Detection of Serum Deoxyribonucleic Acid Methylation Markers: A Novel Diagnostic Tool for Thyroid Cancer

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Context: Serum DNA methylation markers may potentially be useful in diagnosing thyroid cancer and monitoring its recurrence.

Objective: The objective of the study was to assess the utility of serum DNA methylation as a diagnostic test for patients with thyroid nodules and a monitoring test to detect thyroid cancer recurrence in previously treated patients.

Design, Setting, and Subjects: Using real-time quantitative methylation-specific PCR, we analyzed the methylation status of five genes (CALCA, CDH1, TMP3, DAPK, and RARβ2) on 96 bisulfite-treated serum DNA samples isolated preoperatively from either solid thyroid nodule patients or patients in follow-up for history of treated thyroid cancer.

Main Outcome Measure: Diagnostic sensitivity, specificity, and accuracy of serum DNA methylation marker for thyroid cancer were measured.

Results: For the patients with thyroid nodules, when a positive result was defined by a serum methylation level above the appropriately chosen cutoff value for any one of the five genes, the preoperative diagnostic sensitivity for thyroid cancer was 68% (26 of 38), the specificity was 95% (18 of 19), and the overall preoperative diagnostic accuracy was 77%, with positive and negative predictive values of 96 and 60%, respectively. In a subset of patients with cytologically indeterminate thyroid nodules, serum DNA methylation testing could correctly diagnose eight of 11 (73%) cancers and four of four (100%) benign tumors, with a diagnostic accuracy of 80%. We also analyzed these serum DNA methylation markers in 39 previously treated thyroid cancer patients. Among the 10 patients proved to have recurrent disease by conventional measures, seven (70%) were positive on methylation testing. Among the 29 patients who had no corroborating of residual or recurrent disease by conventional studies, six (21%) were positive for serum DNA methylation markers.

Conclusions: We have demonstrated the potential usefulness of serum DNA methylation markers as a novel tool for differential diagnosis of solid thyroid nodules and thyroid cancer recurrence monitoring. (J Clin Endocrinol Metab 91: 98–104, 2006)

FOLLICULAR EPITHELIAL CELL-DERIVED thyroid cancers are the most common endocrine malignancies, with papillary and follicular thyroid cancers (PTC and FTC) accounting for 95% of all thyroid malignancies (1). When diagnosed early, these cancers are generally curable. Consequently, accurate differentiation of malignant from benign thyroid nodules is vital. This is currently accomplished by cytological evaluation of needle-aspirated materials (2). Whereas this cytological assessment is diagnostic in the majority of patients, approximately 15–20% have insufficient cytological findings (3). The procedure also engenders some patient anxiety and causes certain discomfort. Moreover, the cost can be considerable, especially if the procedure is performed under sonographic guidance. Therefore, an alternative diagnostic approach using an accurate blood test would be attractive for evaluating thyroid nodule patients.

Thyroid cancer frequently recurs, causing significant morbidity and even mortality, particularly when extensive metastasis has occurred (4, 5). Therefore, after the initial treatment, it is critical to monitor patients for cancer recurrence, a process that currently depends on serum thyroglobulin (Tg) measurement, radiiodine scans, and other imaging and biopsy techniques (6–8). Because the utility of Tg testing is limited by autoantibody interference in 25% of patients (8, 9), previous studies have examined the potential value of alternative circulating tumor markers, such as Tg mRNA (10). However, clinical use of such markers has been discouraged by their limited sensitivity and specificity (11).

Circulating serum methylated DNA may potentially be a novel diagnostic marker for thyroid cancer. DNA methylation is a process that entails the addition of a methyl group to the fifth carbon position of the cytosine residue in a CpG dinucleotide and usually occurs in CpG islands located in the 5′-flanking promoter regions of genes (12). Aberrant gene methylation is common in human cancers, such as the genes for calcitonin (CALCA) (13), E-cadherin (CDH1) (14), tissue
inhibitor of metalloproteasine 3 (TIMP3) (15), death-associated protein kinase (DAPK) (16), and retinoic acid receptor-β2 (RARβ2) (17) in various human cancers, including thyroid cancer (18, 19). Many of these genes are tumor suppressor genes, and their epigenetic silencing is believed to play an important role in tumorigenesis. Because aberrant DNA methylation usually occurs somatically in cancers and can be released into blood circulation, it can serve as a specific diagnostic blood test for cancer (20). Examples include circulating methylated DNA for diagnostic blood test for cancer (20). Examples include cir-
can be released into blood circulation, it can serve as a specific

Patients and Methods

Patients, blood collection, and DNA isolation

Based on a protocol approved by our institutional review board and with appropriate patient consenting, blood was collected from patients at the Johns Hopkins Hospital and the Johns Hopkins Bayview Medical Center. A total of 96 patients were recruited for this study, including 57 patients from whom blood was drawn during the clinical follow-up for their history of treated thyroid cancer. Among the 57 thyroid nodule patients, 39 patients subsequently underwent total thyroidectomy for thyroid nodules diagnosed cytologically as cancer or suspicious for cancer or causing compressive symptoms, and the remaining four patients with solitary thyroid nodules that were cytologically confirmed to be benign adenomas were not operated. The patients were confirmed to have solid nodules by either preoperative ultrasoundography or postoperative pathology examination. Among the operated patients, postoperative histopathological analysis confirmed thyroid cancer in 38 patients (31 PTC and 7 FTC) and benign solid thyroid neoplasms (adenoma and nodular hyperplasia) in 15 patients. Therefore, a total of 19 benign cases were included for the study. Among the 39 patients in follow-up for their history of treated thyroid cancer (37 PTC and 2 FTC), at the time of blood draw, 10 patients had recurrent thyroid cancer and 29 patients had no evidence of cancer recurrence as documented by conventional tests, including recombinant human (rh)TSH-stimulated Tg and radiiodine body scan, positron emission tomogra-

Primers and probes were designed to specifically amplify the bisulfite-converted genes in serum was most prevalent, whereas methylation of the rest was hardly detectable in serum. We subsequently focused on these five genes and expanded their testing to the whole series of the patients recruited in the present study. Each sample was measured in duplicates or triplicates. The primers and probes used for these genes and the internal reference gene, β-actin, are summarized in Table 1 as previously described (25–27). Fluorogenic QMSP assays were carried out in a reaction volume of 20 μl on a 384-well plate in an Applied Biosystems 7900 sequence detector (PerkinElmer, Foster City, CA). The reaction mixture contained 600 nm forward and reverse primers; 200 nm probe; 0.75 U platinum Taq polymerase (Invitrogen); 200 μM each of dATP, dCTP, dGTP, and dTTP; 16.6 mM ammonium sulfate; 67 mM Trizma; 6.7 mM MgCl2; 10 mM mercapto-

| Genes | Forward primer sequence (5’-3’) | Probe sequence (5’-3’) | 6FAM-DNA sequences-6FAMRA | Reverse sequence (5’-3’) |
|-------|--------------------------------|------------------------|---------------------------|------------------------|
| β-Actin | TGG TGA TGG AGG AGG TTT AGT AGG T | ACC ACC ACC CA ACC A A T A TA C C A T A A C A | A C A C A C A C | ACC CAA TAA ACA CTA CTC TCC |
| CALCA | GGT TTG GGA GTA TGA GGC TGA CG | ACC CCA ATA CAC AAC A A C | TCC CCA CCA CCA | TCC TAA CTA A A C |
| CDH1 | AAT TTT AGG TTA GAG GGT TAT CCG GT | GGC CCA CCC GAC TCA G | A A C A | TCC CAA A A GA AAC T AA GA C |
| TIMP3 | GCC TGG GAG GTT AAG GGT GTT | AAG TCC CCA CTC GCC CGA C A | CTC TAA CAA ATT ACC GTA CG | TCC CCA CAA A A C |
| DAPK | GGA TAG TCG CAG GTA AGT A GTC | GGT TGG | TGG TAA CCG GAA CAC CAG | CCG TCC CAA A A C |
| RARβ2 | GGG ATT AGA ATT TTT TAT GGC AGT TG | TGG CCA GAA CGC GAG CGA T | TCC TAA CCA | TCC CCC GAC GAT ACC CAA C |

Sensitivity and specificity

Thyroid cancer diagnostic sensitivity of serum DNA methylation markers was calculated as the number of cases with positive methylation

Bisulfite treatment of DNA

DNA samples were subjected to bisulfite treatment as described previously (24). Briefly, 1–2 μg of DNA in a volume of 20 μl were denatured by incubation with 0.2 M NaOH for 20 min at 50 C, followed by incubation in 500 μl of a freshly prepared solution containing 10 mM hydroquinone and 3 mM sodium bisulfite for 3 h at 70 C. The treated DNA sample was then desalted through an affinity chromatography column (Wizard DNA clean-up system, Promega, Madison, WI), treated with 0.3 M NaOH for 10 min at room temperature, and precipitated with ethanol. The bisulfite-modified genomic DNA was resuspended in 30 μl of the EDTA-Tris buffer described above and stored at −80 C.

Real-time quantitative methylation-specific PCR (QMSP)

Bisulfite-modified DNA was used as the template for fluorescence-based QMSP (Taqman) as previously described (25). Briefly, the primers and probes were designed to specifically amplify the bisulfite-converted gene of interest. To determine the relative levels of methylation in each sample, the values of the gene of interest were compared with the values of the internal reference gene (β-actin) to obtain a ratio, which was then multiplied by 1000 for convenient tabulation [i.e. (target gene/β-actin) × 1000]. Because the amount of serum DNA obtained was limited, we first tested serum DNA methylation in a subgroup of thyroid cancer patients on a large panel of genes that were known to be methylated in primary thyroid tumors (Refs. 18 and 19 and our unpublished data). These included HIC1, P16, RASSF1A, MINT31, GSTP1, TGFBR1, TG1, CALCA, CDH1, TIMP3, DAPK, and RARβ2 genes in serum was most prevalent, whereas methylation of the rest was hardly detectable in serum. We subsequently focused on these five genes and expanded their testing to the whole series of the patients recruited in the present study. Each sample was measured in duplicates or triplicates. The primers and probes used for these genes and the internal reference gene, β-actin, are summarized in Table 1 as previously described (25–27). Fluorogenic QMSP assays were carried out in a reaction volume of 20 μl on a 384-well plate in an Applied Biosystems 7900 sequence detector (PerkinElmer, Foster City, CA). The reaction mixture contained 600 nm forward and reverse primers; 200 nm probe; 0.75 U platinum Taq polymerase (Invitrogen); 200 μM each of dATP, dCTP, dGTP, and dTTP; 16.6 mM ammonium sulfate; 67 mM Trizma; 6.7 mM MgCl2; 10 mM mercapto-

| Gene | Forward primer sequence | Probe sequence | Reverse primer sequence |
|------|-------------------------|----------------|------------------------|
| β-Actin | TGG TGA TGG AGG AGG TTT AGT AGG T | ACC ACC ACC CA ACC A A T A TA C C A T A A C A | A C A C A C A C | ACC CAA TAA ACA CTA CTC TCC |
| CALCA | GGT TTG GGA GTA TGA GGC TGA CG | ACC CCA ATA CAC AAC A A C | TCC CCA CCA CCA | TCC TAA CTA A A C |
| CDH1 | AAT TTT AGG TTA GAG GGT TAT CCG GT | GGC CCA CCC GAC TCA G | A A C A | TCC CAA A A GA AAC T AA GA C |
| TIMP3 | GCC TGG GAG GTT AAG GGT GTT | AAG TCC CCA CTC GCC CGA C A | CTC TAA CAA ATT ACC GTA CG | TCC CCA CAA A A C |
| DAPK | GGA TAG TCG CAG GTA AGT A GTC | GGT TGG | TGG TAA CCG GAA CAC CAG | CCG TCC CAA A A C |
| RARβ2 | GGG ATT AGA ATT TTT TAT GGC AGT TG | TGG CCA GAA CGC GAG CGA T | TCC TAA CCA | TCC CCC GAC GAT ACC CAA C |
markers divided by the total number of cases with cancer. The specificity was calculated as the number of benign cases with negative methylation markers divided by the total number of cases with benign neoplasm.

Results

Real-time QMSP on serum DNA

We performed QMSP on the five genes, CALCA, CDH1, TIMP3, DAPK, and RARβ2, in serum DNA isolated from patients with various thyroid tumor-related clinical backgrounds. To ensure the quantity and quality of DNA samples before they were applied to real-time QMSP analysis on these genes, each sample was subjected to β-actin gene amplification analysis first. Shown in the first (left upper) panel of Fig. 1 is a representative of real-time PCR amplification of β-actin on a serum DNA sample and on the control DNA samples (isolated from leukocytes in normal subjects). The remaining panels in Fig. 1 show representative QMSP amplification of the five genes and positive control samples (in vitro methylated leukocyte DNA), serum DNA samples from patients with thyroid cancer, and serum DNA samples from patients with benign thyroid neoplasm. The difference in methylation level among these three situations is illustrated in Fig. 1, as reflected by the difference in PCR cycles needed to reach the same signal level. The frequency of detectable methylation and the level of methylation of each gene for patients with thyroid cancer or benign neoplasm are shown in Tables 2 and 3 and Fig. 2. Among the five genes examined, CALCA showed the highest frequency and level of methylation, followed by CDH1, and TIMP3, DAPK, and RARβ2 showed the lowest frequencies and levels in methylation.

Test of the specificity and sensitivity of serum DNA methylation markers in distinguishing benign thyroid nodules from thyroid cancers

Taking the advantage that we had preoperative blood samples from patients with thyroid nodules who had confirmed postoperative histological diagnoses, we focused first on the utility of serum DNA methylation markers in distinguishing thyroid cancers from benign thyroid nodules, using the former to investigate the diagnostic sensitivity and the latter the specificity. As shown in Fig. 2 and Table 3, methylation of the TIMP3 and RARβ2 genes in serum DNA occurred only in patients with thyroid cancer but not in patients with benign thyroid nodules. Overlap in methylation level between thyroid cancer and benign thyroid neoplasm was seen in each of the remaining three genes, most prominently in CALCA, less so in CDH1, and least in DAPK gene (Fig. 2 and Table 3). As shown in Table 3, with appropriate cutoff values, with more weight toward specificity from sensitivity, excellent diagnostic specificity, ranging from 95 to 100%, was obtained for each of the five genes. The sensitivity of each individual gene was relatively low. However, with these cutoff values of methylation, the overall diagnostic sensitivity of the five genes for thyroid cancer, when at least one of the five genes was positive, rose to 68% (26 of 38), the overall specificity was 95% (18 of 19), and the overall preoperative diagnostic accuracy was 77%, with positive and negative predictive values of 96 and 60%, respectively.

Usefulness of serum DNA methylation markers in evaluating thyroid nodules with indeterminate cytology

Because indeterminate cytological finding on a thyroid nodule is currently a problematic diagnostic dilemma in the evaluation of thyroid nodule patients (3), we wondered whether serum DNA methylation testing could help with this special clinical situation. To this end, we applied the five genes as a panel with the above-defined cutoff methylation values to a subset of 15 thyroid nodule patients with indeterminate cytology. None of the four patients subsequently proven histologically to have benign neoplasm was positive for any of the five serum DNA methylation markers, whereas eight of the 11 patients (73%) with thyroid nodules later proven to be thyroid cancers had positive serum methylation markers, involving one or more of the five methylation markers.

Test of the utility of serum DNA methylation markers in the monitoring of thyroid cancer recurrence

We also examined the serum methylation markers of the five genes described above in a group of 39 patients who had a history of thyroid cancer treated with total thyroidectomy and radioiodine ablation in the past and were being followed up at our endocrine clinic. Among these, 10 patients had thyroid cancer recurrence or ongoing thyroid cancer disease, as evidenced by persistently elevated Tg, positive radioiodine body scan, positive biopsy studies, and/or other positive imaging studies (e.g. positron emission tomography/computed tomography scan). The remaining 29 patients had no evidence of cancer recurrence based on conventional clinical and laboratory studies, including negative rTSH-stimulated Tg and radioiodine body scan. When the five genes as a panel with the cutoff values of methylation described above (Table 3) were applied to the 10 patients with cancer recurrence, seven of them (70%) had positive test results, involving one or more of the five genes. Among the three recurrent cases that missed the detection by serum methylation test, two had detectable Tg levels of 1.5 and 2.3 ng/ml, respectively, without other evidence of cancer recurrence (e.g. with negative radioiodine body scan). The third one had a Tg level of 11.5 ng/ml with an ongoing cancer mass in the neck. The seven recurrent patients that were detected by serum methylation markers all had more aggressive evidence for cancer recurrence. Among the 29 patients without evidence for cancer recurrence based on conventional testing, 23 (79%) had negative serum DNA methylation marker testing for any of the five genes, but six (21%) also had positive results involving one or more of the four genes of CALCA, CDH1, TIMP3, and DAPK, with five of the six cases involving only TIMP3 and DAPK genes.

Discussion

Current clinical practices for diagnosis, treatment, and postoperative monitoring of patients with thyroid cancer are relatively effective. However, there remain limitations in assessing the nearly 300,000 thyroid nodule patients seen annually in the United States (28) and in following up the also nearly 300,000 survivors of thyroid cancer (29). These clas-
classical problems in thyroid cancer medicine have long been the focus of studies with the goal to find novel and effective diagnostic serum markers. In our studies, the potential value of detecting gene methylation in circulating DNA as diagnostic markers to address the shortcomings of current diagnostic testing and monitoring for thyroid cancer has been addressed.

Numerous serum markers have previously been explored,
including, for example, serum soluble IL-2 receptor (30), Tg mRNA (10), RET/PTC1 mRNA (31), tissue polypeptide-specific antigen (32), thyroperoxidase protein (33), and thyroperoxidase mRNA (34). Among these, serum Tg mRNA detection has drawn particular attention since the initial report by Ditkoff et al. (10). Unfortunately, variability in diagnostic specificity and sensitivity of serum Tg mRNA, reflecting the unstable nature of RNA, apparent existence of an extrathyroidal Tg mRNA source, and loss of Tg expression in some recurrent thyroid cancers, have limited the clinical utility of this test (11, 35).

There are several reasons to support the hypothesis that serum DNA methylation might be a useful diagnostic marker for thyroid cancer. First, gene methylation commonly occurs in human cancers, including thyroid cancer (Refs. 18, 23, 36–39, and our unpublished data). Second, methylated DNA has been shown to be commonly dislodged from cancers and released into serum (20), in which it has previously been shown to reflect presence of other human cancers (21–23). Third, DNA, unlike mRNA, is a stable molecule, offering the promise of greater test stability. Fourth, DNA methylation can be quantitatively analyzed using precise quantitative methylation-specific PCR, which has now become a well-established and widely available technique.

In this first study applying serum DNA methylation markers to thyroid cancer testing, we have confirmed the measurability of serum DNA methylation markers and preliminarily defined the specificity and sensitivity of a set of such markers for diagnosis and detecting recurrence of thyroid cancer. Using the five genes, CALCA, CDH1, TIMP3, DAPK, and RAR\(_B2\) for differential diagnosis of thyroid nodules with retrospectively defined cutoff values, we were able to identify positive gene methylation in about 70% of cancerous nodules, whereas methylation levels were beneath the cutoff level in 95–100% of benign thyroid nodules (Table 3). Among a subset of patients with cytologically indeterminate thyroid nodules, serum DNA methylation similarly identified about 70% of the subsequently confirmed thyroid cancer, whereas all cytologically indeterminate nodules that proved to be benign lacked methylation marker positivity.

The current first-line test to detect residual or recurrent thyroid cancers is serum Tg measurement, a technique that has several limitations: the presence of Tg autoantibodies in 25% of thyroid cancer patients (9), the need for TSH stimulation to achieve optimal Tg sensitivity (40), and loss of Tg expression in some recurrent thyroid cancers (41). It is important to see that most (70%) of the recurrent thyroid cancer patients were positive for disease existence on serum DNA methylation testing on the five genes. It is interesting to see that 21% of the patients at follow-up for their history of thyroid cancer were also positive for disease existence on serum DNA methylation testing when conventional studies, including rhTSH-stimulated serum Tg and radioiodine body scan, did not reveal cancer recurrence. Loss of Tg production and negative radioiodine body scan are not uncommon in recurrent thyroid cancers and even false-negative rhTSH-stimulated serum Tg or radioiodine body scan are sometimes seen in recurrent thyroid cancer (40–43). We therefore cannot be certain that in the present series those patients with positive serum DNA methylation markers but without conventional evidence for thyroid cancer recurrence truly had no ongoing disease. It remains to be seen whether serum DNA methylation testing may identify this group of patients as having a special risk for progression into overt thyroid cancer recurrence. Given the high specificity (95–100%) of the serum DNA methylation markers to exclude thyroid cancer, it may be reasonable to follow vigilantly these patients at least clinically or perhaps with more aggressive studies such as a positron emission tomography scan to be more certain on the patient’s thyroid cancer status. It remains also as a possibility that nonthyroidal cancer may exist in some of these patients that were positive for serum methylation markers as these markers were seen also in other types of cancer.

Two factors might contribute to the differences in serum methylation markers between malignant and benign thyroid tumors: a greater extent of gene methylation in thyroid cancers than in normal tissue or benign neoplasms and the possibility that thyroid cancer cells, or DNA fragments from cancer cells, are more readily dislodged and released into the blood than benign thyroid nodule cells. The former postulate is supported by previous and recent studies showing generally a high level of gene methylation in thyroid cancers than in benign thyroid neoplasms (19, 39). The latter postu-

### TABLE 2. Frequencies of serum DNA methylation in various genes in thyroid tumor patients [percent (number of methylation-positive cases/total cases)]

| Genes   | Thyroid cancer | Benign thyroid tumor |
|---------|----------------|----------------------|
| CALCA   | 87 (33/38)     | 63 (12/19)           |
| CDH1    | 68 (26/38)     | 47 (9/19)            |
| TIMP3   | 21 (8/38)      | 0 (0/19)             |
| DAPK    | 32 (12/38)     | 11 (2/19)            |
| RAR\(_B2\) | 32 (12/38)       | 0 (0/19)             |
| All five genes | 95 (36/38) | 74 (14/19) |

### TABLE 3. Sensitivity and specificity of serum DNA methylation in various genes for thyroid tumors [percent (positive methylation/total cancer cases, sensitivity; negative methylation/total benign cases, specificity)]

| Genes   | Sensitivity | Specificity | Cut-off values | Ranges of methylation |
|---------|-------------|-------------|----------------|-----------------------|
|         | Cancer      | Benign      |                |                       |
| Individual genes |              |             |                |                       |
| CALCA   | 29 (11/38)  | 100 (19/19) | 38.0           | 0.000–430.677          | 0.000–37.021 |
| CDH1    | 24 (9/38)   | 100 (19/19) | 7.0            | 0.000–63.595           | 0.000–6.978  |
| TIMP3   | 21 (8/38)   | 100 (19/19) | 0.0            | 0.000–181.576          | 0.000–0.000  |
| DAPK    | 32 (12/38)  | 95 (18/19)  | 0.003          | 0.000–19.059           | 0.000–3.881  |
| RAR\(_B2\) | 32 (12/38)       | 100 (19/19) | 0.0            | 0.000–16.868           | 0.000–0.000  |
| All five genes | 68 (26/38) | 95 (18/19)  | N/A            | N/A                   | N/A         |
late is consistent with previous findings that peripheral blood Tg mRNA and TSH receptor mRNA were mostly associated with thyroid cancer, but not benign thyroid nodules or normal thyroid glands (44, 45). This is also supported by the pattern of serum methylation markers in the present study that showed a clear dissociation between cancer and benign thyroid nodules, although benign primary thyroid tumors also harbored methylation in these genes (19). The apparent detectable methylation of some of these tumor suppressor genes in normal thyroid tissues in the previous study may represent contamination from tumor tissues as tissue microdissection was not performed in this study (19). It should be noted that the benign thyroid tumors included in the analysis in this study were all solid tumors without cysts; cysts in thyroid nodules, which are usually caused by necrosis, may conceivably tend to shed cells more readily into blood. This notion is supported by our findings in four cystic benign thyroid nodules, three of which had a level of serum DNA methylation markers above the cutoff values set for cancer detection (data not shown). This suggests that serum DNA methylation markers of the genes analyzed in the present study may have limited usefulness in patients with cystic thyroid nodules.

In summary, we have explored the diagnostic utility of serum DNA methylation markers in both differential diagnosis of thyroid nodules and monitoring of treated thyroid cancer patients for recurrence. With five genes examined, we were able to achieve a reasonably high sensitivity and excellent specificity in identification of thyroid cancers from benign thyroid nodules. We have also demonstrated that these methylation markers can be used to diagnose recurrent thyroid cancer with a relatively high sensitivity. A limitation in early assessment of the accuracy of any novel diagnostic test is the need to define retrospectively the criteria for a positive test, which is putatively associated with presence of disease. More definitive demonstration of the sensitivity and specificity of serum DNA methylation markers for thyroid cancer detection will require application of prospectively defined normal and abnormal ranges to a large population of thyroid nodule and cancer patients. Use of additional serum DNA methylation markers may improve the accuracy of this novel mode of testing. Nonetheless, this first application of serum DNA methylation markers to thyroid cancer diagnostic testing demonstrates that, in principle, the technique may hold promise for enhancing management of patients with thyroid nodules and cancers.
Acknowledgments

Received August 10, 2005. Accepted October 26, 2005.
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This work was supported by a research grant from the Flight Attendant Medical Research Institute and a Johns Hopkins Clinician Scientist Award (to M.X.). M.B. was from Fundacao Antonio Prudente/Ludwig Institute for Cancer Research, Sao Paulo, Brazil, and was supported by CAPES Scholarship BEX 2516/03-9.

The authors have no conflict of interest.

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