Development and preliminary clinical validation of a high sensitivity assay for cardiac troponin using a capillary flow (single molecule) fluorescence detector

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Background: The European and American Cardiology Societies (ESC/ACC) have established cardiac troponin (cTnI) as the gold standard for diagnosis of myocardial infarction (AMI) and risk stratification for adverse cardiac events. The ESC/ACC recommends a cTnI cutoff at the 99th% of the normal range with an assay imprecision (CV) of <10%. Currently none of the commercial assays can detect cTnI in healthy subjects with the requisite precision, therefore many have advocated a cTnI cutoff at the 10% CV value. We developed and evaluated a high-sensitivity cTnI assay and determined its analytical and preliminary clinical performance.

Methods: The Singulex cTnI assay utilizes a 384-well ELISA plate, and the ZeptX™ Digital Molecule Counting (DMC) System. This assay uses a monoclonal capture antibody and a fluorescently-tagged affinity-purified goat anti-cTnI detecting antibody. After washing, the fluorescently-tagged antibody is chemically released into each well. An aliquot is pumped into the analyzer. Individually-labeled antibodies are measured during capillary flow by setting the interrogation volume such that the emission of only 1 fluorescent molecule is detected in a defined space following laser excitation. With each signal representing a digital event, this configuration enables extremely high analytical sensitivities. Total fluorescent signal is determined as a sum of the individual digital events. Each molecule counted is a positive data point with hundreds to thousands of DMC events/sample. The limit of detection for the Singulex cTnI assay was determined by the mean +3 SD method. The normal range was determined on a population of 88 apparently healthy subjects. This assay was correlated to the Bayer Centaur on 130 samples from patients admitted with chest pain. We also examined 47 serial samples from 18 patients who presented to the ED with a diagnosis of AMI. In this latter group, all had initial Centaur cTnI results that were <0.35 ng/mL (10% CV output), and 12 were <0.1 ng/mL (99th%). The cTnI concentration was positive on all subsequent serial samples from these patients on the Centaur, establishing the diagnosis of AMI.

Results: The analytical sensitivity of the Singulex assay was 1 pg/mL. The precision was 10% at 4 and 12 pg/mL. The reference population exhibited a normal distribution. The 99th percentile was determined to be <7 pg/mL. The linear regression between Singulex (y) and Centaur (x) was: y = 0.113x + 0.048, r²=0.937. In the 3 cases that had initial Centaur cTnI value between 0.1 and 0.35 ng/mL, all were positive for Singulex. In the 12 cases that had initial Centaur cTnI value <0.1 ng/mL, 5 of 12 cases were positive. The prospective use of the Singulex assay would have detected 53% more AMI cases than the Centaur when the admission sample was tested.

Conclusion: The use of a highly sensitive and precise cTnI assay will enable detection of AMI earlier than with existing cTnI assays. A higher number of patients at risk for adverse cardiovascular events may be detected. The increased sensitivity was achieved by counting individual fluorescent emission events by the ZeptX analyzer.

Precision, recovery and linearity for cTnI

Precision -- Interplate precision, n=20
Avg (pg/ml) 12 95
Std dev 1.4 10.1
%CV 12 11

Recovery (serum) Spiking 5, 15, 45, and 135 pg/mL TnI standard into pooled serum that was TnI immunodepleted

Serum Dilution % of expected
1:2 79
1:4 87
1:8 96

Linearity Pooled human serum was spiked with a moderate concentration of TnI and diluted with calibrator diluent.

Study 2

Of the 50, 36 were within the 99th%. There were 14 samples which were abnormal on the ZeptX system and normal by the Centaur. Outcomes analysis is necessary to determine if these subjects had minor (ischemic) injury.

Conclusions

• The ZeptX system is a highly sensitive capillary flow laser-induced fluorescence immunosassay analyzer
• The Singulex cTnI assay has 10-20 fold higher sensitivity than the Bayer Centaur
• The LoD was 1.7 pg/mL and the 99th% for a healthy population was 7 pg/mL
• For nSTEMI patients, ZeptX detected cTnI earlier than Centaur
• A high sensitivity cTropinin-I assay may enable more patients to be identified at high short-term cardiovascular risk (study to be conducted)