A Highly Sensitive Thyroglobulin Assay Has Superior Diagnostic Sensitivity for Recurrence of Differentiated Thyroid Cancer in Patients Undergoing TSH Suppression

TO THE EDITOR: In a recent paper (*1*), Ma et al. considered explanations for the occasional discordant findings between serum thyroglobulin and ^{131}I whole-body scanning. They listed several possible reasons for false-positive and false-negative thyroglobulin values in the follow-up of patients with differentiated thyroid cancer and emphasized the important role of thyroglobulin as a tumor marker in differentiated thyroid carcinoma (DTC) and as an indicator of the effectiveness of surgery and ^{131}I therapy (*2–4*). The authors gave an overview of some of the available literature as part of their assessment of the causes of discordance between thyroglobulin results and ^{131}I whole-body scanning, with particular emphasis on different methods of thyroglobulin measurement (*5,6*).

Although the possibility of measuring thyroglobulin by enzyme-linked immunosorbent assay (ELISA) was mentioned, only immunoradiometric assays (IRMAs) and radioimmunoassays were considered in any detail. In addition, the improved sensitivity of thyroglobulin measurements under exogenous or endogenous thyroid-stimulating hormone (TSH) stimulation was emphasized and the problems of thyroglobulin measurement in the presence of thyroglobulin autoantibody (TgAb) and high-dose hook effects (*7–9*) considered.

However, Ma et al. (*1*) stated that an important reason why falsely negative thyroglobulin values occur is that the functional sensitivity of the thyroglobulin method used is not high enough to detect thyroglobulin secretion by small amounts of thyroid tissue when TSH is suppressed. It is therefore disappointing that several studies (*10–13*) using a highly sensitive thyroglobulin ELISA were not mentioned. The ELISA in question (manufactured by RSR Ltd.) has a functional sensitivity of 0.03 ng/mL (international reference preparation, CRM 457), and the first technical and clinical evaluations in 2001 showed that thyroglobulin can be measured reliably and simply (*12*). Thyroglobulin values in sera from patients with DTC ($n = 24$, 17 of whom showed some evidence of recurrence) and from healthy blood donors ($n = 48$) agreed with those obtained by conventional IRMA (SELco Tg IRMA; Medipan Diagnostica GmbH; functional sensitivity, 0.6 ng/mL) ($r = 0.99$ and 0.98, respectively). Analysis of samples from 167 patients treated successfully for DTC (94 with papillary carcinoma and 73 with follicular carcinoma) showed that 139 were negative for TgAb and that, of these, 106 (76%) had thyroglobulin levels measurable by ELISA (0.03 ng/mL or greater). In contrast, only 7 (5%) of these 139 sera had thyroglobulin levels measurable by IRMA (0.6 ng/mL or greater). The assay should have significant advantages for patient care; the thyroglobulin level after initial ablative treatment usually is measurable rather than undetectable. Furthermore, any increases in serum thyroglobulin levels, which may herald relapse, can become detectable earlier.

The ELISA is a 2-step assay and is consequently essentially free of high-dose hook effects (*13*). Furthermore, to ensure that only endogenous thyroglobulin is detected in the assay, samples from DTC patients before and after TSH stimulation have been studied. In another investigation (*10*), serum thyroglobulin was measured serially in 126 TgAb-negative patients with treated DTC while they were undergoing TSH suppression. At the beginning of this retrospective analysis, all 126 patients were in remission and thyroglobulin was detectable by ELISA in 92 (73%; range, 0.03−0.8 ng/mL). Over the following 4 y, thyroglobulin levels remained essentially unchanged (i.e., any increases were less than 2 times the thyroglobulin level at the start of the study) in 121 (96%) of these 126 patients, and all 121 patients remained well. In 5 patients, thyroglobulin levels increased to more than 2 times the starting thyroglobulin level over the study period, and in 4 of these 5, DTC recurred. The fifth patient in this group remained well as evidenced by extensive diagnostic imaging, although his serum thyroglobulin level continued to increase and could be stimulated by TSH. These results suggest that, without using endogenous or exogenous TSH stimulation, serial measurements of low levels of thyroglobulin by ELISA in treated patients with DTC enable detection of recurrence 6–12 mo earlier than would be possible using a conventional thyroglobulin IRMA. These observations were subsequently confirmed by Görges et al. (*11*), who were able to show a 5- to 15-mo earlier detection of DTC recurrence when using the highly sensitive thyroglobulin ELISA than when using thyroglobulin IRMA.

With regard to the occurrence of high-dose hook effects in different thyroglobulin assays, 2-step IRMA methods that include a preincubation of the patient sample before addition of tracer do not show such a problem in routine use even when thyroglobulin concentrations are as high as 100,000 ng/mL. In contrast, 1-step assays do tend to show high-dose hook effects at higher thyroglobulin concentrations, that is, greater than 2,000 ng/mL. Whether this effect occurs in a particular assay at a particular thyroglobulin concentration depends on the features of the assay. For example, one assay can show a high-dose hook effect at 2,000 ng/mL and another one at 20,000 ng/mL (*13*). However, a falsely low thyroglobulin level can be revealed by using a recovery test (*14*), although Ma et al. (*1*) did not mention this possibility and actual problems with high-dose hook effects are rare in routine practice. As an example, a study in our own clinic using a 1-step IRMA (*14*) found a high-dose hook effect with serum from only 1 of 356 consecutive DTC patients visiting the clinic. This study (*14*) also showed the importance of TgAb measurement with sensitive specific assays in follow-up of DTC patients, although recovery tests were performed. The prevalence of positive TgAb (measured by immunoprecipitation assay (*15*)) was 7.6% in the whole study population, 6.6% in the subgroup of tumor-free patients, and 11.8% in the remaining patients with tumor recurrence. TgAb findings were positive in a significantly higher percentage of patients with local or metastatic disease than in tumor-free patients ($P < 0.001$). Seven of 68 patients with tumor recurrence but a thyroglobulin level less than 1.5 ng/mL (CRM 457) tested positively for TgAb. Furthermore, no patients with a thyroglobulin level greater than 1.5 ng/mL (CRM 457) tested positively for TgAb or demonstrated disturbed recovery test ($P < 0.001$ and $P < 0.05$, respectively). Consequently, a positive TgAb finding pointed to a suspicion of tumor recurrence in patients with negative thyroglobulin findings, in agreement with other reports (*16,17*).

The thyroglobulin ELISA (*10–13*) is about 20-fold more sensitive than conventional thyroglobulin assays and can measure serum thyroglobulin reliably and simply in the follow-up of treated DTC patients without exogenous or endogenous TSH stimulation. Serial measurements of low levels of thyroglobulin by ELISA enable detection of recurrence 6–12 mo earlier than would be possible using a conventional thyroglobulin IRMA. A prospective study is now needed to extend these initial observations.

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REPLY: We greatly appreciate the comments of Dr. Zöphel et al. concerning the sensitivity of enzyme-linked immunosorbent assay (ELISA), the occurrence of high-dose hook effects, and recovery testing for serum thyroglobulin measurement. They noted that the high-dose hook effects in 2-step ELISA for thyroglobulin assays occurred theoretically but not in daily routine (*1*), as was supported by the report that the high-dose hook effects occurred in only 1 of 356 consecutive differentiated thyroid carcinoma (DTC) patients in the whole study population (*2*). Furthermore, they speculated that recovery testing could reveal a falsely low thyroglobulin concentration leading to a false sense of safety in patient management. Generally, we agree with their comments. No current thyroglobulin method is perfect. There can be a 4-fold between-method variability that precludes the use of different thyroglobulin methods for serial monitoring of DTC patients (*3*). Therefore, the functional sensitivity of thyroglobulin methods, including ELISA, is reported differently. We also acknowledge that the retrospective study by Zöphel et al. (n = 126) suggests that serial measurements of low levels of thyroglobulin by ELISA in treated patients with DTC enable detection of recurrence (without using thyroid-stimulating hormone stimulation) 6−12 mo earlier than would be possible using a conventional thyroglobulin immunoradiometric assay (*4*). However, as the authors put it, a prospective study is now needed to confirm their observations. According to the abstract of the paper by Görges et al. (*5*), we cannot identify the immunoenzymatic assay as ELISA. As for recovery testing for serum thyroglobulin measurement, recently it was reported that the potential for thyroglobulin autoantibody (TgAb) interference is only weakly related to the TgAb concentration, and even low levels have the potential to interfere (*6–8*). Therefore, guideline 46 of the National Academy of Clinical Biochemistry states that "recovery tests do not reliably detect TgAb and should be discouraged and eliminated" (*6*). Although TgAb interference with serum thyroglobulin measurements, especially when made by immunometric assay methodology, is likely to remain a problem for the foreseeable future, serial TgAb concentrations are, fortunately, a useful surrogate tumor marker test for monitoring the disease status of TgAb-positive DTC patients (*9*).

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