washed, the Vitros Signal Reagent was added to the well, and the luminescence was measured (ALOKA luminometer). The IgG fraction of serum samples from the patient and from five control individuals was purified with a MAbTrap Kit (Amer sham Biosciences), and the IgG concentration was adjusted to 4.0 g/L. The purified IgG (0.08 mL) was incubated at 4 °C for 24 h with 0.08 mL of PBS alone or with T2, T3, or T4 (Sigma; at 4570, 42, and 768 nmol/L, respectively) dissolved in PBS. Each sample was mixed vigorously with 1.2 mL of polyethylene glycol (PEG; 125 g/L), centrifuged at 2800 g for 30 min, aspirated, and washed with 1.2 mL of PEG (125 g/L). After the precipitates were dissolved in 0.001 mol/L hydrochloric acid (0.04 mL) and neutralized by equal amounts of 0.001 mol/L sodium hydroxide, T2 and T3 were measured by the FT3 assay and T4 by the FT4 assay. The concentrations of T2 and T3 were expressed as T3 equivalents. Each sample was analyzed in duplicate. The ability of the purified IgG to bind T2, T3, or T4 was defined as the difference between the FT3 or FT4 assay result and the respective blank value and is reported as the δT2, δT3, or δT4 value.

The ratios of FT3 and FT4 concentrations in PEG-treated samples to those in untreated samples were significantly lower for the patient than for 37 other patients (Table 1 in the online Data Supplement); therefore, immunoglobulins in the patient’s serum interfered with both the FT3 and FT4 assays. FT3 and FT4 values in the mixtures of serum with sheep IgG, bovine globulin, and gelatin did not differ significantly from those in the mixtures of serum and PBS only, suggesting that heterophilic antibodies and anti-gelatin antibodies did not cause the high FT3 and FT4 values.

When we examined the patient’s IgG binding with Vitros FT3II and FT4 assay wells, the luminescence generated by the patient’s serum was higher than that of the 5 control individuals (Table 1 in the online Data Supplement). This suggested that the patient’s IgG bound to T2- and T3-gelatin.

The FT3 and FT4 concentrations in purified IgG and in treated samples of purified IgG (6) were below the lower detection limits of the Elecsys assays, suggesting an absence of T3 and T4 contamination in the IgG fractions. The patient’s δT2 and δT3 values were higher than those of the 5 control individuals, but the δT4 value was within 2 SD of the values for the 5 controls (Table 1 in the online Data Supplement). This finding implies that the patient’s IgG interacted with T2 and T3 but not with T4. The cross-reactivity of the anti-T2 antibody with T2 in the Vitros FT3II assay was very low, whereas the patient’s δT2 value was evidently higher than that of 5 control individuals. We conclude that T2 was bound to the patient’s IgG.

Because anti-gelatin antibodies in the patient’s serum were not recognized, we suggest that the interfering substance was antibodies to T2 and T3. As the interfering antibodies did not interfere with the Elecsys FT3 assay, the interfering antibody in the patient’s serum may recognize T2 and T3 conjugates used in the Vitros ECI FT3 and FT4 assays, as reported for a labeled-antibody assay (7). We suggest that this interference in the Vitros ECI FT3 and FT4 assays arose from antibodies to T2, T3, or their conjugates.

References


Underestimation of Serum 25-Hydroxyvitamin D by the Nichols Advantage Assay in Patients Receiving Vitamin D Replacement Therapy

To the Editor:

Measurement of 25-hydroxyvitamin D (25OHD) is used to assess both vitamin D status and the response to exogenous replacement therapy (1). Inaccurate assays may place patients at risk of either under- or overreplacement, with potential adverse consequences (1, 2).

Significant interassay and interlaboratory variations in measured 25OHD values have been reported (3, 4). The international Vitamin D Quality Assessment Scheme (DEQAS) monitors the performance of vitamin D assays in >100 laboratories in 18 countries (4). Recently, the analysis of samples containing only 25OHD3 was reported by DEQAS to be within 7% of the target value (as determined by gas chromatography–mass spectrometry) for 5 of the 6 methods reported, with a degree of operator-dependent variability. The Nichols Advantage assay, however, yielded consistently higher results, averaging ~31% higher than the mean of
the other methods (4). Conversely, in 2 samples containing mostly 25OHD2, the Nichols assay consistently underestimated 25OHD compared with other assays, such as the DiaSorin RIA (4).

In March 2004, we recruited 50 patients with hypovitaminosis D (43 female; mean age, 53 years; range, 29–82 years) to assess the effects of replacement therapy. The baseline vitamin D concentration was <17 nmol/L in 28 patients and 18–35 nmol/L (mean, 24.5 nmol/L) in 22 patients. Each patient received a single intramuscular injection containing 300 000 IU of ergocalciferol. Serum samples were obtained at baseline and 6, 12, and 24 weeks later. The concentration of 25OHD was measured with the Nichols Advantage automated assay at St. George’s Hospital (London, UK). Several samples were stored at −20 °C and subsequently analyzed blind with the DiaSorin assay at West Park Hospital, Epsom (Surrey, UK).

Comparison of DEQAS data for the laboratories at St. George’s and West Park Hospitals confirmed the reported higher values (4) for the Nichols (mean 25OHD concentration, 63 nmol/L) compared with the DiaSorin assay (mean 25OHD concentration, 55.5 nmol/L) for 28 paired nonstudy samples for which 25OHD was likely to be derived from vitamin D3.

The main source of 25OHD at baseline was hard to determine. In general, the source is assumed to be sunlight-derived vitamin D3, but for some patients, limited sunlight exposure, or without reduced skin exposure, or reduced dermal capacity to synthesize vitamin D3 may mean that dietary vitamin D2 is more important (5–12). In all of the posttreatment samples, vitamin D2 was assumed to comprise the main source of measured 25OHD.

Paired 25OHD results for the Nichols Advantage and DiaSorin assays were obtained for 10 pretreatment baseline samples and for 4 samples at 6 weeks, 19 samples at 12 weeks, and 14 samples at 24 weeks after ergocalciferol treatment. The mean baseline 25OHD concentration obtained with the Nichols assay was significantly lower (24.7 nmol/L; range, 17.0–35.0 nmol/L) than that obtained with the DiaSorin assay (34.8 nmol/L; range, 19.0–63.0 nmol/L; P = 0.041, Wilcoxon signed-rank tests), as were the individual results for 8 of 10 samples. That is consistent with the predominant source of baseline 25OHD in these patients being vitamin D2, given the DEQAS data (4). In the posttreatment samples, an increase in 25OHD concentration compared with baseline was found with both assays, but the mean values from the DiaSorin assay were significantly higher than the Nichols values at both 12 weeks (54.7 and 30.9 nmol/L, respectively; P < 0.001, Wilcoxon signed-rank test) and 24 weeks (56.4 and 34.5 nmol/L, respectively, P = 0.001, Wilcoxon signed-rank test). Furthermore, in every posttreatment sample, the DiaSorin assay yielded a higher 25OHD concentration than the Nichols Advantage assay. A difference plot indicated that the disparity between the 2 assays was greater at higher concentrations (Fig. 1).

We conclude that the Nichols assay underestimates 25OHD in patients treated with ergocalciferol and that the increasing disparity between the studied assays at higher concentrations may lead to unnecessary increases in replacement therapy and potential toxicity.

References

Fig. 1. Difference between 25OHD concentrations (nmol/L) determined by the DiaSorin and Nichols Advantage assays (DiaSorin – Nichols; y axis) compared with the mean of the 2 assays (x axis) in 37 serum samples from 32 patients at 6 (■), 12 (▲), or 24 (▼) weeks after parenteral ergocalciferol therapy.
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An author of one of the studies cited in the above letter and a representative of Nichols Institute Diagnostics respond:

To the Editor:

In their letter, Leventis et al. compare methods for 25-hydroxyvitamin D [25(OH)D] measurements. In light of recent reports, and given the complexities of accurately measuring the physiologically relevant species among metabolites of vitamin D, we consider that the title and the conclusions of their letter may be potentially misleading. One of the methods they studied is based on antibody recognition and isotopic detection (DiaSorin), whereas the other is based on vitamin D-binding protein recognition and chemiluminescence detection (Nichols Institute Diagnostics). These important differences may be responsible for the different results. We communicated to Nichols Advantage assay users in customer bulletins issued on June 15, 2004, and December 6, 2004, that our method gives lower results than do liquid chromatography–mass spectrometry and other assays, particularly in patients treated with vitamin D2. In those bulletins, we advised customers that samples tested with the assay may yield results lower than actual 25(OH)D values because of potential underrecovery of 25(OH)D2. We also advised our customers of a study showing that, after treatment of patients with 25(OH)D2, the assay gave measured increases in 25(OH)D concentrations (1). In their letter, Leventis et al. also indicate that in the posttreatment samples, both of the assays that they studied detected an increase in 25(OH)D concentration compared with baseline. Representatives of the Vitamin D External Quality Assessment Scheme, in a Technical Brief published in this journal (2), indicated that the Nichols Advantage assay does detect 25(OH)D2, although with lower absolute values.

We do not dispute that different vitamin D assays may yield different results. One possibility is that the natural vitamin D-binding protein in the Nichols Advantage assay quantifies the physiologically relevant metabolite(s) of vitamin D3 better than does an antibody-based assay. This has led us to start investigating the identities of metabolites generated by vitamin D2 using HPLC and mass spectrometric methods. A recent report by Heaney et al. (3) is noteworthy in that it reflects substantially more rapid metabolism or clearance of the vitamin D3 metabolites. Further research is necessary to uncover the identities of vitamin D metabolites and their clinical significance. Until this is done, conclusions such as those drawn in the letter of Leventis et al. should be considered as preliminary and premature.

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References


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Detrimental Effect of Formaldehyde on Plasma RNA Detection

To the Editor:

Recent studies have shown that detection of fetal DNA or RNA in maternal plasma is useful for prenatal investigation of certain fetal genetic traits (1, 2) or pregnancy-associated complications (3, 4). Fetal DNA has been shown to amount to 3.4%–6.2% of the total DNA in maternal plasma (5). Thus, the reliability of circulating fetal nucleic acids analysis is dependent on the ability to sensitively and specifically detect and distinguish such fetal molecules from a background of maternal nucleic acids. Hence, methods that enable enrichment of the proportion of fetal nucleic acids in maternal plasma would, in theory, facilitate robust analysis of circulating fetal nucleic acids. Dhallan et al. (6) recently explored the use of formaldehyde for the enrichment of circulating fetal DNA. The authors reported marked increases in the proportion of fetal DNA in maternal blood samples preserved with formaldehyde. Although controversies exist regarding the effect of formaldehyde on fetal DNA enrichment (7, 8), we nonetheless wanted to investigate whether form-