Clinical Chemistry

April 2016 Audio Summary

This is the April 2016 issue of Clinical Chemistry, Volume 62, Issue 4.

ON THE COVER Cancer Cells. This image depicts circulating tumor cells (CTCs) in peripheral blood. CTCs, which originate in primary tumors, recurrences, or metastases, are biomarkers for non-invasively measuring the evolution of tumor genotypes during treatment and disease progression. CTCs are important because the majority of deaths from cancer are linked to the development of disseminated metastases. Published studies have shown that CTCs can be isolated in patients at relatively early stages of tumor growth. Most current methods are based on epithelial cell adhesion molecule (EpCAM) detection, but numerous studies have demonstrated that EpCAM is not a universal marker for CTC detection. Are there alternatives to EpCAM? Yes. This issue of *Clinical Chemistry* contains a review article that describes the most recent EpCAM-independent methods for enriching, isolating, and characterizing CTCs, based on physical and biological characteristics, and points out their main advantages and disadvantages.

**Plasma branched-chain amino acids and incident cardiovascular disease in the PREDIMED trial**
Miguel Ruiz-Canela et al.

This study examined the role of branched-chain amino acids in cardiovascular disease. The investigators hypothesized that higher levels of baseline branched-chain amino acids were associated with a higher risk of cardiovascular disease and that a Mediterranean Diet intervention might counteract this effect. To address these questions they developed a case-cohort study within the PREvencion con DIeta MEDiterranea trial (also known as the PREDIMED trial). They found higher concentrations of baseline branched-chain amino acids to be associated with increased risk of cardiovascular disease, especially stroke. Although a Mediterranean-style diet had a negligible effect on 1-year changes in branched-chain amino acids, it might counteract the harmful effects of branched-chain amino acids on stroke.

**Increased remnant cholesterol explains part of residual risk of all-cause mortality in 5414 patients with ischemic heart disease**
Anne-Marie K Jepsen et al.

Despite statin therapy many patients with ischemic heart disease experience a new myocardial infarction. This is called residual risk. The authors of this study hypothesized that elevated remnant cholesterol in such patients could explain increased all-cause mortality, and thus residual risk. They therefore measured and calculated remnant cholesterol at baseline in 5414 patients with ischemic heart disease and found that elevated remnant cholesterol explained part of the residual risk of all-cause mortality. This points toward a need for randomized trials to examine the effect of remnant cholesterol reduction in patients with ischemic heart disease.

**Interaction of Galectin-3 Concentrations with the Treatment Effects of β-Blockers and RAS-Blockade in Patients with Systolic Heart Failure: A Derivation-Validation Study from the TIME-CHF and GISSI-HF Trial**
Sandra Sanders-van Wijk et al.

Galectin-3 is a fibrosis marker that predicts prognosis in heart failure. Several drug therapies in heart failure target fibrosis. This study investigated whether galectin-3 at baseline could predict the response to heart failure drugs - beta-blockers, renin angiotensin system-blockade and mineralocorticoid antagonists - in terms of preventing hospitalization and death in 850 patients from 2 clinical heart failure cohorts in a derivation-validation manner. Patients with low galectin-3 responded better to lower doses of beta-blockers whereas patients with high galectin-3 responded better to higher doses of renin angiotensin system-blockade. Results for mineralocorticoid antagonists were inconsistent. Galectin-3 may be useful in selecting patients who benefit most from beta-blockers and renin angiotensin system-blockade.

**Different susceptibility of BNP and proBNP to the cleavage by neprilysin: the N-terminal part does matter**
Alexander G. Semenov and Alexey G. Katrukha

BNP is cleaved by neprilysin. However, the susceptibility of intact proBNP to cleavage by neprilysin is unknown. The authors of this study incubated BNP and proBNP with neprilysin for different time periods and BNP-immunoreactivity was analyzed using two sandwich immunoassays. Both glycosylated and nonglycosylated proBNP were resistant to degradation by neprilysin. The BNP assay utilizing antibodies specific to the region 11-17 was much less susceptible to BNP cleavage by neprilysin compared with the immunoassay utilizing antibodies specific to the region 14-21. Thus, modulation of neprilysin activity by specific inhibitors may not greatly influence the circulating levels of immunoreactive BNP which is mostly represented in heart failure by proBNP.

**High-Sensitivity Cardiac troponin I is a strong predictor of cardiovascular events and mortality in the AGES-Reykjavik community based cohort of older individuals**
Ingunn Thorsteinsdóttir et al.

The authors of this paper investigated the predictive power of a high-sensitivity troponin I assay for death and cardio-vascular events in a large, elderly population and found the assay results to be predictive of major adverse events in both men and women at concentrations far below any of the cut-points for myocardial disease. These findings provide a basis for future prospective clinical trials for determining whether high sensitivity measurements of troponin I can be applied in the prevention of cardiovascular disease and coronary heart disease.

**Prognostic Value of Serial Changes in High-Sensitivity Cardiac Troponin I and T Over 3 Months Using Reference Change Values in Hemodialysis Patients**
Yader Sandoval et al.

Serial changes in cardiac troponin in hemodialysis patients have uncertain clinical implications. This study evaluated associations of adverse outcomes in hemodialysis patients with reference change value data and tertile concentrations for cardiac troponin I and cardiac troponin T measured by high sensitivity assays. Changes over 3 months in high sensitivity cardiac troponin I and high sensitivity cardiac troponin T that exceeded reference change values identified patients at greater risk of all-cause mortality, and for high sensitivity cardiac troponin I were also predictive of sudden cardiac death. Among patients with middle tertile cardiac troponin concentrations, cardiac troponin I changes that exceeded reference change values provided additive prognostic value for both sudden cardiac death and all-cause mortality, whereas for cardiac troponin T provided additive prognostic value only for all-cause mortality.

**Minimizing matrix effects for the accurate quantification of 25-hydroxyvitamin D metabolites in dried blood spots by LC-MS/MS**
David Kvaskoff et al.

The authors of this study previously published an assay for the major circulating form of vitamin D (25-hydroxyvitamin D3) in pediatric dried blood spots. Since the publication of this assay the authors have substantially improved assay throughput, analytical sensitivity, stability, and matrix effects. The improvements in this assay should be helpful to the clinical chemistry community, especially researchers using dried blood spots for quantitative analysis of other compounds.

**Systematic Evaluation of Sanger Validation of NextGen Sequencing Variants**
Tyler F Beck et al.

Next-generation sequencing variants are often returned to patients or participants, and the current standard of care is to validate these variants using Sanger sequencing, which is costly and time-consuming. In this study, the authors performed a systematic evaluation of Sanger-based validation of Next-generation sequencing variants using data from the ClinSeq® project. Of over 5,800 Next-generation sequencing variants, 19 were not validated using Sanger sequencing. Seventeen of those variants were subsequently validated using optimized sequencing primers. The validation rate was 99.965% for Next-generation sequencing variants using Sanger sequencing. The authors conclude that Sanger validation has limited utility, and should not be performed routinely.