encourages physician scientists to bring more clinical specimens into research fields to bridge between basic science and clinical medicine.

References


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Comparison of the Abbott IMx Tacrolimus I and Tacrolimus II Assays

To the Editor:

Tacrolimus (FK-506, Prograf, Fujisawa Pharmaceutical Co.), first approved in the United States for liver transplants, is now widely used in other organ transplants (1). Its immunosuppressive potency is 10- to 100-fold greater than cyclosporine A. Because subtherapeutic concentrations are associated with organ rejection and high concentrations are associated with nephrotoxicity, neurotoxicity, and opportunistic infections, therapeutic drug monitoring of FK-506 is well-accepted and is a common laboratory practice (2).

Currently, there is no consensus on the therapeutic range for FK-506. Substantial amounts of the drug are present intracellularly, with a red blood cells-to-plasma ratio >4:1. In addition, clinical effects correlate better with whole blood concentrations than with serum or plasma concentrations (3). Therefore, routine laboratory assays determine concentrations on whole blood. The whole blood therapeutic range was originally thought to be 15–25 μg/L, but has subsequently been modified at most US centers to 5–20 μg/L (1). Several methods are available for monitoring the concentration of FK-506. These include bioassays, radio-receptor assays, HPLC, ELISA (Instar Corp.), and microparticle enhancement immunoassay (MEIA; Abbott Diagnostics) (4). HPLC, the
reference method, is generally used for research purposes only. The ELISA, although more sensitive than MEIA (detection limit, 0.2 vs 1.5 μg/mL), is a time-consuming method, taking >4 h of a technologist’s time per batch. Furthermore, the manufacturer of the ELISA method recommends running the calibration curve with every batch. These factors make ELISA inconvenient for routine use and expensive for small hospital laboratories, which run only a few samples at a time. The MEIA (Abbott IMx) is the most commonly used assay for FK-506 (5). This is in part because of the ease of instrument handling and the stability of the calibration curve over time.

In the Abbott Tacrolimus I assay, the analytical detection limit is 5.0 μg/mL. In light of the downward shift in the therapeutic ranges, Abbott has developed a new assay (Tacrolimus II) for FK-506. The Tacrolimus II assay is more sensitive than the Tacrolimus I assay, with an analytical detection limit of 1.5 μg/mL. In the process of switching from the Tacrolimus I assay to the Tacrolimus II assay, we compared these assays. To avoid the questions of sample stability and drug metabolism, FK-506 was assayed the same day by both methods. Each sample was done in duplicate by each method. Both methods use a similar protocol of organic extraction and assay by MEIA except for the ratios of sample to extraction buffer. The Tacrolimus I assay uses 100 μL of whole blood and 200 μL of extraction buffer. The Tacrolimus II assay uses 150 μL of whole blood and 150 μL of extraction buffer.

We found that the two assays yielded significantly different values for FK-506. In our laboratory, the Tacrolimus II assay gave significantly lower values (P <0.001) than the Tacrolimus I assay. Fig. 1 shows the comparison of the two assays, with a slope of 0.80 and intercept of -1.7. We do not believe that this bias is because of laboratory error, but think that this is a real bias based on these facts: (a) In each assay, the samples were run in duplicate and the duplicates agreed within an average of 5.5% and 6.8% for Tacrolimus I and II assays, respectively; (b) in all the assays, the controls were within manufacturer-stated ranges; and (c) CVs for the controls of the two assays were comparable. The CVs for the Tacrolimus I assay at 14, 25, and 41 μg/L were 11.0%, 9.0%, and 6.6%, respectively, and the CVs for the Tacrolimus II assay at 5, 10, and 23 μg/L were 11.2%, 8.2%, and 10.1%, respectively.

To discover if the lower values given by the Tacrolimus II assay are because of lower recoveries, we added 60 μg/L calibrator from the Tacrolimus I assay to four negative blood samples, to get a drug concentration of 20 μg/L. The average recovery on these four samples by the Tacrolimus II assay was 80%, compared with 102% by the Tacrolimus I assay. These lower recoveries in the Tacrolimus II assay seem to be the cause of lower values in the newer assay. We think that the change in sample-to-extraction buffer ratio in the newer assay produces incomplete drug extraction from red blood cells. When this report was under review, similar differences between the Tacrolimus I and II assays were reported (6, 7). In the first report, the bias was throughout the range studied; in the second report, the Tacrolimus I assay gave higher values at the lower concentrations because of a significant intercept value.

In conclusion, compared to the Tacrolimus I assay, there is a significant negative bias with the Abbott Tacrolimus II assay. At least part of this bias seems to be because of poor drug extraction. For better therapeutic drug monitoring, physicians should be made aware of this bias.

References

Increased Concentrations of Cardiac Troponin I Are Equivalent to Increased Cardiac Troponin T in Identifying Chest Pain Patients at Short-Term Risk of Myocardial Infarction

To the Editor:
A recent paper by Christenson et al. (1) presented a comparative analysis of cardiac troponin I (cTnI) and T (cTnT). Their objective was to stratify patients for short-term risk of an acute coronary event on the basis of initial marker concentration as determined within 3.5 h of ischemic symptoms. The authors suggest that cTnT is a better predictor than cTnI on the basis of comparisons of the area under the curve (AUC) of ROC curves for cTnT (AUC = 0.68) and cTnI (AUC = 0.64). However, although weakly significant (P = 0.0375), the 95% confidence interval (CI) for the two areas overlap almost completely (95% CI for cTnI, 0.56–0.72; 95% CI for cTnT, 0.6–0.75). The fact that the samples were not tested for the two analytes simultaneously, but >1 year apart, could easily account for this small difference.

Using cutoffs of >0.1 ng/mL (0.1 μg/L) for cTnT and >1.5 ng/mL (1.5 μg/L) for cTnI, the authors found 90.4% concordance between the two assays. After correction for samples falling within the 95% CI around the cutoffs, the overall concordance rose to 93%. Of the 66 cTnT-positive/cTnI-negative patients, 7.5% died and 70% suffered from an acute myocardial infarction (AMI). The authors use this as evidence that cTnT is more sensitive than cTnI for risk stratification but neglect to present data recalculating the cTnI sensitivity/specificity profile using a cutoff of 0.4 ng/mL (0.4 μg/L), which has been demonstrated to be the more appropriate concentration for cTnI in risk stratification of chest pain patients (2). The authors cite that the cutoffs used are in accordance with the manufacturer’s specifications; however, the Dade Stratus® Cardiac Troponin-I package insert (3) describes in detail the multicenter, outcomes-based study of Antman et al. (2), where 0.4 ng/mL (0.4 μg/L) was used as a cutoff. The insert also states that in two separate studies, the 97.5 percentile distribution of ostensibly healthy individuals (n = 156) and patients with chest pain but confirmed not to have AMI (n = 149) was 0.4–0.6 ng/mL (0.4–0.6 μg/L).

Both the Christenson et al. study (1) and a previous report describing the use of cTnT in risk assessment of patients with myocardial ischemia (4) used a high-risk population in whom >72% of the patients were diagnosed as having an AMI. The patient population upon which both studies were based came from the GUSTO IIa thrombolytic trial (5), which studied the efficacy of thrombolytic therapy (streptokinase or tissue plasminogen activator–alteplase) for those who had ST-segment elevation in randomization with administration of either the anticoagulant heparin or hirudin (4, 5). An analysis of the electrocardiographic (ECG) classifications reveal that 435 of 755 (58%) of that population had ST-segment elevations sufficient to qualify them for thrombolytic therapy. On the basis of WHO criteria, these patients were diagnosed with AMIs based on chest pain and ECG ST-segment elevation and did not require any biochemical marker for initial AMI diagnosis. Regardless of cTnI or cTnT values at presentation, 83% of the patients had a cardiac event, defined as death, AMI, or revascularization therapy (1). In their study, the single best predictor of 30-day mortality was not cTnT, but the ECG categorization (P = 0.045 for cTnT and 0.019 for ECG).

In clinical studies using the recently developed Chiron Diagnostics ACS:180 cTn assay, which is calibrated to the Dade Stratus cTn assay, the cTnI concentrations in 158 ostensibly healthy patients were <0.1 ng/mL (0.1 μg/L), and the median values in 73 unstable angina patients were 0.8 μg/L and 7.6 μg/L in 130 confirmed AMI patients (data on file, Chiron Diagnostics Corp.). These findings corroborate the use of lower cutoffs for cTnI in risk stratification. Why the authors did not simply recalculate the comparative data using this lower cutoff for cTnI is questionable, because they clearly were aware of the Thrombolitics in Myocardial Infarction (TIMI IIIB) trial and the subsequent cTnI substudy (2, 6).

The use of cTnT as a predictor of short-term acute coronary events in chest pain patients based on marker values on presentation has now been demonstrated in a number of studies (1, 2, 7, 8). The TIMI IIIB substudy specifically analyzed cTnI concentrations on the most difficult to diagnose chest pain population, patients with no or transient ST-segment elevation or depression who are not candidates for acute thrombolytic therapy (2). By using the lower 0.4 μg/L cutoff, this study on 1404 patients showed that patients presenting between 0 and 24 h after chest pain onset with a cTnT concentration above the cutoff were 3.8-fold more likely to die within 42 days of presentation compared with patients with concentrations <0.4 μg/L. When the initial measurement was taken after at least 6 h after onset of chest pain, consistent with the temporal profile of increased cTnT in the majority of AMI patients, the risk ratio of mortality at 42 days was 9.5-fold higher in patients with cTnT concentrations >0.4 μg/L. In terms of overall risk of a short-term acute cardiac event, this population was at much lower risk compared with the population described in the GUSTO IIa studies (1, 4, 5). Comparisons show that the death rate was 2.7-fold...