



Saint Luke's Regional Laboratories Clinical Laboratory Letter



October 2011

Welcome to our new Pathologist

Saint Luke's Pathology is excited to announce that Suparna Nanua, MD PhD, has joined our group. After practicing medicine for four years, Dr. Nanua received a PhD in Microbiology and Immunology from Wayne State University. She then completed two postdoctoral fellowships at the University of Michigan and Texas Tech Health Science Center before undertaking a clinical pathology residency and hematopathology fellowship at Washington University in Saint Louis.

Dr. Nanua is available for consultation in laboratory hematology including bone marrow interpretation, flow cytometry analysis, coagulation testing and molecular diagnostics. She has already assumed the medical directorship of the laboratory at Saint Luke's East Hospital.

BNP to NT-ProBNP Conversion

B-type natriuretic peptide, which is also called brain-type natriuretic peptide (BNP), was first isolated from brain, but later discovered to be a cardiac hormone. Ventricular cardiac myocytes constitute the major source of BNP related peptides. Only small amounts of BNP are stored within the cytoplasmic granules of myocytes. Following myocardial wall stress, there is rapid gene expression and de novo synthesis of a natriuretic prohormone. Upon release into the circulation this prohormone is cleaved into the biologically active BNP, which represents the C-terminal fragment, and the biologically inactive N-terminal fragment (NT-proBNP).

Natriuretic peptides have numerous physiological effects including natriuresis/diuresis, peripheral vasodilatation, and inhibition of the renin-angiotensin-aldosterone system (RAAS) and the sympathetic nervous system (SNS). Secretion of natriuretic peptides may limit the degree of vasoconstriction, sodium retention and pathologic remodeling that occurs in patients with heart failure.

The circulating half-life of NT-proBNP is 120 minutes which is six times longer than BNP. In

healthy individuals, plasma concentrations of BNP and NT-proBNP are similar. However, in patients with left ventricular dysfunction, plasma NT-proBNP is approximately four to six times higher than BNP, even though both molecules are secreted in equimolar proportions. Most of our knowledge regarding the value and performance of BNP and NT-proBNP testing has been derived from studies of patients presenting with acute dyspnea to the emergency department. In general, levels of BNP and NT-proBNP are directly related to the severity of heart failure symptoms and to the severity of the cardiac abnormality.

Numerous commercial assays for BNP and NT-proBNP are available. BNP values obtained with different methods are not comparable and no equation has been devised that reliably allows for the conversion of BNP to NT-proBNP levels or vice-versa. BNP has classically had two cutoff points; a lower one with a high negative predictive value to reliably exclude heart failure as the cause of dyspnea and a second higher one with a positive predictive value to rule in heart failure as the cause of dyspnea. The cutpoints for BNP have been 100 and 400 pg/mL irrespective of age.

The International Collaborative for NT-proBNP recommends age-stratified cutoff points for NT-proBNP (Am Heart J. 2005;149:744-50). For patients <50, 50 to 75, and >75 years of age, the optimal plasma NT-proBNP cutoffs for diagnosing heart failure are 450 pg/mL, 900 pg/mL, and 1800 pg/mL respectively. Overall, these cutoffs yield a sensitivity and specificity of 90 and 84 percent, respectively. Across the entire population, NT-proBNP levels below 300 pg/mL are optimal for excluding a diagnosis of heart failure, with a negative predictive value of 98 percent.

Variability in peptide measurements must be considered when interpreting serial NT-proBNP results. Intraindividual biologic variation as well as analytic variation contribute to total variation. Total variability determines the percentage change needed to demonstrate a significant difference in results over time. An 11% decrease in NT-proBNP

is considered to be a significant within day change, a 25% decrease is considered to be a significant day to day change and a 47% decline is required for a significant week to week change (AH Wu. Am Heart J 2006;152:828). During hospitalization, only two NT-proBNP levels are recommended; an initial value and a pre-discharge value to assess response to therapy. Plasma BNP cannot be measured during administration of nesiritide (BNP 1-32), since nesiritide is detected as an increase in plasma BNP concentration. In contrast, the NT-proBNP assay does not detect nesiritide and can be used to monitor response to this therapy.

BNP is cleared from plasma by binding to the natriuretic peptide receptor and by proteolysis. In contrast, NT-proBNP is mainly cleared by renal excretion. Since NT-proBNP is cleared by the kidney, plasma concentration is often elevated in patients with renal insufficiency alone, whether or not they have clinically diagnosed heart failure. Insufficient data is available to establish clear cut-off values for NT-proBNP in patients with renal failure. One study has suggested that a cutoff of at least 1200 pg/mL should be used to diagnose heart failure in patients 50 to 75 years of age with a GFR between 30 and 60 mL/min per 1.73 m² (J Am Coll Cardiol 2006;47:91). NT-proBNP is unreliable in patients with GFR <30.

Both BNP and NT-proBNP are elevated in many disorders besides heart failure and renal failure. Examples include left and right ventricular hypertrophy, valvulopathy, atrial fibrillation, myocarditis, acute coronary syndrome, congenital heart disease, cardiac surgery, pulmonary hypertension, pulmonary embolism, severe anemia, and sepsis. Falsely negative results may occur in patients with right heart failure, mild heart failure and chronically treated heart failure. Obesity causes down regulation of the natriuretic peptide system, resulting in lower BNP and NT-proBNP levels.

BNP is stable in whole blood with EDTA at room temperature for only 24 hours, whereas NT-proBNP is stable for at least 72 hours and requires no additives. The increased stability of NT-proBNP makes it more suitable for outpatient testing. Testing is performed daily. Specimen requirement for NT-proBNP is a green top tube of blood. On October 26, NT-proBNP will replace BNP at almost all of the laboratories within the Saint Luke's Hospital Health System.

HCV Viral Load Update

HCV viral load measurement is used to monitor antiviral treatment response in infected individuals. In May, the FDA approved two new Direct Acting Antiviral (DAA) drugs for treatment of patients with HCV genotype 1 who are treatment-naïve or previous non-responders to conventional therapy.

Clinical trials for these new drugs, boceprevir and telaprevir, utilized a Roche Cobas TaqMan HCV version 2.0 test to measure viral load and assess treatment response. The assay has a lower limit of quantitation (LOQ) of 25 IU/mL and lower limit of detection (LOD) of 15 IU/mL for HCV genotype 1. This compares to an LOQ of 43 IU/mL and LOD of 7-13 IU/mL for genotype 1 with the previous (v1.0) Roche HCV viral load assay.

During the FDA approval process, an LOQ of 25 IU/mL became a drug label recommendation for assessing sustained viral response (SVR) at 24 weeks after end of treatment. This value can only be obtained with the v2.0 test, although both Roche assays meet guidelines for assessing viral load and treatment response at all other clinical decision points.

Saint Luke's Regional Laboratories recently evaluated the Roche Cobas TaqMan HCV Test v2.0 for quantitation of HCV viral load. Parallel testing of twenty samples with viral loads ranging from 139 to 18,300,000 IU/mL demonstrated 98% agreement between assays ($R^2 = 0.9831$). The table shows additional comparison data from 48 samples at or below LOQ and LOD for both assays. As illustrated, only one sample (2%) yielded more information when tested with the version 2.0 assay.

HCV v2.0	HCV v1.0	
	<43 IU/mL	Not Detected
25-43 IU/mL	1 (2%)	0
<25 IU/mL	0	12 (25%)
Not Detected	0	35 (73%)

In early October Saint Luke's Regional Laboratories converted viral load testing from Roche Cobas TaqMan version 1.0 to the version 2.0 assay in order to provide the recommended assessment of SVR post DAA therapy. Sample requirements remain the same. Testing is performed twice weekly.