The Paradox in Translational Medicine

To the Editor:

Advances in laboratory sciences have raised expectations of discovery of clinically useful biomarkers, but few such new tests have appeared to date. Hortin et al. (1) highlighted several challenges in the translation of promising markers into clinical laboratory tests. Translational medicine, which is currently defined as the translation of basic research into practical clinical applications, has great potential to develop and deliver new tools that may assist prevention, diagnosis, and treatment of disease (2). In the field of laboratory medicine, the transfer of promising research assays to daily laboratory practice is a challenge that may take several years and involves many sequential processes: development and validation of clinical assays, release of reagents and systems by diagnostic companies, evaluation of the analytical and clinical performances of the commercial assays in the field, and reliable implementation into clinical practice through training laboratory professionals and refining interpretation and utilization of the new information by all medical personnel (3).

Major emphasis is currently placed on uncovering theoretical and methodological difficulties that would explain why only a minority of experimental research has made the transition to the clinical domain and produced new diagnostic tests (1, 4). This inefficiency is attributable not only to obvious and well-recognized drawbacks, such as methodological biases in animal experimentation and differences between animal and human pathophysiology, but also to additional scientific, financial, ethical, regulatory, and practical hurdles (3). Ideally, experimental and clinical research share the same targets of improving disease understanding and cost-effective decision-making. In reality, however, these pursuits frequently evolve through parallel rather than coordinated tracks. Much of the failure in this translation arises from the lack of effective communication between clinicians and researchers; a collaborative rather than competitive relationship between the two groups will be beneficial (3).

Translational investigations typically rely on large research consortia and population-based plasma banks that couple biomarker information with longitudinal observational data. Basic research, however, usually includes specific hypothesis-driven studies that have small sample sizes and are conducted by independent academic or industry researchers (2). Because of industry support of some basic research in academic institutions, some medical schools, especially the smaller ones, might need to make compromises for fear that companies would suspend funding. Some companies may take ownership of results although academic researchers have generated them. Biased reporting of the clinical benefits of various diagnostic or therapeutic methods may occur because some agreements signed between academic institutions and diagnostic or drug companies require confidentiality of data, often depending on the outcome. As a consequence, negative results may not be emphasized or submitted to medical journals for publication.

Two other problems merit mention. One is the limited funding for developing new diagnostic products in some specific research fields, because industry tends to seek products with a safer return on investment. Finally, there is a serious concern regarding reliability and reproducibility of results because of sample handling; preanalytical variability may influence profiles of some biomarkers. Therefore, the most feasible and standardized collection procedures should be established before results of a novel and promising diagnostic technique such as high-throughput proteomics can be transferred to daily laboratory practice (5).

Few solutions have been proposed to bridge the gap between basic research and clinical and laboratory practice. Accordingly, translational medicine requires reorganization in a thoughtful cooperative manner between basic researchers, clinicians, laboratory professionals, and manufacturers; it should be more clinically driven and supported by funding independent from any potential conflict of interest.

Grant funding/support: None declared. Financial disclosures: None declared.

References


Giuseppe Lippi1* Mario Plebani2 Gian Cesare Guidi1

1 Sezione di Chimica Clinica Dipartimento di Scienze Morfologico-Biomediche Università degli Studi di Verona Verona, Italy

2 Dipartimento di Medicina Laboratorio Università di Padova, Padova, Italy

* Address correspondence to this author at: Istituto di Chimica e Microscopia Clinica, Dipartimento di Scienze Morfologico-Biomediche, Università degli Studi di Verona, Ospedale Policlinico G.B. Rossi, Piazzale Scuro, 10, 37134 Verona, Italy. Fax 0039-045-8201889; e-mail ulippi@tin.it.

DOI: 10.1373/clinchem.2007.087288

Primary Hyperoxaluria Remains Undiagnosed in Patients with Hyperoxaluria and Recurrent Urolithiasis

To the Editor:

Primary hyperoxaluria (PH) is a metabolic disorder caused by deficiency...
of either the liver-specific enzyme alanine:glyoxylate aminotransferase (AGT) for PH type 1 (PH1) or glyoxylate reductase/hydroxyypyruvate dehydrogenase (GR/HPR) for PH type 2 (PH2). PH increases endogenous oxalate production, leading to the development of renal stones and nephrocalcinosis, which may result in end-stage renal disease (ESRD) (1). The 1st step in the diagnostic process involves measurement of urinary oxalate, glycolate, and/or l-glycerate, followed by enzymatic analysis of AGT (in liver) or GR/HPR (in lymphocytes), or genetic testing of the AGXT gene for PH1 or the GRHPR gene for PH2. Extensive metabolic screening is rarely performed in adults who present with stone disease because PH disease presentation usually occurs in childhood, not in adulthood. Nevertheless, more than one-third of PH1 is diagnosed in adult patients in the Netherlands (2). Early diagnosis and treatment is of utmost importance in PH, because conservative therapy can prevent renal insufficiency (2), particularly pyridoxine therapy in PH1 (3). If treatment is not initiated in a timely manner, ESRD will ensue in these patients. Therefore, adequate diagnostic measures should be taken after the first clinical signs and symptoms have evolved.

We performed a comprehensive search among all nephrologists in the Netherlands (2) and discovered a relatively high prevalence of PH in the Netherlands. The high number of adult patients who were diagnosed only after the development of ESRD (59% for adults) indicates that our search method may have missed patients with PH and prompted us to design a strategy to identify more patients with PH. During a period of 8 years, in one routine academic hospital laboratory, we traced all patients with hyperoxaluria and performed further metabolic and genetic investigation to diagnose or exclude PH.

Since 1995, urinary oxalate has been measured at the Laboratory for General Clinical Chemistry at the Department of Clinical Chemistry in the Academic Medical Center. Until 2003, results of urinary oxalate screening revealed hyperoxaluria in 32 of 150 patients, according to the reference interval we determined with a 24-h urine collection study performed in this laboratory. In 25 of these 32 patients (those we were able to contact) we performed analyses of urinary oxalate, glycolate, and l-glycerate in fresh 24-h urine by ion chromatography (Dionex) and gas chromatography. Hyperoxaluria was detected in 6 patients (clinical characteristics as listed in Table 1). One patient had PH1, confirmed by detection of a Gly170Arg mutation on the so-called minor allele of the AGXT gene. The urinary oxalate excretion rate was only mildly increased because this patient used pyridoxine at the time of urinalysis. The other 5 patients had urine glycolate and l-glycerate excretion rates that were within the reference intervals and therefore in these 5 patients the diagnosis of PH was rejected and no further testing was performed. Four of these 5 patients had developed urinary tract symptoms after the onset of intestinal disease, diagnosed as secondary hyperoxaluria related to malabsorption. The 5th patient did not have evidence of PH or secondary hyperoxaluria and therefore this case was classified as idiopathic hyperoxaluria.

In this study, we searched for PH in a specific patient group in which we suspected that PH may have been undiagnosed (2). Apparently, our strategy led to the discovery of a patient with previously undiagnosed PH. In view of the very low prevalence of PH in populations studied so far, the finding of a new PH patient in a cohort of 150 patients is remarkable and confirms that PH remains undiagnosed in some adult patients, and that the prevalence of this disease may be higher than previously estimated (2). The cases we describe in this report show that PH can be diagnosed only with an immediate and complete diagnostic work-up, a procedure that provides the best opportunity to prevent further renal damage. Therefore, episodes of urolithiasis, nephrocalcinosis, recurrent urinary tract infections, or unexplained decline of renal function require assessment of urinary oxalate to exclude PH. We strongly suggest that performing the same screening strategy in other hospital laboratories will detect more PH patients who may benefit from early recognition and treatment.

As previously reported, hyperoxaluria, dehydration, and other comorbidities may lead to renal involvement (4). Citrate administration decreases the risk of stone formation (5), but this treatment was not used in 5 of the cases we investigated, an omission that may have placed them at higher risk for renal involvement. Therefore, all available conservative measures to decrease the risk of stone formation should be taken in patients with any type of hyperoxaluria.

Grant/funding support: None declared. Financial disclosures: None declared.

Table 1. Characteristics of patients with hyperoxaluria.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age at inclusion, years</th>
<th>Age at first symptoms, years</th>
<th>Symptoms</th>
<th>Urinary oxalate, mmol/mol creatinine</th>
<th>Comorbidity</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>59</td>
<td>2</td>
<td>UNI&lt;sup&gt;b&lt;/sup&gt;</td>
<td>60</td>
<td></td>
<td>PH1</td>
</tr>
<tr>
<td>2</td>
<td>85</td>
<td>34</td>
<td>U</td>
<td>80</td>
<td></td>
<td>IH</td>
</tr>
<tr>
<td>3</td>
<td>55</td>
<td>51</td>
<td>UNR</td>
<td>70</td>
<td>Sb</td>
<td>SH</td>
</tr>
<tr>
<td>4</td>
<td>40</td>
<td>31</td>
<td>UNR</td>
<td>200</td>
<td>Cd</td>
<td>SH</td>
</tr>
<tr>
<td>5</td>
<td>63</td>
<td>48</td>
<td>UI</td>
<td>130</td>
<td>Sb,Cd</td>
<td>SH</td>
</tr>
<tr>
<td>6</td>
<td>69</td>
<td>50</td>
<td>UE</td>
<td>100</td>
<td>Sb</td>
<td>SH</td>
</tr>
</tbody>
</table>

<sup>a</sup> Reference value, 0–54 mmol/mol creatinine.

<sup>b</sup> Abbreviations: Symptoms: U, urolithiasis; N, nephrocalcinosis; R, renal insufficiency; I, urinary tract infections; R, renal insufficiency; E, end-stage renal disease; Comorbidity: Sb, short bowel after intestinal resections; Cd, Crohn’s disease; Diagnosis: PH1, primary hyperoxaluria type 1; IH, idiopathic hyperoxaluria; SH, secondary hyperoxaluria.
Quality Assessment of CoaguChek Point-of-Care International Normalized Ratio Monitors: A Note of Caution

To the Editor:

In a recent publication Poller et al. (1) described quality assessment (QA) of CoaguChek (CUC) point-of-care (POC) international normalized ratio (INR) monitors and compared 2 procedures. The study compared the proportion of 523 CUC monitors having unsatisfactory performance as indicated by either a >15% deviation of INR results from the INR assigned to 5 lyophilized QA plasmas or a >15% deviation from the overall median of INRs obtained on the monitors included in the survey. The INRs assigned to QA plasmas had been derived from a full calibration in compliance with WHO-recommended procedures and a local mean normal prothrombin time.

The CUC device is calibrated by the manufacturer for use with non-anticoagulated whole blood. Patients using the device analyze whole blood and not plasma. Furthermore, the calibration procedure employed by Poller et al. (1) used whole blood samples to derive the International Sensitivity Index for assigning INRs to the lyophilized plasma samples. We believe that caution is required when interpreting data obtained on lyophilized plasma samples for devices calibrated for whole blood, and that results should not be assumed to be representative of results that would have been obtained for whole blood analysis.

We recently reported results for 6 years (1996–2002) of experience with external QA (EQA) of CUC and CUC-S devices, comparing >30 test samples analyzed by up to 175 centers (2). We have continued to perform EQA surveys and recently analyzed data on 8 different freeze-dried plasma samples obtained in 2006 from >800 centers, grouping results according to the lot number of test strips used for analysis. For several hundred test-strip lots used for a series of surveys, no significant difference was observed between results obtained with different lots, with 4 exceptions. On these 4 occasions, results with 1 lot number were >10% different from the median of results with all other lots. For 2 of these lots, test strips were available for further investigation. Native whole blood samples from warfarinized patients were analyzed to assess whether the discrepancy observed in lyophilized plasmas was also present when whole blood samples were analyzed. On the 1st occasion a series of lyophilized plasmas and native (non-anticoagulated) blood samples were analyzed with test strips from 2 different lots, including those for which the lot number was associated with the discrepancy identified in survey data. The plasma and whole blood samples were from different patients. For whole blood analysis, we collected samples by syringe, immediately applied them to strips from both lots, and analyzed them with 2 monitors. The mean INRs with the 2 lots were 3.08 and 3.79 (n = 12, 23% difference, P < 0.001) for lyophilized plasmas. For whole blood the mean INRs with the 2 lots were 2.75 and 2.96 (n = 10, 8% difference, P < 0.01).

On the 2nd occasion the mean INR of 10 lyophilized National External Quality Assessment Scheme (NEQAS) plasma samples was 3.97 with the lot number under investigation compared to a mean of 3.08 for samples analyzed by NEQAS participants using strips from multiple lots (29% difference, P < 0.001). Native blood samples from 14 patients were analyzed with the lot number under investigation and a 2nd lot number (which survey data had shown to be in agreement with other lots). The mean INRs for whole blood samples were 2.92 and 3.01 (3% difference, not significant) for these 2 lots. Thus the difference present when lyophilized plasmas were analyzed was absent when native whole blood was tested.

The reason for the different results for test strip lots for whole blood and lyophilized plasmas is unknown, but our data indicate that findings based on lyophilized plasma cannot be safely extrapolated to whole blood without supporting evidence.

On the basis of their study results, Poller et al. (1) concluded that a proportion of CUC monitors in current everyday use for dosage control gave unsatisfactory results. Our experience suggests that although this conclusion may be true for lyophilized plasmas, it should not be assumed that the same conclusion applies to whole-blood samples.

We believe that EQA for POC INR devices should be mandatory, as it is for laboratory INR methods. For large multicenter surveys (1, 2), EQA cur-

References


Christiaan S. van Woerden1*
Jaap W. Groothoff1
Frits A. Wijburg1
Hans R. Waterham2
Ronald J.A. Wanders1
Marcel J.W. Janssen3
Marinus Duran2

Departments of 1 Pediatrics and 3 Clinical Chemistry Laboratory of Genetic Metabolic Diseases Academic Medical Center University of Amsterdam Amsterdam, The Netherlands

2 Department of Clinical Chemistry and Hematology VieCuri Medical Center Venlo, The Netherlands

* Address correspondence to this author at: Department of Pediatrics, Academic Medical Center, G8-205, P.O. Box 22660, 1100 DD Amsterdam, The Netherlands. Fax 31-20-6917735; e-mail c.s.vanoerden@amc.nl.

DOI: 10.1373/clinchem.2007.090688
ently involves the use of lyophilized plasmas and can successfully identify genuine problems (2). EQA is useful to assess imprecision, to reassure users about the comparability of their results with those obtained by users of the same devices, and to promote good QA practices. We advise caution in the interpretation of such results. When such devices are calibrated only for analysis of whole blood, results for plasma cannot be used to assess accuracy without supporting evidence derived from whole blood analysis.

Grant/funding support: None declared. Financial disclosures: None declared.

References

Steve Kitchen*
Dianne Kitchen
Ian Jennings
Tim Woods
Isobel Walker

UK NEQAS Blood Coagulation
Rutledge Mews
Sheffield Teaching Hospitals NHS Foundation Trust
Sheffield, United Kingdom

* Address correspondence to this author at: UK NEQAS Blood Coagulation, Rutledge Mews, 3 Southbourne Rd., Sheffield Teaching Hospitals NHS Foundation Trust S10 2QN, United Kingdom. Fax 44-0-114-271-2149; e-mail Steve.kitchen@sh.nhs.uk.

DOI: 10.1373/clinchem.2007.088435

The authors of the article cited above respond:

To the Editor:
The European Concerted Action on Anticoagulation (ECAA) includes the founder and former long-term organizer of the UK National Exter-

nal Quality Assessment Scheme (NEQAS) and the WHO International External Quality Assessment Scheme in blood coagulation as well as current European national external quality assessment (EQA) organizers.

We strongly endorse the 2 main points made by Kitchen and his UK NEQAS colleagues, that EQA for point-of-care international normalized ratio (INR) devices should be mandatory and caution is required when interpreting data from lyophilized plasma samples analyzed with whole blood prothrombin time (PT) monitors. During the past several years, the ECAA and its successor, European Action on Anticoagulation (EAA), have published data on both of these concerns in more than 60 reports, including 4 in Clinical Chemistry.

With regard to plasma/whole blood differences, the ECAA initially studied the reliability of use of plasma samples in calibrating the CoaguChek monitor (1) with an international sensitivity index (ISI). An optimum formulation of calcium chloride for recalcification of plasma on the CoaguChek and TAS monitors was developed for ISI calibrations and EQA. In full multicenter ISI calibrations at 10 centers, the plasma/whole blood ISI difference was thereby reduced to 1%–6% with various lots of CoaguChek test strips, but small differences between whole blood and plasma persisted. The ECAA studies also revealed previously unsuspected differences in mean ISI with different CoaguChek test strip lots (2). In full calibrations performed at 3 centers, 1 lot gave a 13% ISI difference (1.51) from the mean of 3 others (1.74). A similar interlot difference was detected with both whole blood and plasma. The ECAA EQA plasmas in our 2006 Netherlands national field study (3) also showed evidence of interlot differences. The relatively low detection rate of interlot problems reported by Kitchen and coworkers for the investigation of up to 70 different lots, of which only 4 showed >10% difference from the median INR, can be regarded only as evidence of the limitations of the UK NEQAS approach and the need for more specific EQA.

Kitchen and coworkers challenge our use of the whole blood ISI to certify the INR of ECAA EQA plasmas. The whole blood ISI was preferred because of the small but constant difference in ISI between plasma and whole blood that persisted even with our modified recalcification. Thus adoption of the whole blood INR certification seemed preferable because this method gives less deviation from the certified values without affecting the underlying principles of EQA, which are more concerned with variability than absolute truth.

The UK NEQAS is designed to cover the whole range of PT testing systems. Therefore it would be difficult to provide a similarly precise analysis of performance to that contained in the EC-approved Technology Implementation Plan designed specifically for the EQA of the CoaguChek. The ECAA Technology Implementation Plan specifies that users of point-of-care testing (POCT) monitors should test them with EQA plasmas at intervals of not >6 months (or whenever there is a change of the lot of test strips).

ECAA surveys showed that the POCT PT monitors are less precise than traditional methods and that a minimum of 5 INR-certified EQA plasmas tested on the same day was required in an exercise to provide a reliable EQA of CoaguChek monitors. Sets of 5 ECAA EQA plasmas are therefore provided to users with diluent and calcium chloride. A 15% or more deviation from certified INR on 1 or more test plasmas in the set of 5 is classified as “unsatisfactory performance” (4). Immediate EQA is thus provided for CoaguChek users.

Traditional UK NEQAS analysis is slower and different depending on deviation from the overall median of all participants in an exercise and taking weeks or months to provide the results for a user. The ECAA/ECAT Netherlands study in our 2006 report showed that the ECAA method of rapid, “on the spot” analysis by percentage deviation from certified INR values gave results similar to those of traditional UK
Grant/funding support: The work was supported by grants from the EC Commission (SMT4-CT98-2269 and QLG4-CT-2001-02175) and from the Manchester Thrombosis Research Foundation. Financial interests: None declared.

References


Leon Poller1*
Jorgen Jespersen2
Michelle Keown1
Saied Ibrahim1
Armando Tripodi3

1 European Action on Anticoagulation Central Facility University of Manchester Manchester, United Kingdom

2 Department of Clinical Biochemistry Ribe County Hospital in Esbjerg Esbjerg, Denmark

3 Bianchi Bonomi Hemophilia and Thrombosis Centre University and Institute di Ricovero e Cura a Carattere Scientifico Maggiore Hospital Milan, Italy

* Address correspondence to this author at: European Action on Anticoagulation, Central Facility, Faculty of Life Sciences, University of Manchester, 3,239 Stopford Bldg., Oxford Rd., Manchester M13 9PT, United Kingdom. Fax 44-161-275-5316; e-mail ecaa@manchester.ac.uk.

DOI: 10.1373/clinchem.2007.089227

Interlaboratory Reproducibility of Isoelectric Focusing in Oligoclonal Band Detection

To the Editor:

Current criteria for the diagnosis of multiple sclerosis (MS), an inflammatory neurological disease commonly affecting young adults, include cerebrospinal fluid (CSF) analysis to detect oligoclonal IgG bands (OCB) (1). CSF analysis methods vary substantially, however, and experts in MS and CSF diagnostic techniques addressed the need for standardization led by compiling recommendations (2). External quality control schemes are fundamental steps in standardization processes, particularly in the field of isoelectric focusing (IEF), the recommended technique for OCB detection (1–4), because many IEF steps may be difficult to standardize (5).

Data from our previous OCB quality control survey showed that participating centers concurred in OCB-positive and OCB-negative sample identification, but differed in the numbers of OCBs found (5). We assumed that this lack of reproducibility could lead to false-negative/positive results in critical CSF samples, i.e., samples with few and weak bands. Accordingly, we aimed to produce a more comprehensive survey by involving more centers and by including critical samples.

We asked the 20 laboratories that participated in the 2006 OCB Quality Control Survey performed by the Italian Association for Neuroimmunology to blindly analyze freshly collected paired CSF and serum samples from 4 patients (controls A–D) with clinically isolated syndrome, a disorder that converts into MS in ~50% of cases (1). IgG concentrations in the samples were provided. Laboratories were asked to interpret the IEF, and to report the number of bands observed. All participants used IEF with immunoblotting for IgG, in accordance with recommended procedures (2, 3, 5). IEF was performed with agarose/polyacrylamide gels from the following suppliers: Helena (n = 9), homemade (n = 4), Pharmacia (n = 3), Amer sham (n = 2), Sebia (n = 1), and Cambrex (n = 1).

Results for control A were OCB-negative in 15 centers, and OCB-positive with a mirror pattern (i.e., identical OCB in CSF and serum) (4) in 5 centers. All 20 centers identified CSF OCB in controls B and C, but additional serum bands were found in controls B (12 centers) and C (8 centers). For control D, 13 centers found a few CSF bands, the remaining centers found none; control D was accordingly considered a critical sample. Minimum and maximum (median) band numbers in control samples were as follows: A [0–6, (0), serum; 0–6, (0) CSF], B [0–15, (3), 3–26, (13)], C [0–8 (0), 5–20, (9)], D [0–5, (0), 0–7, (2)] (Kappa statistic for interobserver agreement was not significant for each control). Fig. 1
shows the 4 controls as detected in the center whose reported band numbers were the nearest to the median values in each control.

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 band patterns 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 respondents indicating OCB-positive and 35% OCB-negative results; such variation is important for the correct diagnosis of MS.

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.

Grant/funding support: None declared. Financial disclosures: None declared.

Fig. 1. Paired CSF and serum (ser) samples used as controls (A–D) in the survey, as analyzed by one center.

IEF was in agarose gel and staining of nitrocellulose paper after affinity-mediated protein blotting was IgG-specific. Arrows indicate OCBs.
ereference 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 OCD 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 (4). 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 (3). As assays become more sensitive, increasing numbers of MIs will be detected, but an increased prevalence of myocardial injury not related to ischemic pathologies will also be seen (5). 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.

Grant/funding support: We thank Sheryl Sullivan, Jody Parsells, Michele Steinmann, and Theresa Tubbs (all from Ortho-Clinical Diagnostics) for their assistance and for the partial financial support provided by OCD for this study. Financial disclosures: F.S.A. has both consulted for and received research grant support from Abbott, Beckman, and Ortho-Clinical Diagnostics.

References


Fred S. Apple* MaryAnn M. Murakami

Hennepin County Medical Center
Minneapolis, MN

*Address correspondence to this author at: Hennepin County Medical Center, Clinical Laboratories P4, 701 Park Ave., Minneapolis, MN 55415. Fax 612-904-4229; e-mail apple004@umn.edu.

DOI: 10.1373/clinchem.2007.087718

Increased Human Chorionic Gonadotropin Due to Hypogonadism after Treatment of a Testicular Seminoma

To the Editor:

Alpha-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 re instituted 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 re instituted 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).
hCG concentrations increase with age, and with our assay values up to 10 IU/L can be observed in postmenopausal women (2). This increase is caused by increased pituitary gonadotropin secretion and is suppressed by hormone replacement therapy (3). hCG concentrations also increase in elderly men, but values exceeding 2 IU/L are rare (2).

Testicular cancer and its treatment, particularly cytotoxic chemotherapy, may cause gonadal suppression leading to hypogonadism, which is mostly transient (4) and may lead to increased serum concentrations of LH and FSH, which normalize with testosterone replacement therapy (1). Increased hCG immunoreactivity after treatment of testicular cancer has been described previously but was ascribed to cross-reaction of LH and the free beta subunit of hCG together. With other assays, hCG >5 IU/L will most likely be observed in male patients with severe hypogonadism.

Thus we determined that the increased serum hCG was caused by pituitary hCG secretion in response to hypogonadism.

With our hCG-assay the upper reference limit in healthy males younger than 50 years is 0.7 IU/L and in men older than 50 years it is 2.1 IU/L (2). In our patient, the hCG concentration exceeded this limit, although it did not exceed 5 IU/L, which is a commonly used decision limit. Concentrations up to 32 IU/L have been observed in postmenopausal, hypogonadal women (5) but not in men, possibly because of a physiological difference between men and women or differences in calibration and broader assay specificity, i.e., detection of hCG and the free beta subunit of hCG together. With other assays, hCG >5 IU/L will most likely be observed in male patients with severe hypogonadism.

The case we describe shows that a moderate increase of serum hCG is a physiological reaction to hypogonadism, which is common in testicular cancer patients because of the disease and may occur in the absence of relapse. To avoid unnecessary and potentially harmful chemotherapy, it is important to remember that moderate increases in hCG in men treated for testicular cancer are not always caused by cancer relapses.

Grant/funding support: Finska Läkaresällskapet.

Financial disclosures: None declared.

References