of extracellular cysteine proteases. In melanoma we found significant increases ($P = 0.02$) in the cystatin C concentration among patients with metastatic disease and smaller increases in patients with primary melanoma (Fig. 1), indicating the up-regulation of cystatin C in later events of tumor progression. In colorectal cancer, serum concentrations of cystatin C were significantly increased ($P < 0.0001$) in patients at all Dukes stages, correlating weakly with patient age and gender (unpublished data). The correlation between cystatin C and creatinine serum values (7), however, was much weaker in cancer patients than that reported for healthy controls, strongly support the results of our studies, which involved 401 patients included in previous studies (3), suggesting the influence of nonrenal factors on the concentration of cystatin C in malignant sera. The creatinine values, not significantly changed in cancer patients, suggest that patients’ renal function had not been altered at the time of sample collection.

In our opinion the number of patients included in previous studies was too low to provide relevant information about changes in the cystatin C serum concentration during malignant progression. The results of our studies, which involved 401 patients with colorectal cancer, 97 patients with melanoma, and 124 healthy controls, strongly support the need for further evaluation of cystatin C as a marker for glomerular filtration rate determination, at least in cancer patients, to determine its potential for use in clinical practice.

References


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Anti-Thyrotropin Antibody Interference in Thyrotropin Assays

To the Editor:

We read with interest the paper by Despre’s and Grant (1) on antibody interference in thyroid assays. Thyroid hormone autoantibodies, heterophile antibodies, and rheumatoid factors are certainly the main sources of artifacts. As mentioned by the authors, anti-thyrotropin (anti-TSH) antibodies are more uncommon but may nevertheless deserve additional comments.

The existence of anti-TSH antibodies in patient sera has been reported after injections of bovine TSH (2, 3). The antibodies also appear in autoimmune thyroid diseases such as Graves disease, Hashimoto thyroiditis, silent thyroiditis, and subacute thyroiditis (4–7), and nonthyroid autoimmune disease (6). In sera from patients with Graves disease, the possibility that thyrotopin receptor antibodies (TRAbs) may be anti-idiotypic antibodies against anti-TSH antibodies or that anti-TSH antibodies may be anti-idiotypic antibodies against TRAbs is controversial (8–12). Most of the reported anti-TSH antibodies reacted against bovine TSH; however, some also reacted against human TSH (4, 12–14). The results of published studies on anti-TSH antibody interference in TSH assays concerned mainly RIAs. In those cases, depending on the assay design and the antibody specificity, interference may yield lower or increased values. Increased results were found with the double antibody technique (4, 5, 13, 15–17). Single antibody techniques with polyethylene glycol (PEG) precipitation yielded low values (14, 15). Fewer results have been reported with the widely used, “sandwich” immunoassays (IMAs). IMA results have been found to be lower (5) or similar to double antibody results (6). Moreover, different IMA kits may yield discrepant values (14).

We previously reported (18) TSH concentrations that we measure (19) with eight different third-generation IMAs in four serum samples that contained anti-TSH antibodies as determined by increased precipitation of protein-bound 125I bovine or human TSH. Two samples from patients with autoimmune thyroid disorders (Graves disease and postpartum thyroiditis) contained only anti-bovine TSH antibodies. The results of the different TSH kits were not grossly discrepant, ranging from 0.36 to 0.60 mIU/L and from 2.9 to 4.7 mIU/L for the two samples, respectively. The other two sera contained both anti-bovine and anti-human TSH antibodies. In the first case, our suspicion was aroused because the high serum TSH contrasted with an apparently healthy clinical picture. The second case was from a euthyroid woman who had given birth to two children with trans...
sient neonatal hyperthyrotropinemia, a case similar to those presented in previous reports (17, 20). This patient’s serum contained neither stimulating nor blocking TRAbs. The thyroid-stimulating TRAbs were checked by measuring the stimulating activity (cAMP production) of the serum on thyroid cells. Blocking TRAbs were ruled out because, after preincubation of the serum with thyroid cells followed by two wash steps, added TSH displayed normal stimulating activity. This serum blocked the stimulating activity of TSH on thyroid cells only when TSH and the serum were incubated simultaneously. We considered that this serum contained anti-TSH antibodies capable of inhibiting the stimulating activity of TSH. Measurements with the different kits yielded, for these two euthyroid patient sera containing anti-human TSH antibodies, TSH results that were highly discrepant, ranging from 2.2 to 36.6 mIU/L and from 2.1 to 13.9 mIU/L, respectively [see Sapin et al. (18) for details]. Of 12 values, 9 were in the hypothyroid range. As measured by the BeriLux kit, estimates of TSH concentrations in these samples were within the health-related reference interval after immunoglobulins were eliminated through pretreatment with 250 mL/L PEG and centrifugation for 30 min at 4 °C (18). After this treatment, the mean TSH recovery was 66% in control sera without anti-TSH antibodies. This decreased recovery value was taken into account when the results after PEG treatment were calculated (1.2 mIU/L for both patients).

Our observations underline the possibility of misinformation in TSH assays because of anti-TSH antibodies, even with recent kits. For the first two patients, variation between kits could be explained in part by the bias we observed between the results of the different kits in euthyroid control patients (19, 21). The median ranged from 1.0 to 1.9 mIU/L. This is definitely not the case for the last two patients. During this study, which has spanned >5 years, 300 000 TSH determinations have been performed by the two laboratories in which these four cases were found. The incidence of anti-TSH antibodies may exceed 4 in 300 000 because some cases may have been missed. However, the incidence of TSH results showing a gross discordance either with the clinical status of the patient or between methods because of anti-TSH antibodies could be only 2 in 300 000.

Despite the forthcoming preclusion of bovine TSH injections (22) and despite the fact that recombinant human TSH (Thyrogen) does not seem to be a potent immunogen (23), anti-bovine and anti-human TSH antibodies may both be spontaneously present in the sera of thyroidal ill or clinically healthy patients. Moreover, it is worth noting that anti-TSH anti-bodies have been consistently reported to be a source of interference in TRAB assays, yielding negative TRAB values (5–7, 12, 13, 24). This interference frequently contributed to the discovery of anti-TSH antibodies.

This work was supported by the Hôpitaux Universitaires de Strasbourg. We thank H. Bornet for the anti-human TSH antibody determinations and A.M. Madec for measuring the stimulating and blocking activities on thyroid cells. We also thank N. Heider for carefully reviewing the manuscript.

References
1. Despres N, Grant AM. Antibody interference in thyroid assays: a potential for clinical misinforma-
3. Melmed S, Harada A, Hershman JM, Krishnamurthy GT, Bland WH. Neutralizing antibod-
5. Ochi Y, Nagamune T, Nakajima Y, Ishida M, Kajita Y, Hachiya T, Ogura H. Anti-TSH antibo-
6. Akamizu T, Mori T, Kasagi K, Kosugi S, Miyamoto M, Nishino K, et al. Anti-TSH antibody with high specificity to human TSH in sera from a patient with Graves’ disease: its isolation from, and interaction with, TSH receptor antibo-
8. Sain A, Shah R, Singh A, Silver L. Erroneous thyroid-stimulating hormone radioimmunoassay results due to interfering antibovine thyro-
9. Frohman LA, Baron MA, Schneider AB. Plasma immunoreactive TSH: spurious elevation due to antibovine TSH which cross-react with human TSH. Metabolism 1962;31:
   834–40.
11. Sapin R, D’Herbomez M, Gasser F, Wemeau JL, Schlenger JL. Analytical limitations of thyro-
   198–202.
   690–15.
13. Bachelot I, Barbe G, Orgiazzi J, Halimi S, Auto-
    anticorps anti-TSH: étude chez une mère et son nouveau né [Abstract]. Ann Endocrinol (Paris) 1997;
   58:188–91.
14. Sapin R, d’Herbomez M, Gasser F, Schlenger JL, Wemeau JL. Evaluation de sept trousses de dosage immunométrique de TSH avec mar-
    queur luminescent. Immunoanal Biol Spéc 1996;
15. Emerson CH, Colzani R, Braverman LE. Epithe-
    lial cell thyroid cancer and thyroid stimulating hormone–when less is more [Editorial]. J Clin Endocrinol Metab 1997;82:9–10.
16. Ramírez L, Braverman LE, White B, Emerson CH. Recombinant human thyrotropin is a po-
    tent stimulator of thyroid function in normal subjects. J Clin Endocrinol Metab 1997;82:
   2836–9.
17. Akamizu T, Ishii H, Mori T, Ishihara T, Ikekubo K, Imura H. Abnormal thyrotropin binding immu-
    noglobulins in two patients with Graves’ disease. J Clin Endocrinol Metab 1984;59:
   240–5.
Evaluation of Two Automated Immunoassays for Measurement of Free Deoxypyridinoline in Urine Using Analytical Goals Derived from Biological Variation

To the Editor:

Free deoxypyridinoline (fDPD) is increasingly used as a specific marker of bone resorption (1). To date, the concentration of fDPD in urine has been measured using cumbersome HPLC or manual microtiter-based ELISA procedures, which require ~4 h to perform (2). Recently, two rapid, fully automated chemiluminescent immunoassays were developed: by Chiron Diagnostics for the ACS:180® analyzer and by DPC® for the Immulite® analyzer. Both immunoassays use a competitive format involving the same monoclonal anti-fDPD antibody from Metra Biosystems (3, 4). If sufficiently reliable, these assays can accommodate increasing testing demands with both controlled operating costs and dramatically reduced turnaround time. This study assessed their analytical performance for the routine measurement of fDPD in our clinical laboratory. In particular, as suggested previously (5), goals for precision and accuracy were based on the biological variation of fDPD excretion in urine from healthy premenopausal women, and the results obtained during the evaluation were compared with these to assess acceptability (6).

All measurements on the two instruments were performed according to the recommendations of the manufacturers by the same trained technician. Linearity in the working ranges of the tests (ACS:180, 2–350 nmol/L; Immulite, 7–300 nmol/L) was good (r = 0.999 for the ACS:180; r = 0.998 for the Immulite). The data for the imprecision study, which used two pooled human urines, are summarized in Table 1. Eighty-one urine samples (first morning void) with fDPD concentrations ranging from 13 to 184 nmol/L were assayed, using the Pyrilinks®-D (Metra Biosystems) method as the reference (x) and the two automated procedures (y) in a correlation study. The following results were obtained: ACS:180 = 0.96 (±0.03)x – 0.8 (±3.0); S\textsubscript{xy} = 12.9 nmol/L; r = 0.9552; and Immulite = 0.99 (±0.03)x + 9.8 (±2.8); S\textsubscript{xy} = 12.1 nmol/L; r = 0.9629.

When biology is used to set analytical goals, desirable imprecision (CV) is less than or equal to one-half of the average within-subject biological variation (i.e., for urinary fDPD, total CV ≤6.7%), and desirable inaccuracy (bias) is less than or equal to one-quarter of the group (within-subject plus between-subject) biological variation (i.e., for urinary fDPD, average bias ≤5.5%) (6). From our experimental results, we conclude that the ACS:180 assay is probably accurate but is too imprecise for the between-day evaluation and that the Immulite assay shows good precision but also has a significant, constant positive bias. The final considerations depend on medical needs (7). Because the bone markers are useful adjuncts in monitoring patients and not in screening for bone disorders, low imprecision (at least as good as the goal) is required, whereas some degree of inaccuracy is probably less important (8).

We acknowledge the expert technical assistance of Cristina Serena. We also thank Chiron Diagnostics (Cassina de’ Pecchi, Milano, Italy) and Medical Systems (Genova, Italy) for the generous loan of instruments and reagents to carry out the study.

Table 1. Imprecision of the evaluated immunoassays.

<table>
<thead>
<tr>
<th>Mean fDPD</th>
<th>Within-run CV (n = 20)</th>
<th>Between-day CV (n = 10)</th>
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<tbody>
<tr>
<td>ACS:180</td>
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<tr>
<td>82 nmol/L</td>
<td>4.1%</td>
<td>9.0%</td>
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<tr>
<td>304 nmol/L</td>
<td>2.6%</td>
<td>4.2%</td>
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<tr>
<td>Immulite</td>
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<tr>
<td>78 nmol/L</td>
<td>5.6%</td>
<td>3.9%</td>
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<tr>
<td>289 nmol/L</td>
<td>4.7%</td>
<td>3.2%</td>
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References


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