



CADhs TEST

Physician Information Guide for HART CADhs Test

HART CADhs Overview

The HART CADhs multi-protein blood test diagnoses the likelihood of coronary artery disease (CAD), defined as having $\geq 70\%$ stenosis in a major coronary artery.

The HART CADhs 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. The 927 subjects were randomly split into a training set (70%, n=636) and a holdout internal validation set (30%, n=275). 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 atherosclerosis. Candidate panels of proteins and clinical variables were generated using computerized Machine Learning methods. In the holdout internal validation set, using the same multi-protein panel and algorithm, **HART CADhs** had an area under the receiver operating curve (AUC) of 0.85 for $\geq 70\%$ obstruction in a major epicardial vessel. On a 5-point score, a score of 5 had a positive predictive value (PPV) = 97%; a score of 1 had a negative predictive value (NPV) = 89%.¹

During external validation using a cohort of 241 patients presenting to the Emergency Department with suspicion for acute myocardial infarction (MI) from the University of Hamburg's Biomarkers in Acute Cardiac Care (BACC) Study population, the **HART CADhs**[®] clinical/biomarker panel generated an area under the receiver operating curve (AUC) of 0.86 for stenosis. On a 5-point score, a score of 5 had a positive predictive value (PPV) = 97% ("**HART CADhs**[®] External Validation Set").

When **HART CADhs**[®] was applied to a subset of 65 patients from the BACC study with 'Indeterminate/Gray Zone' hs-cTnI values for myocardial infarction, the panel generated an AUC of 0.88 for obstruction. On a 5-point score, a score of 5 had a positive predictive value (PPV) = 100%.

HART CADhs Multi-Protein Panel

Three final proteins (**↓ Adiponectin, and ↑ Kidney Injury Molecule-1 (KIM-1), ↑ hsTroponin I**) were identified, each of which either added significantly to model discrimination and/or calibration for predicting epicardial coronary artery stenoses $\geq 70\%$. Additionally, three clinical variables (sex, age, and prior percutaneous coronary intervention) were identified which added additional model discrimination and/or calibration.

These biomarkers represent pathophysiological pathways that affect glucose and fatty acid metabolism (Adiponectin); cardiorenal syndrome and vascular inflammation (Kidney Injury Molecule (KIM)); and cardiac ischemia or stress (Troponin). Those with severe CAD had lower concentrations of adiponectin and higher concentrations of KIM-1 and hsTroponin I at baseline.

Adiponectin, secreted from adipose tissue, is involved glucose level regulation and fatty acid breakdown. In the heart, low levels of adiponectin are associated with increased inflammation, endothelial adhesion, and lipid accumulation in macrophage foam cells.² Adiponectin blood levels are inversely correlated with percent body fat and triglycerides,³ and low levels of adiponectin increase risk of developing insulin resistance, metabolic syndrome, diabetes, CAD and CAD progression.^{4,5} Additionally, low adiponectin levels have been found in patients with endothelial dysfunction, hypertension,⁶ dyslipidemia, atherosclerosis,⁷ ischemic heart disease⁸ and increased myocardial infarction risk.⁹ Accordingly, in HART CADhs, decreased plasma levels of adiponectin were associated with coronary artery obstruction.

Kidney injury molecule-1 (KIM-1), a marker of cardio-renal syndromes/ischemia, vascular inflammation, 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.¹⁰ It has also been demonstrated that KIM-1 serves as a plasma biomarker of kidney injury.^{11,12} KIM-1 levels were predictive of myocardial infarction,^{13,14,15} stroke, heart failure, and decompensated renal failure in patients after coronary artery bypass graft surgery. Recently, KIM-1 was shown to be independently associated with cardiovascular disease events, including myocardial infarct, stroke and cardiac death,^{16,17,18} as well as peripheral artery disease.¹⁹ Accordingly, in HART CAD, elevated plasma levels of KIM-1 were associated with coronary artery obstruction.

Cardiac troponins (cTn) are components of the thin filament of the sarcomere of cardiac muscle regulating excitation–contraction coupling in the heart.^{20,21} Owing to their superior sensitivity and cardiac tissue specificity compared with intracellular cardiac enzymes or creatine kinase-MB mass, cardiac troponin T or I (cTn) are now considered the preferred biomarkers for the diagnosis of myocardial injury.^{22,23}

Use of high-sensitivity (hs) troponin assays allow more accurate and earlier detection of myocardial infarction (MI).^{24,25} Higher analytical sensitivity increases the number of patients with analytically true positive cTn results due to non-ST-elevation MI, but also due to numerous acute or chronic diseases in the absence of overt ischemic heart disease.

HART CADhs Risk Score and Report

Prevenico and MGH researchers then developed a HART CADhs Risk Score, scaled from 1 to 5.

The scores were then divided into three risk ranges:

Lower Risk for Scores 1-2

Moderate Risk for a Score of 3

Higher Risk for Scores 4-5

Lower Risk (Green)

A score of 1 had a mean stenosis of ~20% and 9% risk of having a $\geq 70\%$ obstruction in at least one epicardial coronary artery

A score of 2 had a mean stenosis of ~40% and 21% risk of having a $\geq 70\%$ obstruction in at least one epicardial coronary artery

Moderate Risk (Yellow)

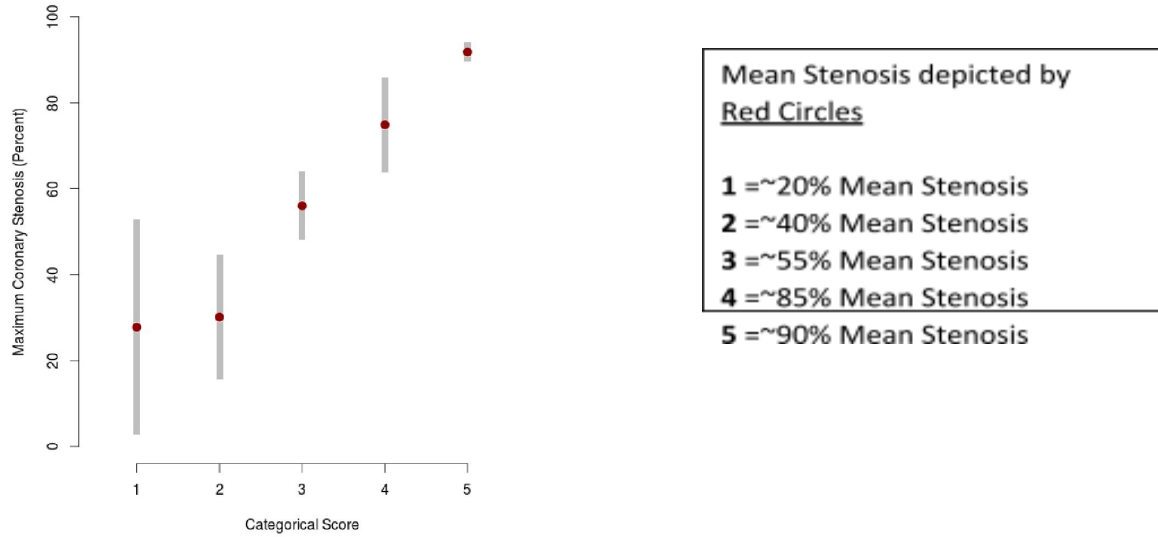
A score of 3 had a mean stenosis of ~55% and 46% risk of having a $\geq 70\%$ obstruction in at least one epicardial coronary artery

Higher Risk (Red)

A score of 4 had a mean stenosis of ~85% and 85% risk of having a $\geq 70\%$ obstruction in at least one epicardial coronary artery

A score of 5 had a mean stenosis of 90% and 93% risk of having a $\geq 70\%$ obstruction in at least one epicardial coronary artery

The HART CADhs Risk Scores can identify those at higher risk which merit consideration for aggressive invasive or medical management therapy while considering less aggressive measures in lower risk patients.²⁶



HART CADhs Mean Stenosis and Risk for the Presence of Obstructive ($\geq 70\%$) Coronary Artery Disease (CAD)		
Score	Mean Stenosis	Risk of CAD
5	~90%	93%
4	~85%	85%
3	~55%	46%
2	~40%	21%
1	~20%	9%



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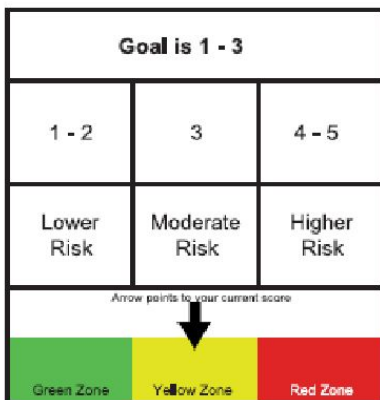
HEIGHT
71
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176
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HART CADhs SCORES

Past Results				Recent Results
				08/03/2020
				3



Risk for Coronary (Heart) Artery Disease*

When a patient has a significant obstruction/plaque/blockage of coronary (heart) arteries, they are at higher risk of having a heart attack.

Patients with Lower Risk Scores (Range 1 - 2; Green Zone) had: Lower Likelihood of having significant obstruction/plaque/blockage in at least one major coronary (heart) artery that is responsible for providing blood flow to the heart.

Patients with Moderate Risk Scores (Range 3; Yellow Zone) had: Moderate likelihood of having significant obstruction/plaque/blockage in at least one major coronary (heart) artery that is responsible for providing blood flow to the heart.

Patients with Higher Risk Scores (Range 4 - 5; Red Zone) had: Higher risk of a significant obstruction/plaque/blockage in at least one major coronary (heart) artery that is responsible for providing blood flow to the heart.

* Cian P, McCarthy, MB, BCH, BAO; Johannes T, Neumann, MD; Sam A, Mitchell, BA; Nasrien E, Ibrahim, MD; Hanna K, Caggini, MD, MPH; Nils A, Sorenson, MD; Sarah Schaefer, MD; Tanja Zeller, PhD; Craig A, Magaret, MS; Grady James, PhD; Rhonda F, Rhyne, BPharm, MBA; Dirk Westermann, MD; James L, Januzzi Jr, MD. J Am Heart Assoc. 2020;9:e017221. DOI: 10.1161/JAHA.120.017221

This test was developed and performance characteristics were determined by Alphas Genomics LLC and this test is not FDA approved/cleared. The laboratory is regulated under CLIA as qualified to perform high-complexity testing. Results are not for use as the sole means for clinical diagnosis or patient management.

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Additional information is available on Prevencao's website at <http://www.preveniomed.com>

For Questions

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