The main finding of the survey was the unacceptably large interlaboratory variation not only in OCB numbering, as already ascertained in 2005 (5), but also in qualitative reporting of the OCB pattern and in differentiating OCB-positive from OCB-negative samples. Misinterpretation of control A as a mirror pattern leads to a misleading suspicion of systemic inflammation/disease. Similarly, but with less impact on diagnostic workup, the exceedingly high number of serum bands found by some centers in controls B and C indicates acute, rather than chronic (as in the case of OCB unique to CSF) inflammatory disorders of the central nervous system. More important was the variation in OCB identification in control D, with 65% of survey responses indicating OCB-positive and/or neurological background seemed absolutely critical and may be substantially responsible for unacceptably large interlaboratory variation not only in OCB was the unacceptably large interlaboratory variation.

The participation of new centers that lack specialized sections for CSF analysis [general laboratories (GL)] probably accounts for the worsening in results between the 2005 (2 of 11 GLs) and 2006 (8 of 20 GLs) surveys. The interlaboratory differences were not gel dependent (data not shown). Possible remaining causes include (a) misinterpretation of artifactual bands, which derive from a nonhomogeneous pH gradient, as true bands (OCB-negative controls should help identify gradient-dependent bands), which would yield erroneous mirror patterns and exceedingly numerous OCB; (b) insufficient IEF skills, which could lead to underestimation of OCB (OCB-positive and hemoglobin controls should be built into IEF protocols); and (c) poor blotting and staining skills, which could distort OCB interpretation.

Our findings indicate that CSF analysis for OCB detection should be performed by experienced laboratories carefully selected by neurologists (2). Inadequate technical training and/or neurological background seem to be substantially responsible for unreliable OCB detection. Analysis of CSF samples with few and weak bands is absolutely critical and may yield false-negative results, even in experienced laboratories. Through educational support and external quality control schemes, scientific associations involved in CSF analysis play essential roles in promoting quality.

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References

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erence value for cardiac troponin I (cTnI) and T (cTnT) for the detection of myocardial injury (1, 2). In the presence of ischemia, cTn above the 99th percentile has become the cornerstone for the definition of acute myocardial infarction (MI) in non-ST-elevation MI (1, 2), leading manufacturers of in vitro diagnostic tests to improve low-end analytic sensitivity and precision. We determined the 99th percentile values for 3 2nd-generation cTnI assays in serum and/or plasma.

After receiving appropriate institutional review board approval, we obtained frozen (-20 °C up to 30 days) serum samples from 2992 apparently healthy volunteers. Because this group of samples was collected for another study, data on donor age and sex were unavailable, a study limitation. We also obtained 2000 plasma (heparin) samples from a separate group of healthy individuals, 75% male, age 18–66 years. These fresh specimens were refrigerated for 24–48 h before analysis. cTnI concentrations were measured by use of the following US Food and Drug Administration–cleared assays: the Ortho-Clinical Diagnostics (OCD) Vitros Troponin I-ES assay on the Vitros ECi /ECiQ System, the Abbott Diagnostics Architect STAT Troponin-I on the i2000SR System, and the Beckman Coulter Access Accu TnI on the Access system. Results were reported to 3 decimals for the Abbott and OCD assays and to 2 decimals for the Beckman assay. All specimens were analyzed on the 3 different assays on the same day for serum or plasma. According to package inserts, limits of detection were ≤0.010 μg/L for the Abbott, 0.01 μg/L for the Beckman, and 0.012 μg/L for the OCD assay. As determined in the current study, 99th percentiles for plasma (heparin) were 0.012 μg/L (n = 224) for Abbott, 0.04 μg/L (n = 254) for Beckman, and 0.031 μg/L (n = 2000) for OCD. We determined 99th percentile reference cutoffs by nonparametric statistics following Clinical and Laboratory Standards Institute guidelines C28-A2. Our laboratory has documented <10% cTnI variability within specimens for cTnI in serum and plasma frozen at ≤-20 °C for 30 days.

Fig. 1 shows the histogram distributions for serum cTnI by OCD (top), Beckman (middle), and Abbott (bottom) assays. The 99th percentiles were 0.034 μg/L, 0.04 μg/L, and 0.025 μg/L, respectively. Maximum concentrations were OCD 0.108 μg/L, Beckman 0.31 μg/L, and Abbott 1.124 μg/L (confirmed by repeat analysis; assay results for the same specimen were 0.01 μg/L for Beckman and 0.003 μg/L for OCD). The skewed histograms demonstrated that 88% (n = 2637) of the OCD, 98% (n = 2930) of the Abbott, and 33% (n = 979) of the Beckman assay results were below the limit of detection. Each assay also showed different numbers of samples (a) between the limit of detection and 99th percentile limits [Abbott n = 33 (1%), OCD n = 319 (11%), Beckman n = 1994 (67%)] and (b) greater than the 99th percentile [Abbott n = 29 (1%), Beckman n = 19 (1%), OCD n = 36 (1%)]. Only 3 samples had cTnI concentrations greater than the 99th percentile according to all 3 assays. Similar observations were found for plasma, with a 99th percentile of 0.031 μg/L for OCD and 0.04 μg/L for Beckman (histograms not shown because of nonsignificant differences with serum). The Abbott assay was not analyzed for plasma, because of lack of funding and inadequate volumes. There were no significant cTnI differences between samples from donors of different sexes or across donor age by decade, with age limited to 66 years.

Our findings represent the largest database for 99th percentile values determined on 2nd generation cTnI
assays for serum and plasma. The nonsignificant differences between cutoffs for serum and plasma and between assays are encouraging regarding the ease of interpretation by clinicians and laboratories. Also encouraging were improvements in the analytical sensitivity of these newer-generation assays. Interestingly, only 3 samples were identified as above the 99th percentile by all 3 assays. The mechanisms responsible for this lack of agreement are not known, but antibody differences and the cTnI epitopes recognized on the different circulating cTnI forms are the most likely causes. The discrepancies between assays in regard to individual samples identified as above the 99th percentile raise questions as to whether healthy individuals can be better characterized for use in defining reference limits. A prototype cTnI assay has shown sensitivity to 0.001 μg/L with gaussian-distributed results. As assays become more sensitive, increasing numbers of MIs will be detected, but an increased prevalence of myocardial injury not related to ischemic pathologies also will be seen. The latter may complicate schemes for selecting healthy individuals when defining reference limits. The 99th percentile reference cutoffs derived here in 2 large apparently healthy populations for 3 2nd-generation cTnI assays should prove useful in clinical practice.

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References


To the Editor:

Alfa-fetoprotein (AFP) and serum human chorionic gonadotropin (hCG) are reliable markers of testicular cancer, and treatment of a relapse is often initiated on the basis of marker increase alone. Slightly increased hCG concentrations have occasionally been misinterpreted to indicate a relapse, leading to inappropriate chemotherapy (1). We describe a seminoma patient in whom a relapse was suspected 10 years after therapy because the patient had increased hCG concentrations found to be caused by hypogonadism-induced pituitary hCG secretion.

A 27-year-old man underwent left radical orchectomy and adjuvant radiotherapy for stage I testicular seminoma in the early 1990s at Helsinki University Central Hospital. The patient had a preoperative serum hCG of 0.5 IU/L (upper reference limit 0.7 IU/L) and AFP <1 IU/L (upper reference limit 9 IU/L). Atrophy of the nonmalignant testicle was suspected on the basis of preoperative ultrasound findings, but the serum testosterone concentration, 10.2 nmol/L, was within the reference interval (10–38 nmol/L), whereas follicle-stimulating hormone (FSH) concentration was increased, at 28 IU/L (reference interval 1–7 IU/L), suggesting partially compensated hypogonadism. One year later, examinations revealed a subnormal serum testosterone concentration and azoospermia. At this point the patient’s hCG had increased to 3.7 IU/L, FSH to 50 IU/L, and luteinizing hormone (LH) to 20 IU/L (reference interval 1–9 IU/L). Testosterone replacement therapy was administered, but the patient discontinued its use within a few weeks. During the next 2.5 years, when he did not receive replacement therapy, the serum concentration of hCG remained slightly increased. Intramuscular testosterone replacement therapy was reintroduced 3.5 years after surgery, and serum concentrations of hCG, FSH, and LH normalized. Approximately 9 years after surgery, the patient stopped the testosterone medication because of acne. His hCG gradually increased to 4.5 IU/L, and this finding led to suspicion of a tumor relapse. Serum testosterone was 2.9 nmol/L, FSH 62 IU/L, and LH 31 IU/L, indicating hypogonadism. Testosterone therapy was reinitiated and hCG, FSH, and LH concentrations decreased rapidly (Fig. 1). Follow-up consisting of radiographic imaging, serum tumor marker determinations, and clinical examinations was discontinued a few months later, almost 11 years after primary therapy. Apart from the increasing serum hCG concentration, there were no other signs of relapse during follow-up.

The pituitary is a source of hCG, and low serum concentrations can be detected with sensitive assays in most healthy men and women (2, 3).