Physician Information Guide for HART CVE

HART CVE Overview

The HART CVE (Cardiovascular Events) multi-protein biomarker blood test identifies individuals at risk for developing a major adverse cardiovascular event (MACE)—myocardial infarction (MI), stroke or cardiovascular death—within the next year.

The HART CVE panel and algorithm were developed using Machine Learning (a subset of AI) and a cohort of 927 subjects from Massachusetts General Hospital's (MGH's) CASABLANCA study[1]. The 927 subjects were randomly split into a training set (70%, n=649) and a holdout internal validation set (30%, n=278). All work for protein biomarker and clinical variable selection and development of a prognostic algorithmic model were done exclusively on the training set, in accordance with the Institute of Medicine's guidelines for omics-based (e.g. proteomics or genomics) test development. In addition to 109 proteins, more than 250 clinical variables were used for their potential clinical relevance to myocardial infarction, stroke and cardiovascular death. Candidate panels of proteins and clinical variables were generated using computerized Machine Learning methods. In MGH's holdout internal validation set, using the same multi-protein panel and algorithm, **HART CVE** had an area under the receiver operating curve (AUC) of 0.79, with a 97% negative predictive value (NPV) for low-score patients. In an external validation at University of Hamburg, **HART CVE** had AUC of 0.86. At the optimal cutoff, the panel had a Sensitivity = 86.4%; Negative Predictive Value = 99.4% on the University of Hamburg cohort[2].

HART CVE Multi-Protein Panel

No candidate clinical variables survived the Machine Learning model building process.

The biologic underpinnings of the identified protein biomarkers predictive of myocardial infarction, stroke, and cardiovascular death are significant and represent a unique pathophysiological mix of left ventricular wall stress and myocardial ischemia (↑ NT-proBNP); cardio-renal syndromes/ischemia/injury and vascular inflammation (↑KIM-1); calcification and plaque (↑ osteopontin); and smooth muscle proliferation, inflammation, plaque destabilization via angiogenesis, and left ventricular wall thickening (↑TIMP-1).

N-terminal pro-brain-type natriuretic peptide (NT-proBNP) is best known as a cardiovascular risk marker, with substantial worth as a predictor of risk across a wide range of cardiovascular diagnoses. It is a pro-hormone synthesized and secreted mainly from the ventricular myocardium in response to an increase in left ventricular wall stress and in myocardial

ischemia[3]. Elevated plasma NT-proBNP levels have been reported in patients diagnosed with acute myocardial infarction[4,5]. NT-proBNP independently predicted occurrence of cardiovascular disease in patients presenting healthy at baseline[6] and increased NT-proBNP levels correlated with increased risk of myocardial infarction, stroke, and death[7].

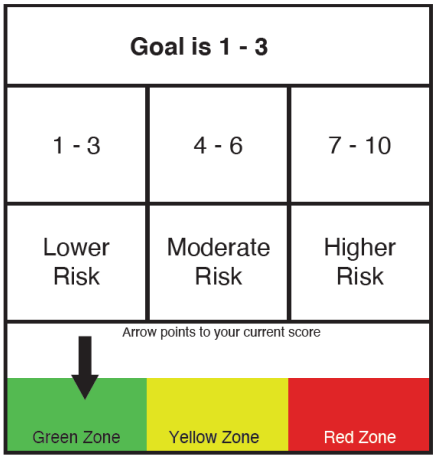
Kidney injury molecule-1 (KIM-1), a marker of cardio-renal syndromes/ischemia and injury, is upregulated in the proximal tubular cells following ischemic injury to the kidney and in chronic kidney disease. KIM-1 is a specific urinary biomarker for kidney injury[8]. It has also been demonstrated that KIM-1 serves as a plasma biomarker of kidney injury[9,10]. KIM-1 levels were predictive of myocardial infarction, stroke, heart failure, and decompensated renal failure in patients after coronary artery bypass graft surgery[11,12,13]. Recently, KIM-1 was shown to be independently associated with cardiovascular disease events and cardiac death[14,15].

Osteopontin (OPN) is a glycoprotein that is synthesized and secreted in many tissues including bone, cardiac tissues, and kidneys. This protein binds calcium and has been found to be associated with calcium deposits in carotid arteries[16]. It has been shown that in a rat model of myocardial infarction, OPN mRNA and protein levels were increased in the heart after injury[17]. In human patients, plasma OPN levels were elevated post-myocardial infarction[18]. In a study of more than 700 men, plasma OPN levels were associated with cardiovascular death[19]. In the PEACE trial, OPN levels were measured in more than 3500 patients and increased levels correlate with increased risk for myocardial infarction, hospitalization, and death[20].

Tissue inhibitor of metalloproteinase-1 (TIMP-1) is a protein that binds to and inhibits the activity of matrix metalloproteinases[21]. TIMP-1 has been associated with smooth muscle proliferation[22], inflammation[23], plaque destabilization via angiogenesis[24], and left ventricular wall thickening[25]. In humans, TIMP-1 levels have shown to increase after myocardial infarction[26] and increasing levels of TIMP-1 positively correlate to risk of myocardial infarction. TIMP-1 also accurately predicted cardiac mortality out to two years[27]. Additionally, the Framingham Heart Study demonstrated that TIMP-1 expression levels positively correlated to an increase in left ventricular mass, wall thickness, and end-systolic diameter, as well as left atrial diameter, which are all indicators of cardiac disease[28].

HART CVE Scoring

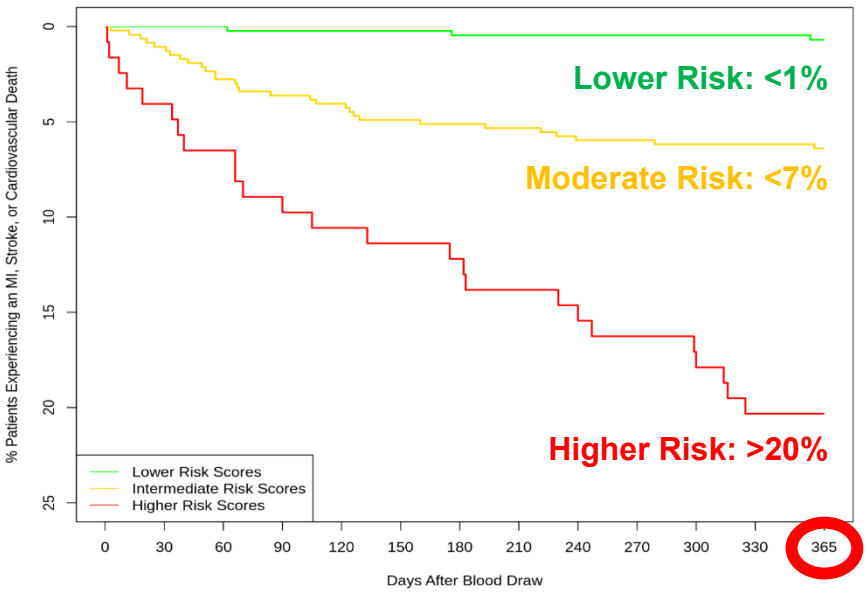
Prevencio and MGH researchers developed a HART CVE risk score scaled from 1 to 10. The scores were divided into three risk ranges: **Lower Risk**; **Moderate Risk; Higher Risk**



Lower Risk (Green Line)
A score from 1-3 indicates a *low likelihood* of developing an adverse MACE event; specifically, a **<1 in a 100 or (<1%) risk** of having a heart attack, stroke, or cardiovascular death at 1 year.

Moderate Risk (Yellow Line)
A score of 4-6 signifies a *moderate likelihood* of developing an adverse MACE event; specifically, a **<1 in 15 or (<7%) risk** of having a heart attack, stroke, or cardiovascular death at 1 year.

Higher Risk (Red Line)
A score of 7-10 indicates a *high likelihood* of developing an adverse MACE event; specifically, a **>1 in 5 or (>20%) risk** of having a heart attack, stroke, or cardiovascular death at 1 year.



Additional information available on the Prevencio website at http://www.prevenciomed.com

For Questions

Please contact Prevencio, Inc., at HART@prevenciomed.com.

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