REX

Laboratory Bulletin

July 2006

UPDATES AND INFORMATION FROM REX PATHOLOGY LABORATORY

Issue Number 117

Revised Troponin Reference Range

Effective July 26, 2006 Rex Laboratory will revise the reference range for troponin I (TPNI), in effect lowering the threshold for both acute myocardial infarct and myocardial injury. The change is prompted by recommendations from a consensus of American and European cardiologists, although we will not be following their recommendations to the letter, for reasons discussed below.[1] The "revised " clinical definition for acute myocardial infarct is a "typical rise and gradual fall (troponin) or rapid rise and fall (CK-MB) with at least one of the following:

- ischemic symptoms;
- development of pathologic Q waves on ECG;
- ST segment elevation or depression on ECG; or
- coronary artery intervention (e.g. coronary angioplasty).[1]

Nothing particularly radical there, but the Committee went on to state that an increase should be defined as a measurement exceeding the 99th percentile of a reference control population and that the imprecision at this level should be a coefficient of variation (CV) ≤ 10%. **Whoa Nellie!**

History of troponin implementation at Rex

In 2000, the Laboratory changed methods for CK-MB testing, and began offering TPNI testing on the Dade Dimension. We had studied a different vendor's troponin assay shortly after release, but found that it was less sensitive, less specific, and had a worse positive predictive value than the CK-MB method we were using at the time. Interest in troponins (both troponin I and troponin T) continued as the enzymes were touted as possessing superior sensitivity and specificity to CK-MB with regard to myocardial cell necrosis. We subsequently evaluated a different (Dade) TPNI method. Prior to making the above changes, we evaluated 150 specimens from 85 patients for TPNI, CK-MB, and myoglobin. The medical records were reviewed and in cases of uncertainty, the patient's physician was consulted for patient follow-up information. Using the manufacturer's suggested reference intervals for "negative" (= 0.4 ng/mL for TPNI, = 3.5 ng/mL for CK-MB), we concluded:

- both assays were excellent markers of cardiac injury;
- TPNI was more sensitive (100% vs. 88.1%) but less specific (90.0% vs. 100%) than CK-MB;
- TPNI had a superior negative predictive value (100% vs. 95.6%) but inferior positive predictive value (83.9% vs. 100%) compared to CK-MB; and
- for optimal performance, serial testing (e.g. q 4-6 hr sampling) is preferable to a single determination, to detect the pattern of rising/falling values.[*2]

We subsequently adopted Dade's reference range and jargon for TPNI, to wit:

- ≤0.4 - 1.5 ng/mL suggests "cardiac injury"
- ≤ 1.5 ng/mL suggests myocardial infarct

To the best of my knowledge, the test has performed relatively well. Despite literature reports to the contrary, we have seen elevated TPNI in patients with chronic renal failure.[2] There have been occasional (anecdotal) reports of patients with "positive TPNIs" with negative cardiac catheterizations, as well as occasional patients with persistently abnormal TPNIs who most likely have some (endogenous or exogenous) interference with the assay.[3] For most patients with chest pain, serial TPNI measurement is adequate for laboratory evaluation of myocardial injury/infarct. (Indeed, some hospitals no longer offer CK-MB testing.) The choice between troponin I and troponin T depends largely on the type of chemistry analyzer used by the laboratory as the performance characteristics are similar. I believe CK-MB can be helpful in problematic cases, particularly if there is a question regarding the timing of the infarct, or re-infarction (as TPNI remains elevated for five to nine days, while CK-MB normalizes after one to two days); or if there appears to be an interfering substance present.

Sensitivity and Precision of TPNI

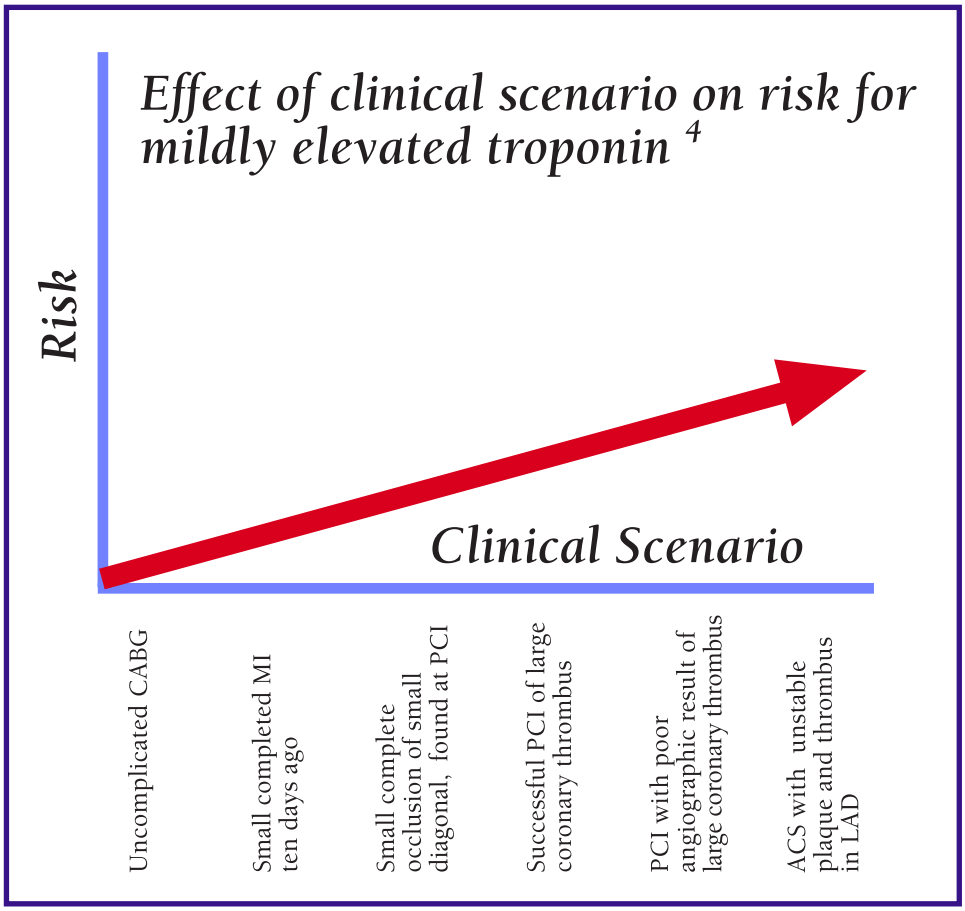
Over time, the sensitivity of troponin assays improved. As physicians gained experience with troponin testing in patients with chest pain, it became clear that even

Rex Pathology Associates, P.A.

Stephen V. Chiavetta, MD	(919) 784-3060	Keith V. Nance, MD	(919) 784-3286
Timothy R. Carter, MD	(919) 784-3058	Vincent C. Smith, MD	(919) 784-3056
John D. Benson, MD	(919) 784-3059	Rhonda Humphrey, Practice Manager	(919) 784-3063
John P. Sorge, MD	(919) 784-3062		

REX LABORATORY SERVICES
Healthcare for life. Your life.

mild elevations over the reference range predicted a worse prognosis. The degree of risk depends on the clinical setting. In the setting of a patient with features suggesting an acute coronary syndrome (ACS), mild elevations presumably reflect small acute microinfarcts in the setting of an unstable plaque. In patients who have just undergone percutaneous coronary intervention (PCI) or coronary artery bypass grafting (CABG), a similar small rise reflects periprocedural injury, and increases the risk of the individual patient to one who has undergone similar treatment without an associated increase in troponin, but the risk of subsequent mortality or morbidity may not be as great as in the patient presenting with ACS (Figure).[4] The common denominator in all of these cases is myocardial cell injury or death, but the clinical setting appears to be important in terms of the overall risk.



modified from White [4]

These findings prompted the push to more precisely define and determine minimal elevations in troponin as stated above. Unfortunately no currently available troponin assays have a CV < 10% at the 99th percentile of the reference population.[4-6] This led to a recommendation that laboratories should employ a cut-off level of < 10% imprecision to separate normal from elevated enzyme levels.[4-6] For the Dade Dimension analyzer used for TPNI at Rex, the manufacturer's studies indicate a lower limit of detection of 0.04 ng/mL and a 99th percentile value of 0.07 ng/mL.(For CK-MB, the lower limit of detection was 0.5 ng/mL with a 97.5th percentile value of 3.1 ng/mL.) A large independent study of major chemistry analyzers validated the manufacturer's claim. In a study population of 696 adults (45% male, 55% female, 58% white, 31% black, 5% Asian, 2% Hispanic, 2% Native American, and 2% other), the Dimension findings were validated. The actual 99th percentile value for TPNI was 0.06 ng/mL, while the 99th percentile value for CK-MB was 3.9. Significantly there were no statistically significant differences by race or gender for either assay (although there was a trend for higher CK-MB values in men [4.2 ng/mL] compared to women [3.1 ng/mL] and blacks/Native Americans [4.2/5.1 ng/mL] compared to whites [3.5 ng/mL]).[5] Precision was not evaluated in this study. A subsequent similarly designed study determined that the 10% CV cutpoint for the Dimension was 0.26 ng/mL.[6] The authors noted that no commercially available assays were available with a 10% CV at the 99th percentile. Manufacturers are aware of this challenge and "improved" assays are in the pipeline. Indeed, we recently evaluated an instrument, which was found by the latter study to have a 10% CV at 0.10 ng/mL (but the 99th percentile was also at 0.07ng/mL).

New Cardiac Enzyme Cut-Off Levels at Rex

- No change in CK-MB ("normal = 0 - 5.0 ng/mL")
  - This assay has performed well. The above studies tend to validate the reference range - particularly in view of the findings in African Americans and Native Americans. TPNI is superior for detecting "micro-infarcts'.
- No change in CK-MB index ("normal = 0 - 3.5%")
  - Total CK and CK-MB index aren't necessary in chest pain patients, unless there is a possibility of skeletal muscle injury. We certainly see elevated CK-MBs in patients with rhabdomyolysis. The CK-MB index is helpful in this setting.
- TPNI < 0.1 ng/mL is "normal"; replaces ≤ 0.4 ng/mL
- TPNI ≥ 0.6 ng/mL "suggests myocardial infarct"; replaces ≥ 1.5 ng/mL
  - This is the cut-off currently recommended by Dade for myocardial infarct based on their studies. Given the discussion above, this seems reasonable.
- TPNI between 0.1 - 0.6 ng/mL "suggests myocardial injury"; replaces 0.4 - 1.5 ng/mL)
  - 0.1 ng/mL is the cut-off currently recommended by Dade for "increased risk of adverse prognosis", based on their studies. Given the data cited above, this seems reasonable for now. Recognize that values < 0.3 ng/mL may be "analytical noise" rather than true myocardial injury. Therefore, as always….
- for optimal performance, serial testing (e.g. q 4-6 hr sampling) is preferable to a single determination, to detect the pattern of rising/falling values.
- Correlation with the clinical setting and ECG findings is essential. Any test applied in a setting of low disease prevalence will not "perform as well as advertised".

John D. Benson, MD

References

1. *The Joint European Society of Cardiology/American College of Cardiology Committee. Myocardial infarction redefined - A consensus document of the Joint European Society of Cardiology/American College of Cardiology Committee for the redefinition of myocardial infarction. J Am Coll Cardiol 36:959-967, 2000.*
2. *Benson JD, Johnson J. Cardiac enzymology update. Rex Healthcare Laboratory Bulletin Issue 46, March 2000.*
3. *Benson JD. Analytical error in immunoassays Rex Healthcare Laboratory Bulletin Issue 67, April 2002.*
4. *White HD. Things ain't what they used to be: Impact of a new definition of myocardial infarction. Am Heart J 144:933-7, 2002.*
5. *Apple FS, Quist HE, Doyle PJ, et al. Plasma 99th percentile reference limits for cardiac troponin and creatine kinase MB mass for use with European Society of Cardiology/American College of Cardiology consensus recommendations. Clin Chem 49:1331-6, 2003.*
6. *Panteghini M, Pagani F, Yeo KTJ, Apple FS, et al. Evaluation of imprecision for cardiac troponin assays at low-range concentrations. Clin Chem 50:327-32, 2004.*

** We also commented on the excellent negative predictive value of myoglobin and how that could assist in rapid triage of chest pain patients in the ED, but no one bought into it and that test was quickly discontinued due to lack of interest.*

Bordetella Pertussis

Bordetella are small gram-negative aerobic coccobacilli, which may cause a respiratory illness referred to as pertussis. The development of effective vaccines has substantially reduced the threat of this illness. Infants are at particular risk for pertussis, but the disease also occurs in children (both vaccinated and unvaccinated) as well as adults. The rate of pertussis in the United States peaked in 1930 (265,000 cases, 7,000 deaths) while the nadir was in 1976 (1010 cases, four deaths).[1] Despite continued vaccination, there has been a recent increase in cases (11, 647 cases reported to the CDC in 2003) and the CDC believes only 5-10% of actual cases are recognized/reported.[1] The increase is attributed to increased awareness of the "atypical" presentations of the disease and more sensitive laboratory tests. Pertussis is the most common vaccine-preventable illness reported in children < 5 years old and has been found to be responsible for chronic (one to four weeks) cough in 12-32% of adults.[1] While the mortality rate had approached 50% in the past, it is currently < 0.2%, with an infant mortality rate of 2.4/million live births.[1]

Bordetella pertussis is the microorganism responsible for most cases of pertussis, however *B. parapertussis, B. bronchiseptica,* and *B. holmesii* may produce a similar syndrome, albeit frequently milder in degree. *B. pertussis* is found only in humans, *B. parapertussis* may be found in humans or sheep, and *B. bronchiseptica* is found primarily in animals including dogs, cats, rabbits and swine. *Bordetella* display tropism for respiratory ciliated epithelium and produce virulence factors, including toxins that play a role in the disease. A mucopurulent exudate covers the respiratory mucosa, and obstructs small airways. A carrier state is not recognized, but transient colonization or infection may explain the occasional presence of the organism in asymptomatic individuals.[2] Transmission from human to human occurs by aerosolized respiratory droplets. While the disease is highly contagious (80-90% of susceptible persons develop the disease), it requires relatively close (within a few feet) contact. Most cases occur in late summer or early fall. Following infection or vaccination, immunity decreases after five to 12 years, thus in the United States the primary reservoir of *B. pertussis* is older children and adults. Acellular vaccines have replaced the earlier heat-killed whole-cell vaccines.

"Typical" pertussis has three stages: incubation, catarrhal, and paroxysmal. The incubation period is asymptomatic and usually lasts from seven to ten days (although the range may be from four to 21 days). The catarrhal stage (one to two weeks) is characterized by nonspecific symptoms such as rhinorrhea, sneezing, low-grade fever, and mild cough. With the paroxysmal stage, the patient experiences paroxysmal coughing, with or without the classic inspiratory gasp ("whoop"), and posttussive vomiting. However "atypical" presentations may occur. Infants may experience choking, cyanosis and apnea without the development of a cough, while older children and adults may manifest only a persistent (otherwise unremarkable) cough. Routine

laboratory studies are largely unremarkable, apart from lymphocytosis in non-vaccinated patients. Complications include hypoxia, dehydration, and pneumonia.

Laboratory Diagnosis of Bordetella Infection

Direct detection of the bacteria is the preferred method for diagnosis of acute pertussis. Three standard approaches include direct fluorescence antibody (DFA) microscopy, bacterial culture and polymerase chain reaction (PCR) identification of bacterial DNA from nasopharyngeal source material. DFA suffers from poor sensitivity (30-71%), specificity, and reproducibility.[2] A positive DFA result should be considered as "presumptively positive" and should be accompanied by either culture or PCR assay.[2] Culture provides the most definitive and specific identification, but the sensitivity can be low (15-40%).[1] While some *Bordetella* species may be cultured on routine blood or MacConkey agar, *B. pertussis* is a fastidious organism that requires specialized media for recovery. Regan-Lowe charcoal transport media inoculated at the bedside and refrigerated promptly may permit successful culture of the bacteria. The bacteria may require seven to 12 days of incubation for isolation. PCR detection of bacterial DNA is the most sensitive method for diagnosing acute pertussis (93%).[3] Most PCR methods detect both *B. pertussis* and *B. parapertussis,* and are generally able to distinguish between the two. *B. holmesii* shares homology with *B. pertussis*, thus some cross-reactivity may occur. As PCR may detect dead organisms, it may remain positive longer in the course of the disease than culture and persist following antibiotic therapy. Most authorities now recommend duplicate testing by PCR and culture to achieve maximum sensitivity and specificity.

Serologic testing has been advocated by some as an ancillary technique for diagnosis of acute infection, due to increased sensitivity. [1,2] Theoretically, "acute" and "convalescent" sera could be used to document either seroconversion or a raise in titer. From a practical standpoint, the vague nature of the early symptoms limits the likelihood of "true" acute phase specimens, while many patients may not return for "convalescent" specimens. Furthermore, vaccinated individuals will often have detectable levels of IgG antibody. Maternal IgG antibodies cross the placenta and thus may be detected in infant sera. *Bordetella* IgM antibodies, if present, presumptively suggest acute infection but must be interpreted in the context of the clinical findings. IgM antibodies are generally undetectable after six months.[4] Serology may also be used to evaluate immune response to vaccination or prior exposure.

Testing at Rex

The Laboratory currently sends nasopharyngeal swabs to the NC State Laboratory of Public Health (NCSLPH) for *Bordetella* culture and PCR DNA analysis. Collection kits are available either from the NCSLPH or the Rex Laboratory. The kits **must be kept refrigerated prior to use and following specimen collection.** The kit includes two Dacron tipped swabs, one vial of Regan-Lowe transport media, one microcentrifuge tube, collection instructions and a patient information sheet (DHHS form #T806), which **MUST** be completed prior to sample submission. Calcium alginate swabs must not be substituted for the Dacron swabs, as the former may interfere with PCR analysis. Physicians or nurses are responsible for collecting specimens on inpatients and outpatients (e.g. Emergency Dept.) As a courtesy to Outreach clients, laboratory staff, under pathologist supervision, collect specimens (and complete DHHS form #T806!) on walk-in clients to the main campus laboratory. Positive PCR and culture results are phoned by NCSLPH upon confirmation. There is (currently) no charge for this testing.

Bordetella IgG and IgM serology is available from Mayo Medical Laboratories (Mayo test code 83268). The Outreach charge is $112.

John D. Benson, MD

References
1. *Bocka J. Pediatrics, Pertussis. http://www.emedicine.com/emerg/topic394.htm.*
2. *Loeffelholz MJ. Bordetella. In: Murray PR et al, ed. Manual of Clinical Microbiology, 8th ed. Washington DC: ASM Press; 2003:780-3.*
3. *Mayo Medical Laboratories. Interpretive Handbook. 2005, 123-4.*
4. *Mayo Medical Laboratories. Interpretive Handbook. 2005, 124.*
5. *http://204.211.171.13/Forms/dhhs-t806.pdf (NCSLPH patient information form which must be completed and accompany specimen)*

Rex Healthcare Laboratory (919) 784-3040. Telephone extensions are: Pathologists' Direct Line (3063), Robin Ivosic (Customer Service and Outreach Manager 3053), Elaine Patterson (Core Lab, Blood Bank and Microbiology Manager 3054), Diane Young (Anatomic Pathology Manager 3888).
Client Response Center (919) 784-6000 (phone) (919) 784-6299 (fax)