

## Development and Multi-Center Validation of a Blood-based Gene Expression Test for Diagnosis of Obstructive CAD in Non-Diabetic Patients

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### **Abstract:**

**Background:** The diagnosis of significant CAD in stable chest pain patients without prior known disease follows a varied clinical path, including several non-invasive imaging modalities and the gold standard of coronary angiography. In this study we developed and validated a classifier from whole blood samples, based on the expression of 23 genes to aid in the diagnosis of obstructive CAD in non-diabetic patients.

**Methods:** Total blood RNA was isolated from PAXgene<sup>®</sup> tubes drawn from subjects undergoing coronary angiography participating in the PREDICT multi-center trial. Cases had  $\geq 50\%$  stenosis in  $>1$  major coronary artery; controls had  $< 50\%$  luminal stenosis in any major coronary artery as determined by quantitative coronary angiography. A series of microarray experiments identified 130 genes for which RT-PCR was performed in an independent development set of 640 patients. A 23 gene RT-PCR and demographics based classifier was derived from these data using Ridge regression. The classifier consists of gene-expression terms, gene-expression-based cell type ratios and gender specific age functions. An independent validation cohort of 526 patients (192 cases and 334 controls, using the same case definition) was used to prospectively test the RT-PCR based 23 gene classifier.

**Results:** In the validation cohort ROC analysis yielded an overall AUC = 0.70, ( $p=10^{-16}$ ) with independently significant performance in the male and female subsets, ( $p<0.001$  in each gender). The classifier was significantly additive to clinical variables (Diamond-Forrester method (AUC 0.72 vs 0.66,  $p<0.01$ ) in ROC analysis, and classification of clinically defined disease risk was significantly improved as well ( $p=0.004$ ). For the 23 gene classifier, at a sensitivity threshold

of 85%, the overall specificity was 43%, corresponding to NPV and PPV of 83% and 46%, respectively. In the validation study, 34% of the patients had scores below this threshold.

**Conclusion:** This multi-center prospective study validates a non-invasive whole blood based gene expression classifier for the assessment of obstructive CAD in non-diabetic patients. This test may be useful in the management of stable, non-diabetic chest pain patients without a known history of CAD.