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# A Blood-Based Gene Expression Test for Obstructive Coronary Artery Disease Tested in Symptomatic Nondiabetic Patients Referred for Myocardial Perfusion Imaging

## The COMPASS Study

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***Background***—Obstructive coronary artery disease diagnosis in symptomatic patients often involves noninvasive testing before invasive coronary angiography. A blood-based gene expression score (GES) was previously validated in nondiabetic patients referred for invasive coronary angiography but not in symptomatic patients referred for myocardial perfusion imaging (MPI).

***Methods and Results***—This prospective, multicenter study obtained peripheral blood samples for GES before MPI in 537 consecutive patients. Patients with abnormal MPI usually underwent invasive coronary angiography; all others had research coronary computed tomographic angiography, with core laboratories defining coronary anatomy. A total of 431 patients completed GES, coronary imaging (invasive coronary angiography or computed tomographic angiography), and MPI. Mean age was 56±10 years (48% women). The prespecified primary end point was GES receiver-operating characteristics analysis to discriminate ≥50% stenosis (15% prevalence by core laboratory analysis). Area under the receiver-operating characteristics curve for GES was 0.79 (95% confidence interval, 0.73–0.84; $P<0.001$), with sensitivity, specificity, and negative predictive value of 89%, 52%, and 96%, respectively, at a prespecified threshold of ≤15 with 46% of patients below this score. The GES outperformed clinical factors by receiver-operating characteristics and reclassification analysis and showed significant correlation with maximum percent stenosis. Six-month follow-up on 97% of patients showed that 27 of 28 patients with adverse cardiovascular events or revascularization had GES >15. Site and core-laboratory MPI had areas under the curve of 0.59 and 0.63, respectively, significantly less than GES.

***Conclusions***—GES has high sensitivity and negative predictive value for obstructive coronary artery disease. In this population clinically referred for MPI, the GES outperformed clinical factors and MPI.

***Clinical Trial Registration***—URL: http://www.clinicaltrials.gov. Unique identifier: NCT01117506. **(*Circ Cardiovasc Genet*. 2013;6:154-162.)**

**Key Words:** atherosclerosis ■ computed tomography angiography ■ coronary angiography ■ gene expression ■ myocardial perfusion imaging

The evaluation of patients presenting with chest pain or other symptoms suggestive of coronary artery disease (CAD) is a common clinical challenge. A history and physical examination followed by a stress test, without or with myocardial perfusion imaging (MPI), make up most evaluations. In the United States, MPI is most commonly performed; 6.8 million patients underwent such tests in 2009.[1] Direct referral to invasive coronary angiography (ICA) or computed tomographic angiography (CTA)[2] in place of or after positive stress tests is another common pathway. However, concerns about cumulative radiation exposure from multiple tests,[3–5] the overall low proportion of obstructive CAD in patients referred for ICA,[6,7] and the implications of the Clinical Trials Utilizing Revascularization and Aggressive Drug Evaluation (COURAGE) trial[8] suggesting a more conservative approach make less invasive and non–radiation-based diagnostic alternatives desirable.

**Editorial see p 139**
**Clinical Perspective on p 162**

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We previously developed and validated a peripheral blood gene expression score (GES) to assess obstructive CAD likelihood in nondiabetic patients referred for ICA and analyzed by core-laboratory quantitative coronary angiography (QCA) in the Personalized Risk Evaluation and Diagnosis in the Coronary Tree (PREDICT) study (NCT005617).[9,10] The score algorithm was derived by the use of Ridge regression from 640 patients for whom real-time polymerase chain reaction gene expression data and QCA had been obtained.[9] This algorithm comprises expression values for 23 genes from peripheral blood cells in 6 terms, patient age, and sex as shown in Figure 1. Each term is composed of ratios of highly correlated genes representing a diverse set of inflammatory cell biology, including neutrophil apoptosis, neutrophil-to-lymphocyte ratio, and natural killer-cell activation. There are both sex-specific and common algorithm terms with sex-specific weights. Subsequently, we showed that patients with low GES (≤15) had very low rates of revascularizations and adverse events over 1 year[11] and that the GES appeared to be especially useful in women.[12]

A limitation of the PREDICT study was selection bias inherent in the angiographically referred population,[13] and the accuracy of the GES in a lower-CAD-prevalence population is unknown. Accordingly, we designed the Coronary Obstruction Detection by Molecular Personalized Gene Expression (COMPASS) study to extend this work upstream in the referral path to symptomatic nondiabetic patients referred for MPI using a composite hierarchical anatomic end point of QCA and core-laboratory CTA to define obstructive CAD status in all participants. Thus, COMPASS enables an assessment of GES and MPI performance in a lower- risk population while minimizing selection bias.

## Methods

### Study Design

The COMPASS study was a multicenter, prospective, double-blind, diagnostic clinical study. We enrolled 537 patients at 19 US sites, both community and academic centers (Appendix II in the online-only Data Supplement); of these, 431 patients were evaluable, having completed the protocol prespecified testing: GES, MPI, and ICA or research CTA.

Patients were enrolled from May 2010 to March 2011. The Institutional Review Board at each center or a central Institutional Review Board approved the study, and all patients provided written informed consent. Patients referred for diagnostic MPI stress testing with angina or angina-equivalent symptoms were eligible. Exclusion criteria included history of myocardial infarction (MI) or CAD, acute MI, diabetes mellitus or hemoglobin $A_{1c}$ >6.5%, New York Heart Association class III or IV heart failure symptoms, cardiomyopathy with ejection fraction ≤35%, severe cardiac valvular diseases, systemic infectious or inflammatory conditions, or treatment with immunosuppressive or chemotherapeutic agents at study entry. For patients requiring a research CTA, additional exclusion criteria were atrial fibrillation, known renal insufficiency (creatinine ≥2.0 mg/dL), or severe iodinated contrast allergy.

Peripheral blood was collected before MPI for GES measurements. Subjects with positive MPI underwent ICA on the basis of clinical

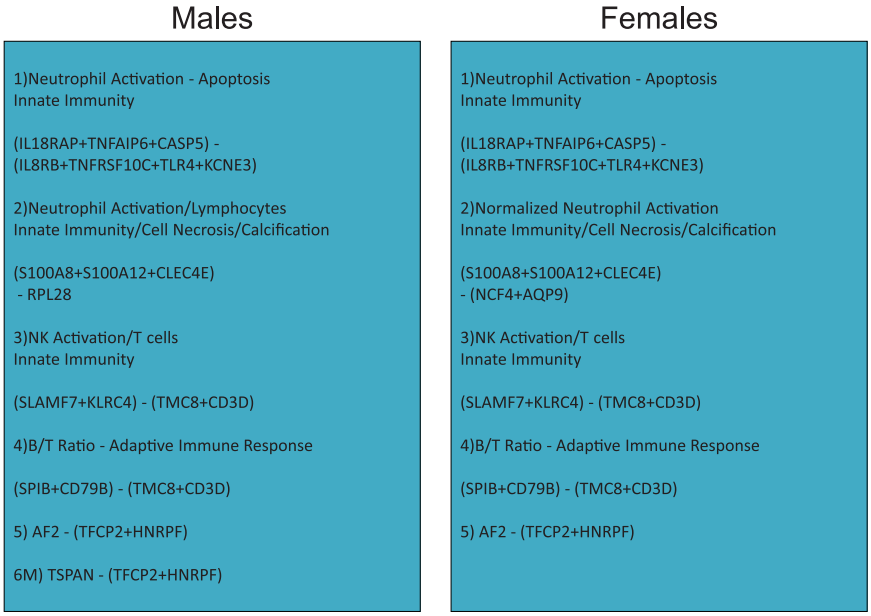


**Figure 1.** Schematic of gene expression score algorithm. The algorithm consists of overlapping gene expression functions for men and women with sex-specific coronary artery disease (CAD) age dependencies. The algorithm gene expression terms and their biological or cellular pathways are shown. The genes symbols are as follows: *IL18RAP*, interleukin-18 receptor-associated protein; *TNFAIP6*, tumor necrosis factor-α–induced protein 6; *CASP5*, caspase-5; *IL8RB*, interleukin-8 receptor β; *TNFRSF10C*, TRAIL decoy receptor 3; *TLR4*, Toll-like receptor-4; *KCNE3*, ISK family potassium voltage-gated channel; *S100A8*, S100 calcium-binding protein 8; *S100A12*, S100 calcium-binding protein 12; *CLEC4e*, C-type lectin domain family 4e; *RPL28*, ribosomal protein 28 light subunit; *AQP9*, aquaporin 9; *NCF4*, neutrophil cytosolic factor 4; *SLAMF7*, SLAM family member 7; *KLRC4*, killer cell lectin receptor family C4; *TMC8*, transmembrane channel-like-8; *CD3D*, CD3-δ; *SPIB*, spi-B transcription factor; *CD79B*, immunoglobulin associated CD79B; *AF2*, AF289562, unknown protein; *TSPAN*, AF161365, unknown protein; *TFCP2*, transcription factor CP2; and *HNRPF*, heterogeneous nuclear riboprotein F. The gene expression score is calculated from median Cp values as follows: raw score=intercept−0.755×($N_{up}$−$N_{down}$)−0.308×sex×($SCA_1$−$Norm_1$)−0.548×(1−sex)×($SCA_1$−Neut)−0.406×($NK_{up}$−$T_{cell}$)−0.137×($B_{cell}$−$T_{cell}$)−0.482×sex×(TSPAN)−0.246 (AF2−$Norm_2$). For men (SEX=1) and women (sex=0), intercept=2.672+0.0449×Age and 1.821+0.123×(Age−60), respectively, with only positive values allowed for women; $N_{up}$=1/3×(CASP5+IL18RAP+TNFAIP6), $N_{down}$=0.25×(IL8RB+TNFRSF10C+TLR4+KCNE3); $SCA_1$=1/3×(S100A12+S100A8+CLEC4E); $Norm_1$=RPL28; Neut=0.5×(AQP9+NCF4); $NK_{up}$=0.5×(SLAMF7+KLRC4); $T_{cell}$=0.5×(CD3D+TMC8); $B_{cell}$=2/3×CD79B+1/3×SPIB; TSPAN=1 if (AF161365−Norm2)>6.27 otherwise 0; and $Norm_2$=0.5×(HNRPF+TFCP2). The final score is transformed to the integer 1 to 40 scale for clinical reporting as described in Methods in the online-only Data Supplement. Adapted from Elashoff et al.[9]

judgment; all others had research CTA. This established anatomic reference data for all patients and attenuated the impact of referral bias on test performance estimates. Patients were followed up for 6 months after index MPI and GES with clinical end points defined as major adverse cardiac events I (MACEs); nonfatal MI, stroke/transient ischemia attack, and all-cause mortality) and revascularization (Appendix III in the online-only Data Supplement).

## Clinical Estimations of CAD Likelihood

The clinical pretest probability of CAD was estimated by 2 methods: the Diamond–Forrester classification[14] and the Morise score.[15,16]

## Stress MPI and Angiography

All subjects underwent single-photon emission computed tomography MPI based on site standard of care with either exercise (78%) or pharmacological (22%) stress, with stress-only imaging in 22% (4% with attenuation correction). Patients were classified as MPI negative (normal or fixed defect interpreted as artifact) or MPI positive (reversible or fixed perfusion defect in any myocardial segment). Site MPI interpretation was used to reflect real-world MPI use and core-laboratory evaluation completed to provide an expert interpretation for secondary analysis (Appendix III in the online-only Data Supplement).

ICA was performed according to institutional protocols, with at least 2 orthogonal views of the major coronary arteries. CTA image acquisition and reconstruction parameters were based on local institutional protocols on ≥64-slice multidetector CT systems. β-Blockade was encouraged to achieve heart rate of ≤65 bpm and sublingual nitroglycerin for vasodilation. For local CTA image analysis, investigators interpreted scans on the basis of a modified 17-segment American Heart Association coronary segmentation model.[17] Each segment stenosis was visually and qualitatively graded (none; minimal [<25%]; mild [25%–49%]; moderate [50%–69%]; severe [70%–99%]; occluded [100%]; nonevaluable).

Core-laboratory evaluations were performed for ICA by QCA and for coronary CTA by 2 independent readers to define obstructive CAD anatomic reference standards (Appendix III in the online-only Data Supplement).

## CAD and Clinical Events Definitions

Obstructive CAD was defined prospectively as ≥1 stenosis ≥50% in a major vessel on QCA (≥1.5 mm) or CTA (≥2.0 mm). If QCA results were obtained, they were used; otherwise, core-laboratory CTA defined obstructive CAD. Patients with obstructive CAD were defined as cases and others as controls for dichotomous analyses. A subset of patients (n=28) with both QCA and core-laboratory CTA were used for intermethod comparisons. Mild CAD was defined as ≥25% to 49% stenosis.

Clinical end points were predefined as all revascularizations and MACEs (nonfatal MI, stroke/transient ischemic attacks, or all-cause mortality) both within 30 days of the index MPI and subsequently during follow-up.

## GES Determination

Venous blood samples were collected before MPI in PAXgene RNA preservation tubes (PreAnalytiX, Valencia, CA) according to the manufacturer's instructions and stored at −20°C. Automated RNA purification, cDNA synthesis, and real-time polymerase chain reaction were performed as described,[10,18] according to Corus CAD protocols in a Clinical Laboratory Improvement Amendments-approved reference laboratory (CardioDx, Inc, Palo Alto, CA). Raw GESs were computed from median expression values for the 23 algorithm genes, age, and sex and linearly transformed to a 1 to 40 scale for reporting (Figure 1; Appendix I in the online-only Data Supplement).[10]

## Statistical Analysis

A prospectively defined analysis plan (Appendix IV in the online-only Data Supplement) was communicated to the external statistician (M.E.W.) before study completion, and primary and secondary analyses were performed starting from individual well real-time polymerase chain reaction data. The primary end point of GES area under

**Table 1. Clinical and Demographic Characteristics of the Patient Cohort***

| Variable | Controls† (n =368) | Cases† (n=63) | All (n=431) | *P* Value |
|---|---|---|---|---|
| Male sex, n (%) | 174 (47) | 51 (81) | 225 (52) | <0.001 |
| White, n (%) | 324 (88) | 59 (94) | 383 (89) | 0.275 |
| Age, y | 55±10 | 62±9 | 56±10 | <0.001 |
| Systolic BP, mm Hg | 129±16 | 136±18 | 130±17 | 0.002 |
| Dyslipidemia, n (%) | 190 (52) | 46 (73) | 236 (55) | 0.003 |
| Symptoms, n (%) | | | | 0.775 |
| Asymptomatic | 1 (0.3) | 0 (0) | 1 (0.2) | |
| Atypical | 212 (58) | 38 (60) | 250 (58) | |
| Nonanginal | 83 (23) | 11 (18) | 94 (22) | |
| Typical | 71 (19) | 14 (22) | 85 (20) | |
| BMI kg/m$^2$ | 30±6 | 29±4 | 30±6 | 0.368 |
| Smoking status, n (%) | | | | 0.011 |
| Current | 52 (14) | 14 (22) | 66 (15) | |
| Former | 101 (27) | 25 (40) | 126 (29) | |
| Never | 215 (58) | 24 (38) | 239 (56) | |
| Aspirin, n (%) | 171 (47) | 41 (65) | 212 (49) | 0.009 |
| Statins, n (%) | 161 (44) | 33 (52) | 194 (45) | 0.256 |
| β-Blockers, n (%) | 67 (18) | 19 (30) | 86 (20) | 0.043 |
| ACE inhibitors, n (%) | 103 (28) | 27 (43) | 130 (39) | 0.030 |

ACE indicates angiotensin-converting enzyme; BMI, body mass index; and BP, blood pressure.

*Results shown for the 431 evaluable patients.

†Case and control status determined by core laboratory with ≥50% maximum stenosis used as the case threshold.

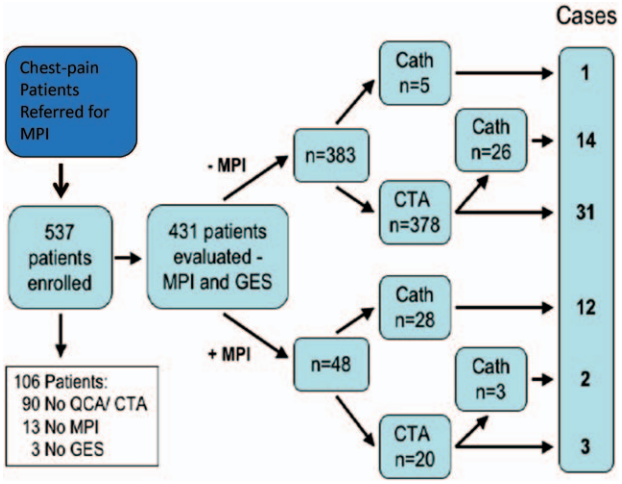


**Figure 2.** Study design and patient flow diagram. Nondiabetic patients without known coronary artery disease referred for myocardial perfusion imaging (MPI) were consented and had blood drawn for gene expression score (GES) before MPI. Positive MPI results were referred for invasive coronary angiography (ICA), if clinically appropriate; all other patients were asked to obtain a research computed tomographic angiography (CTA), yielding anatomic reference data for all patients. If CTA results warranted, patients could be referred for ICA. All enrolled patients and MPI and angiographic results leading to the 63 cases are shown. Patients enrolled but not included in the final analysis set included 3 without GES, 90 without CTA or ICA, and 13 without evaluable MPI scans. Negative MPI scans (89% of total) were largely evaluated by CTA (378 of 383) and led to 46 cases (12% of negative MPIs). Positive MPI scans were evaluated predominantly by ICA (28 of 48) and led to 17 cases (35% of positive MPIs). QCA indicates quantitative coronary angiography.

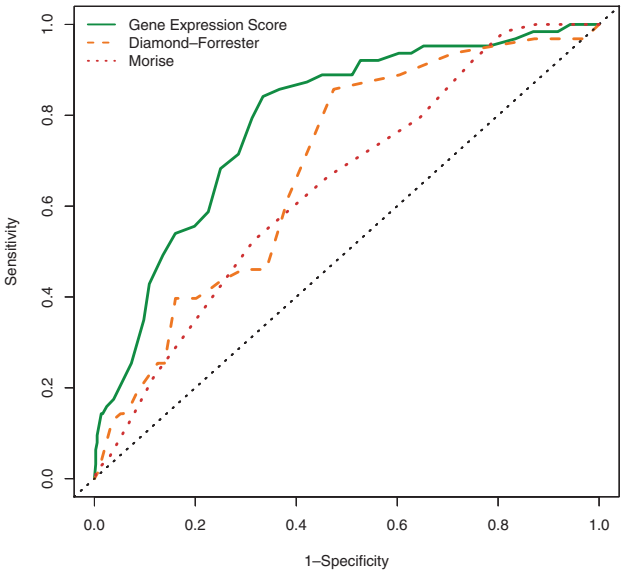


**Figure 3.** Receiver-operating characteristics (ROC) analysis of gene expression score (GES) and clinical factors. ROC curves for a case definition of ≥50% maximum stenosis by either quantitative coronary angiography (QCA) or computed tomographic angiography (CTA) are shown: GES (green solid line), Morise score (yellow dashed line), and Diamond–Forrester score (orange heavy dotted line), with diagonal reference area under the curve (AUC) of 0.50. AUCs for the GES, Morise, and Diamond–Forrester scores were 0.79, 0.67, and 0.69, respectively. All 431 patients were used for the GES and the Morise score; 430 were used for the Diamond–Forrester score because chest pain information was missing for 1 patient.

the curve (AUC) superiority to 0.5 was powered to >90% (2-sided $\alpha$=0.05) with 376 subjects and 62 cases assuming an AUC of 0.70. Sensitivity, specificity, negative predictive value (NPV), and positive predictive value were calculated at a prespecified GES threshold of ≤15 (>15 is GES positive, ≤15 is GES negative) from our previous validation study.[10]

Referral bias correction was performed as described by Diamond.[19]

$$Se = (q)/(p/ASe + q - p)Sp = (p)/(q/ASp + p - q). \quad (1)$$

Se is true sensitivity; Sp is true specificity; ASe is apparent (biased) sensitivity; ASp is apparent (biased) specificity; p is referral rate for positive tests; q is referral rate for negative tests.

All analyses were performed with R, version 2.13 (Hmisc, pROC, ROCR, verification, and SDMTools packages).[20] Unless otherwise specified, univariate comparisons used *t* tests for continuous variables and $\chi^2$ tests for categorical variables. All reported *P* values are 2 sided. Standard methods were used to estimate receiver-operating characteristics (ROC) curves and associated AUCs with the *Z* test to discriminate AUCs from 0.5. For other AUC comparisons, 10 000 bootstrap iterations were performed, and *P* values were estimated from the empirical distribution of bootstrapped AUC differences.[10]

GES correlation with maximum percent stenosis was estimated by linear regression and the Pearson correlation coefficient (*r*). Influence of demographic and clinical factors was assessed with a linear regression model in which the gene expression portion of the GES was the dependent variable and the independent variables were the factors in Table 1 (apart from age and sex, which are incorporated into the GES algorithm).

Reclassification of disease status using the GES in patients after MPI was assessed by net reclassification improvement (NRI)[21,22] using 3 GES categories (low, ≤15; intermediate, 16–27; and high, ≥28). A successful reclassification was defined as a patient without obstructive CAD with positive MPI and a low GES (≤15) or with obstructive CAD and negative MPI with a high GES (≥28). NRI for the GES represents patients correctly reclassified from an incorrect MPI classification minus those incorrectly reclassified by GES from a correct MPI classification. For comparison with clinical factors, the pretest probability was divided into 3 categories: low (<15%), medium (15%–50%), or high (>50%) likelihood.[10]

## Results

### Patient Flow and CAD Prevalence

This study enrolled 537 patients at 19 sites who were clinically referred for MPI and had a blood sample obtained for GES measurement before stress testing, with coronary anatomy assessed by ICA if clinically indicated and by research CTA otherwise (Figure 2). A final cohort of 431 patients was evaluable having completed all prespecified diagnostic tests: MPI, GES, and core-laboratory assessed CTA or ICA. Patient exclusions were attributable primarily to 90 subjects declining a research CTA after a negative MPI.

The clinical and demographic characteristics of this 431-patient cohort are shown in Table 1. Characteristics associated with obstructive CAD were older age, male sex, higher systolic blood pressure, dyslipidemia, smoking, and prescription of aspirin, β-blockers, and angiotensin-converting enzyme inhibitors, whereas symptoms, ethnicity, and body mass index were not. The proportions of patients with low, intermediate, and high Diamond–Forrester CAD likelihoods were 58%, 17%, and 25%, respectively. Obstructive CAD was present in 63 patients (15%): 17 patients with positive MPIs and 46 with negative MPIs (Figure 2). Obstructive disease was identified in 29 patients by QCA and in 34 by core-laboratory

**Table 2. Comparative Summary Statistics of Gene Expression Score, Myocardial Perfusion Imaging, and Clinical Factor Algorithms**

| | Gene Expression Score (n=431)* | Myocardial Perfusion Imaging Site-Read (n=431)* | Myocardial Perfusion Imaging Core-Laboratory (n=371)* | Diamond–Forrester (n=430)* | Morise (n=431)* |
|---|---|---|---|---|---|
| ROC AUC† | 0.79 (0.72–0.84) | 0.59 (0.54–0.65) | 0.63 (0.57–0.70) | 0.69 (0.62–0.75) | 0.65 (0.59–0.74) |
| Sensitivity, %‡ | 89 (78–95) | 27 (17–40) | 36 (24–50) | | |
| Specificity, % | 52 (47–57) | 92 (88–94) | 90 (87–93) | | |
| NPV, % | 96 (93–99) | 88 (84–91) | 88 (84–92) | | |
| PPV, % | 24 (19–30) | 35 (22–51) | 41 (28–56) | | |
| Net reclassification improvement for GES compared with second modality, %§ | N/A | 26 | 11 | 28 | 60 |
| ROC AUC for GES and second modality combined‖ | N/A | 0.81 (0.75–0.86) | 0.81 (0.76–0.87) | 0.79 (0.73–0.85) | 0.81 (0.75–0.89) |

GES indicates gene expression score; MPI, myocardial perfusion imaging; N/A, not applicable; NPV, negative predictive value; PPV, positive predictive value; and ROC AUC, area under the receiver-operating characteristics curve.

*For the GES, site-read MPI, and Morise score, all 431 patients were used. For the Diamond–Forrester classification, 430 patients were used because 1 patient lacked chest pain information. For the core-laboratory MPI, a total of 371 patients were analyzable (Appendix III in the online-only Data Supplement).

†For individual ROC AUCs vs AUC=0.5 and ROC AUC differences between GES and imaging or clinical factors, the point estimate and 95% confidence intervals are shown. $P<0.001$ in all cases except GES vs Diamond-Forrester, where $P=0.0013$.

‡Summary statistics for the GES are shown for a threshold of ≤15.

§All $P<0.001$, except $P=0.13$ for core-laboratory MPI.

‖Comparison of logistic models adding the GES to MPI and clinical factor models.

CTA. Of these, 35 had 50% to 69% stenosis and 28 had 70% to 100% stenosis. Comparing site with core-laboratory reads for angiography and CTA showed a consistent shift to lower percent stenosis in core-laboratory reads, with median shifts of 15% and 22%, respectively. For the 28 patients with both QCA and CTA core-laboratory data, case:control status agreement was 86% (κ=0.72), with only a 1% median stenosis difference between these results ($P$=NS). An additional 92 patients (21%) had mild CAD (25%–49% stenosis).

## GES Performance

The GES (Figure 1) was developed and validated in a series of studies involving >1000 patients.[9,10] In the present study, the GES was a highly significant predictor of obstructive CAD by ROC analysis (AUC=0.79; 95% confidence interval [CI], 0.73–0.84; $P<0.001$; Figure 3 and Table 2). Sensitivity and specificity of the GES were 89% and 52%, respectively, with NPV and positive predictive value of 96% and 24%, with 199 patients (46%) below the prespecified threshold of ≤15. The GES added to clinical factors by both ROC analysis (Figure 3) and NRI using either Diamond–Forrester or Morise classifications (NRI=28% and 60%, respectively; Table 2). The GES was not significantly affected by demographic or clinical covariates, including ethnicity, smoking status, body mass index, dyslipidemia, and systolic blood pressure, or medications (aspirin, statins, β-blockers, and angiotensin-converting enzyme inhibitors; all $P>0.1$; Table I in the online-only Data Supplement).

The GES was significantly correlated with maximum percent stenosis ($r=0.46$; $P<0.001$). The continuous relationship between CAD likelihood and GES is shown for ≥25% and ≥50% stenosis (Figure 4A); a categorical representation using the prespecified GES thresholds of 15 and 28 is shown in Figure 4B.

Patients were followed up for 6 months after index MPI and GES, with 97% (420 of 431) completing follow-up. There were 28 adverse clinical events noted, including 25 revascularizations within 30 days, 1 further revascularization, and 2 MACEs over the next 5 months. A total of 25 of 26 patients with revascularizations and both patients with MACEs had GES >15. The GES was associated with MACEs and revascularization likelihood in a logistic regression model ($P=0.0015$) and showed a sensitivity of 96% and NPV of 99% at a score threshold of ≤15.

## MPI Performance

Local-site MPI scans were reported as positive in 48 of 431 patients (11%) and 51 of 371 patients (14%) by core laboratory with 87% concordance. Site-read image quality was rated as excellent in 210, very good in 72, good in 127, and poor in 22 patients. Overall core-laboratory interpreter certainty was high (279), fair (76), and low (16). MPI was significant in predicting obstructive CAD (≥50% stenosis) by both site and core-laboratory reads (AUC=0.59; 95% CI, 0.54–0.65; and AUC=0.63; 95% CI, 0.57–0.70; $P<0.001$, respectively; Figure 5). For patients with ≥70% stenosis (n=28), these increased to 0.63 and 0.67, respectively, whereas the GES AUC was 0.76. Site-read and core-laboratory MPI had sensitivities of 27% and 36% and specificities of 92% and 90%, respectively; the NPVs and positive predictive values are shown in Table 2. The GES outperformed site-read MPI as a predictor of obstructive CAD by ROC and NRI (ΔAUC=0.19; NRI=26%; both $P<0.001$) and by ROC for core-laboratory MPI (ΔAUC=0.16; $P<0.001$; NRI=11%; $P=0.13$; Figure 5 and Table 2). To further illustrate the relationships between stenosis category (<25%, 25%–49%, and ≥50%), MPI, and GES results, a dot plot for the 371 patients with core-laboratory MPI and GES results is shown in Figure I in the online-only Data Supplement. In the 6-month follow-up, site and core-laboratory MPI were positive in 11 and 14 early revascularizations and 0 and 1 of 3 events/late revascularizations, yielding sensitivities of 39% and 54%, respectively, and NPVs of 96% for both.

To account for potential verification bias on MPI diagnostic accuracy from the 90 patients not undergoing CTA, we performed a sensitivity analysis assuming that these MPI

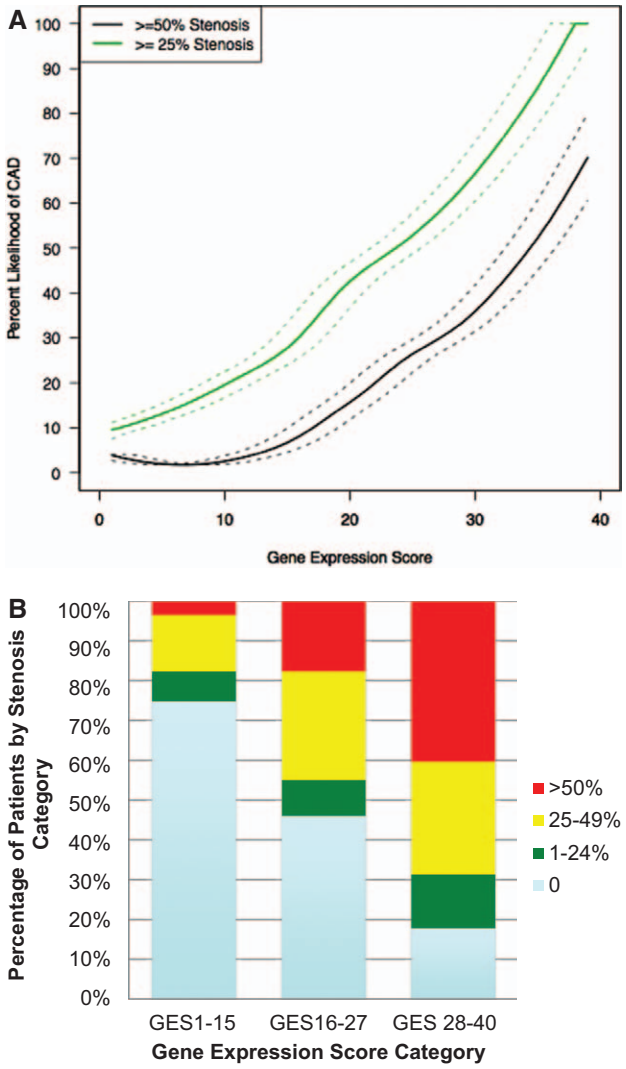


**Figure 4.** **A**, Likelihood of coronary artery disease (CAD) and obstructive CAD as a continuous function of gene expression score (GES). The percent likelihoods of ≥25% stenosis (mild and obstructive CAD) and ≥50% stenosis (obstructive CAD) are indicated by the green and red lines, respectively, as a function of GES, with dashed lines representing 95% confidence intervals. For a given score, the likelihood of mild or greater CAD is higher than for obstructive CAD. **B**, Relationship between stenosis category and GES category. The percentages of patients with 0%, 1% to 24%, 25% to 49%, and ≥50% stenosis are shown in prespecified GES categories of 1 to 15, 16 to 27, and 28 to 40. For these GES categories, the patient numbers are 199 (46%), 165 (38%), and 67 (16%), respectively.

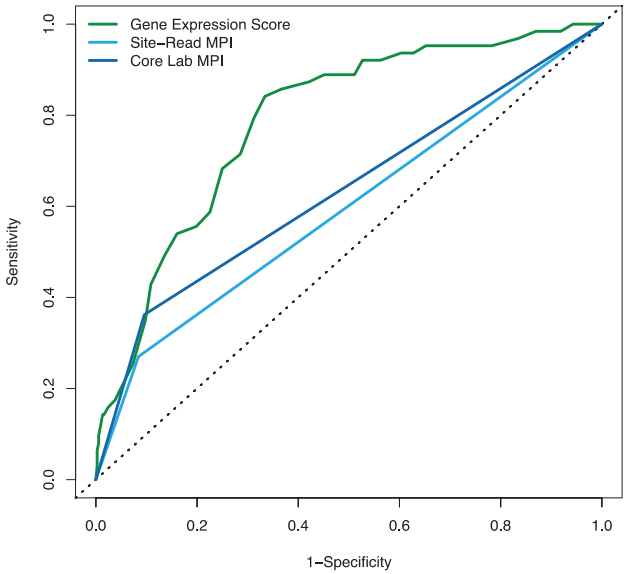


**Figure 5.** Receiver operating characteristics (ROC) analysis of gene expression score (GES) and myocardial perfusion imaging (MPI). ROC curves for a case definition of ≥50% maximum stenosis by either quantitative coronary angiography or computed tomographic angiography are shown: GES (green solid line), site-read MPI (light blue dashed line), core-laboratory MPI (dark blue heavy dotted line), and diagonal reference area under the curve (AUC) of 0.50. The GES, site-read MPI, and core-laboratory MPI AUCs were 0.79, 0.59, and 0.63, respectively. The GES and site-read MPI AUCs were based on 431 patients; the core-laboratory MPI AUC was based on 371 patients (Appendix III in the online-only Data Supplement) for which GES and site-read MPI AUCs were unchanged from the entire cohort.

negatives were all correct (true negatives). This increased the AUC to 0.60 (95% CI, 0.55–0.66) and 0.64 (95% CI, 0.58–0.70) for site and core-laboratory MPI, respectively.

## Discussion

This multicenter, prospective study assessed the diagnostic accuracy of a peripheral blood GES to discriminate obstructive CAD in symptomatic nondiabetic patients clinically referred for MPI, extending our previous work in patients clinically referred for ICA.[10] This study has 4 major findings. First, the GES showed strong discrimination for obstructive CAD (AUC=0.79; 95% CI, 0.73–0.84; $P$<0.001) in this independent, community-based, lower-risk population and was superior to clinical estimates by Diamond–Forrester and Morise scores (ΔAUC=0.10; $P$=0.003; and ΔAUC=0.12; $P$=0.002), respectively. Second, the GES was proportional to maximum percent stenosis, as seen previously.[10] Third, the GES outperformed site-read and core-laboratory MPI for discrimination of obstructive CAD (ΔAUC=0.19 and 0.16; both $P$<0.001). Finally, we demonstrated good agreement between QCA and core-laboratory CTA in case definitions, validating the composite anatomic end point.

The GES is based on peripheral blood cell gene expression levels of 23 genes, age, and sex and reflects changes in peripheral blood gene expression and cell-type distributions in the presence of CAD.[9,10] Clinical practice guidelines for the management of patients with CAD and for revascularization are largely predicated on obstructive CAD; therefore, the prespecified primary end point of the present study was the identification of anatomically obstructive CAD. All patients with GES and MPI results had QCA or core-laboratory CTA to identify obstructive CAD. GES performance was consistent with the PREDICT study validation (AUC=0.79±0.06 versus 0.70±0.04)[10] and similar to the cross-validated estimate of 0.77 from test development.[9] As expected, obstructive disease prevalence in this patient population (15%) was significantly lower than that in the PREDICT study (37%) and in a large angiography registry.[6] This leads to the higher GES NPV in this MPI-referred population (96%) compared with the angiographic population (83%) and a larger proportion of patients with scores ≤15 (46% versus 33%). The optimal GES threshold, maximizing the sum of

sensitivity and specificity, was 19 (sensitivity, 84%; specificity, 67%; NPV, 96%; Table II in the online-only Data Supplement), with 59% of patients below this threshold.

The most common noninvasive imaging modality used in clinical assessment of CAD in the United States is MPI.[23] Thus, this study was designed to assess the GES in this patient population, and a secondary end point was to compare the general community setting performance of MPI with the GES. The 19 sites involved represent a variety of clinical settings, from academic centers to private practices. The GES outperformed MPI by ROC analysis and NRI (Table 2). We previously observed in the angiographic PREDICT study that the GES outperformed site-read MPI by ROC ($\Delta$AUC=0.16; $P<0.001$), but that result was confounded by referral bias of negative MPIs not being referred to ICA.[10] For the 310 patients in the PREDICT validation cohort who had MPI, 72% were positive compared with 11% in COMPASS, suggesting selective patient referral with positive MPIs. However, in both studies, the majority of positive MPIs with low GES were false positives (51 of 57 and 13 of 14, respectively).

## Limitations

First, our study was limited to a relatively small nondiabetic, largely white US population without known CAD, previous revascularization or MI, and known inflammatory or autoimmune disorders but with symptoms suggestive of CAD. Both asymptomatic patients and those with high-risk unstable angina were excluded. Diabetics were excluded on the basis of the observation that peripheral blood gene expression classifiers for CAD in diabetics and nondiabetics are distinct, attributable to either medication effects or differences in underlying pathophysiology.[9] These factors together suggest that the subjects enrolled may have lower disease prevalence and severity than typical outpatient populations without known CAD.

Second, 106 patients from the original population of 537 were excluded from analysis, with the large majority (n=90) of patients with negative MPIs who refused research CTA. As noted above, we required an anatomic gold standard for all patients, not just those with positive MPI. Assuming that all these negative MPIs were correct, site-read MPI AUC increased to only 0.60. In addition, 11 patients were lost to follow-up from the 431 in the evaluable set, which could have influenced MACE and revascularization rates. This is unlikely to be significant because 7 of 11 of these had GES $\leq$15 at baseline and only 1 of 199 with low scores had a revascularization on follow-up.

Third, the GES has high sensitivity and NPV and hence is most suitable as a rule-out test, but 54% of patients had scores >15. These most likely represent patients with nonobstructive CAD but with significant plaque burden and stenosis because the GES was proportional to maximum percent stenosis. As shown in Figure 4B, more than half of the patients with GES >15 had measurable CAD ($\geq$25% stenosis), and this proportion increased with increasing GES. The clinical importance of nonobstructive lesions for disease progression and events was highlighted in the An Imaging Study in Patients with Unstable Atherosclerotic Lesions (PROSPECT) study.[24] Other possible explanations for these higher GES scores without obstructive CAD could be diffuse CAD, atherosclerosis in other vascular beds, or unidentified inflammatory disorders.

Finally, MPI performance in this study was less than expected. Several factors likely contributed to this. First, this study used an anatomic obstructive CAD end point; however, systematic differences would be expected because MPI assesses ischemia. The rationale for an anatomic gold standard was to provide quantitative information across the range of stenosis and because of the prognostic importance of obstructive CAD.[25–27] However, recent studies comparing MPI and CTA-defined anatomy consistently demonstrate that only 30% to 50% of $\geq$50% stenoses result in abnormal MPI,[28–30] lower than cited in the American College of Cardiology 2003 guidelines.[31] Second, this study population was relatively low risk (15% obstructive CAD) and excluded diabetics, inpatients, and those with high-risk symptoms. The mean age of the patient population (56$\pm$10 years) was lower and the frequency of exercise versus pharmacological testing (78%) was greater than those observed in another outpatient-only trial (65 $\pm$12 years and 63% exercise versus 37% pharmacological stress).[32] Whereas ischemia is particularly important in assessing the potential benefit of lesion revascularization and intermediate and long-term prognosis,[31] recent outcome studies of patients undergoing CTA demonstrated a stepwise worsening of prognosis from nonobstructive to obstructive CAD.[26,27] Third, we did not control for inter-reader variability or prespecify a standard image acquisition protocol. Training on specific MPI protocols has been shown to improve inter-reader agreement.[33] A comparison of the GES with other noninvasive imaging modalities such as stress echocardiography or MRI might yield different results.

Finally, studies of cardiovascular imaging modalities, including echocardiography[34,35] and exercise treadmill,[36] correcting for referral bias have reported diagnostic test performance characteristics that vary significantly from those typically reported. Because patients with positive stress-test results are more likely to undergo follow-up ICA, sensitivity and specificity derived from an angiographic population are overestimated and underestimated, respectively. A recent meta-analysis of MPI studies with angiographic end points found a median sensitivity of 81% and specificity of 65%.[37] When we applied a referral bias correction to these data (see Methods),[19] using recent estimates of angiography referral rates for positive (48.2%) and negative (6.2%) MPI results,[38] the unbiased estimates of MPI performance were 35% sensitivity and 94% specificity. These estimates are very similar to the core-laboratory results obtained in this study, which had minimal referral bias by design, and suggest that our results are consistent with the literature after verification bias removal.

## Implications: Atherosclerosis Testing as a Precursor to Ischemia Testing

The correlation of the GES with maximum percent stenosis, the high sensitivity (89%), and the NPV (96%) for obstructive CAD at the prespecified GES threshold of 15 in this symptomatic population with relatively low (15%) CAD prevalence suggest that this test is a highly sensitive measure of coronary atherosclerosis. This is further supported by the GES sensitivity to nonobstructive CAD (Figure 4B). Conversely, MPI had high specificity (92%) for obstructive CAD in this population and measures functional ischemia. Together, these results suggest that MPI could be used to risk stratify the enriched

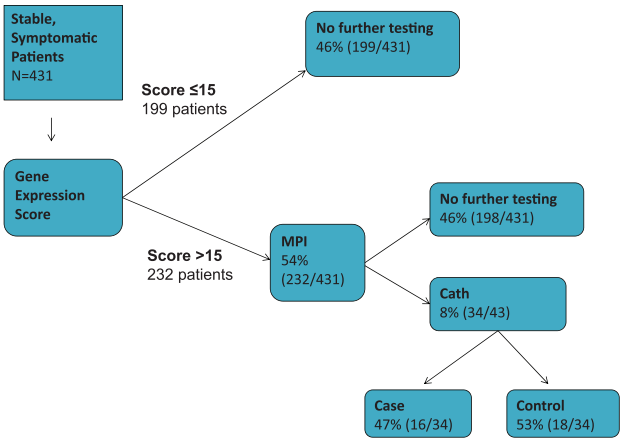


**Figure 6.** Clinical algorithm with sequential use of gene expression score (GES) and myocardial perfusion imaging (MPI). Based on the data in this study, the model shown is suggested. For patients with GES ≤15, no further follow-up is proposed given the high sensitivity and negative predictive value at this threshold. The remaining patients (54%) would undergo MPI, and only those with positive MPIs would be referred for invasive coronary angiography (ICA). Such a clinical algorithm results in a 46% reduction in MPI, a 29% reduction in ICA, and an improvement in ICA yield from 35% to 47%.

population of those with GES above a certain threshold (eg, >15) into those with positive MPI with an ischemic burden or symptom status such that ICA and potential revascularization were warranted and those with negative MPI who would be aggressive medical therapy candidates. Because nonischemic atherosclerotic CAD burden assessed by CTA was shown in the CONFIRM Registry to predict increasing risk of hard cardiac events with increasing nonobstructive CAD,[27] identification and treatment of this group with elevated GES and normal MPI would likely be beneficial. Such a clinical algorithm, illustrated in Figure 6, would result in 46% fewer MPIs and 29% fewer ICA with a higher yield of obstructive disease (47%) based on site-read MPIs (Table III in the online-only Data Supplement.); similar results (45%, 33%, and 49%, respectively) are obtained with core-laboratory MPI (Table IV in the online-only Data Supplement) with a few false-negative GESs with positive MPIs. Given the 6-month follow-up data, in which only 1 patient of the 199 with GES ≤15 had a revascularization, this strategy may have significant clinical utility and safety, yielding more appropriate and targeted cardiac imaging and ICA.

In summary, in this second prospective multicenter validation study of a peripheral blood GES for obstructive CAD in nondiabetic patients, the GES showed significant improvement over clinical estimation of CAD and outperformed MPI in identifying anatomically defined obstructive CAD in symptomatic patients.

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## Disclosures

Drs Rosenberg, Johnson, Daniels, and Elashoff are CardioDx, Inc, employees and have equity interests and stock options in CardioDx, Inc. Drs Rosenberg, Daniels, and Elashoff have filed patent applications on behalf of CardioDx, Inc. Dr Lieu is a consultant for CardioDx, Inc, and has stock options in CardioDx, Inc. Drs Thomas, McPherson, Phelps, and Ladapo were consultants for CardioDx, Inc, and Dr Thomas is a consultant for Astellas Pharma. Dr Douglas reports stock ownership, consulting, and advisory board membership in CardioDx, Inc. Drs Lansky, Voros, and Bateman report research grants from CardioDx, Inc, for core-laboratory activities.

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## CLINICAL PERSPECTIVE

For patients with symptoms suggestive of coronary artery disease, diagnosis can be challenging and is often accomplished by noninvasive imaging, especially myocardial perfusion imaging or computed tomographic angiography, followed by invasive coronary angiography as the gold standard. This diagnostic pathway has associated risks, including procedural complications, radiation exposure, and contrast agent allergy and nephrotoxicity. In this work, a peripheral blood-based gene expression score (GES) for obstructive coronary artery disease, based on 23 genes, age, and sex, previously validated in a population referred for invasive coronary angiography, is tested in symptomatic patients referred for myocardial perfusion imaging. To minimize referral bias, coronary anatomy was defined in all patients by invasive coronary angiography or computed tomographic angiography. For the 431 patients who had GES, myocardial perfusion imaging, and invasive coronary angiography or computed tomographic angiography, 199 (46%) had GES below the prespecified threshold of ≤15, where the GES had a sensitivity, specificity, negative predictive value, and positive predictive value of 89%, 52%, 96%, and 24%, respectively. The area under the curve by receiver-operating characteristics analysis was 0.79, significantly higher than that for myocardial perfusion imaging or clinical predictors. In a clinical model in which the GES was used to rule out further testing in patients with scores of ≤15, a 46% reduction in myocardial perfusion imaging and 29% reduction in invasive coronary angiography could be achieved. Importantly, after a 6-month follow-up, the vast majority of cardiovascular events and revascularizations (27 of 28, 96%) were found in patients with GES >15. These results suggest this noninvasive genomic blood test can play a significant role in reducing noninvasive imaging and invasive coronary angiography in patients with symptoms suggestive of coronary artery disease.

**SUPPLEMENTARY MATERIAL**

eAppendix1 Gene Expression Score Derivation Calculations and Reproducibility

The gene expression score was derived from a series of studies of peripheral blood cell gene expression representing gene discovery by microarrays, algorithm development by RT-PCR, and clinical validation, each representing independent patient cohorts [1-3]. The algorithm comprises the gene expression levels of 23 genes, measured by quantitative RT-PCR, in 6 correlated terms with distinct weighting for men and women (Figure 1), as well as sex-specific age dependent obstructive CAD likelihood functions.

To determine the gene expression score for a patient, each gene expression level was measured in triplicate and the median Cp values used for subsequent calculations, as described below (adapted from 3):

**Algorithm Calculation**.

1) Define $Norm_1$ = RPL28

2) Define $Norm_2$ = (.5*HNRPF + .5*TFCP2)

3) Define $NK_{up}$ = (.5*SLAMF7 + .5*KLRC4)

4) Define $T_{cell}$ = (.5*CD3D + .5*TMC8)

5) Define $B_{cell}$ = (2/3 *CD79B + 1/3 * SPIB)

6) Define Neut = (.5*AQP9 + .5*NCF4)

7) Define $N_{up}$ = (1/3 * CASP5 + 1/3*IL18RAP + 1/3*TNFAIP6)

8) Define $N_{down}$ = (.25*IL8RB + .25*TNFRSF10C + .25*TLR4 + .25*KCNE3)

9) Define $SCA_1$ = (1/3*S100A12 + 1/3*CLEC4E + 1/3*S100A8)

10) Define $AF_2$ = AF289562

11) Define TSPAN = 1 if (AF161365-Norm2 > 6.27 or AF161365=NoCall), 0 otherwise

12) Define SEX= 1 for Males, 0 for Females

13) Define Intercept

a) For Males, INTERCEPT = 2.672 + 0.0449*Age

b) For Females, INTERCEPT = 1.821 + 0.123*(Age-60), if negative set to 0

Define Score = INTERCEPT – 0.755 *( $N_{up}$ - $N_{down}$) – 0.308 *SEX*( $SCA_1$- $Norm_1$) - 0.548 *(1-SEX)*( $SCA_1$- Neut) – 0.406*( $NK_{up}$ - $T_{cell}$) - 0.137* ( $B_{cell}$- $T_{cell}$) - 0.482 *SEX*(TSPAN)- 0.246 ( AF2- $Norm_2$),

14) **Score Transformation**

The endpoint analyses defined were performed using raw algorithm scores. For clinical reporting purposes, as well as ease of presentation, raw scores were transformed into a transformed score with a scale from 1-40 designed for ease of clinical use as follows:

Input is Raw Score

If Raw Score< -2.95, set RawScore = -2.95

If Raw Score> 1.57, set RawScore = 1.57

Raw Score = 2.95 + RawScore

Final Score = RawScore*40/4.52

Round Final Score up to nearest integer

If Final Score is greater than 40, set to 40

If Final Score is less than 1, set to 1

Value obtained is the Final Transformed Score

Reproducibility of GES Measurements

Total process variability was estimated using 895 whole blood control samples from the study period of 2 years. The SD derived from this set of samples was 0.11 Cp units, or slightly less than 1 point on the reported GES scale (0.97 points on the 1–40 reported GES scale, 1.7% change in probability of obstructive disease, see below)[4].

| Type of Variability | Cp SD[1] | GES SD[1] | % Probability Change[2] |
|---|---|---|---|
| **Total Variability** | 0.11 | 0.97 | 1.7% |
| **Intra-Batch Variability** | 0.092 | 0.81 | 1.42% |
| **Clinical Variability** | 1.19 | 10.5 | 18.4% |

[1]SD given in Cp units and gene expression score points (GES).
[2]Percent change in probability of subject having obstructive CAD.
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eAppendix2. COMPASS Clinical Investigators

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eAppendix3. Clinical Follow-up, Myocardial Perfusion Imaging and Angiography Core Laboratory Protocols and Procedures

Prespecified clinical data, including updates to medical histories, medications, office visits and additional cardiac testing were obtained for 6 months after index MPI by research study coordinators who used standardized data collection methods. Data were verified by independent study monitors. Clinical endpoints were pre-defined as all revascularizations and MACE (non-fatal myocardial infarction, stroke/transient ischemic attacks, or all-cause mortality) both within 30 days of index MPI and subsequently. Logistic regression was used to test for the association between the GES as a continuous predictor and MACE/revascularization as a binary endpoint.

Invasive angiography core laboratory results were obtained by QCA in an independent core laboratory (Cardiovascular Research Foundation, New York) as previously described [5]. All lesions causing more than 10% diameter stenosis in vessels >1.5 mm in diameter were evaluated with a computer-assisted algorithm (Medis, Leiden, The Netherlands), which generated the lumen reference diameters and maximum percent stenosis.

Coronary CTA blinded core laboratory reads were performed by Integrated Cardiovascular Research Group (Atlanta, GA) by two independent readers using the same coronary segmentation and visual assessment as the site reads utilized in patients with mild or greater stenosis. A random sampling of 30 normal cases by site-read and core laboratory showed 100% concordance, thus no additional normal cases were evaluated. Two methods of coronary artery segment stenosis were used; an expert visual

interpretation as above and a quantitative analysis. Quantitative analysis was performed based on a 12-segment model in segments greater than 2 mm on a Vitrea FX workstation (Version 2.0), using SurePlaque (Vital Images; Minnetonka, Minnesota), as previously validated for reproducibility [6] and accuracy against QCA and intravascular ultrasound [7]. The quantitative read was used to resolve disagreements between the two independent readers.

MPI core laboratory evaluation was performed by Cardiovascular Imaging Technologies, (Kansas City, MO) to provide a uniform, expert interpretation of the MPI scans. The core laboratory uniformly processed and displayed de-identified images which were interpreted by an independent expert reader with no access to the subject's clinical history or profile. Overall diagnosis (MPI negative (normal or a fixed defect interpreted as artifact) or MPI positive (reversible or fixed perfusion defect in any coronary segments) and segmental perfusion interpretation by the 17-segment model were performed. The core lab rated image quality as excellent, good, fair and poor. Overall interpreter certainty of the core lab interpretation was graded as high, fair and low. A total of 420 subjects had data which was submitted for core laboratory interpretation. Due to a variety of technical issues, data from 28 subjects were not analyzable yielding 392 subjects; of these, 19 were judged not interpretable by the core lab reader resulting in a final set of 371. The MPI core lab CRF is appended below:

CARDIOVASCULAR IMAGING TECHNOLOGIES

**Protocol: COMPASS**
**Blinded Read Case Report Form**

**Study ID:** _____ **Reader: BATEMAN**

## QUALITY ASSESSMENT:

| | Category | Stress Perfusion | Stress Gating | Rest Perfusion | Rest Gating |
|---|---|---|---|---|---|
| Overall Image Quality | Excellent | | | | |
| | Good | | | | |
| | Fair | | | | |
| | Poor | | | | |
| | N/A | | | | |

| | No | Major | Minor | | No | Major | Minor |
|---|---|---|---|---|---|---|---|
| Patient Motion | | | | Diaphragmatic Attenuation | | | |
| Breast Attenuation | | | | Liver/Bowel Overlap/Scatter | | | |
| Other Attenuation: *(free text)* | | | | | | | |

| | LAD | LCx | RCA | | |
|---|---|---|---|---|---|
| Territories with Artifact | | | | Overall Reader Certainty | |

(0=none, 1=minor, 2=major) (3= high, 2=fair, 1=low)

## DIAGNOSTIC INTERPRETATION:

| | | |
|---|---|---|
| Overall | Normal | |
| | Normal With Artifact | |
| | Equivocal | |
| | Abnormal With Artifact | |
| | Abnormal | |
| | Non-Diagnostic | |
| LAD | Normal | |
| | Normal With Artifact | |
| | Equivocal | |
| | Abnormal With Artifact | |
| | Abnormal | |
| | Non-Diagnostic | |
| LCx | Normal | |
| | Normal With Artifact | |
| | Equivocal | |
| | Abnormal With Artifact | |
| | Abnormal | |
| | Non-Diagnostic | |
| RCA | Normal | |
| | Normal With Artifact | |
| | Equivocal | |
| | Abnormal With Artifact | |
| | Abnormal | |
| | Non-Diagnostic | |


## SEGMENTAL PERFUSION INTERPRETATION:

| SEGMENTAL SCORING | Perfusion STRESS | Perfusion REST |
|---|---|---|
| 1. Anterior | | |
| 2. Antero-septal | | |
| 3. Infero-septal | | |
| 4. Inferior | | |
| 5. Infero-lateral | | |
| 6. Antero-lateral | | |
| 7. Anterior | | |
| 8. Antero-septal | | |
| 9. Infero-septal | | |
| 10. Inferior | | |
| 11. Infero-lateral | | |
| 12. Antero-lateral | | |
| 13. Anterior | | |
| 14. Septal | | |
| 15. Inferior | | |
| 16. Lateral | | |
| 17. Apical | | |

Attenuation Correction: Yes No

| Perfusion Score Key | |
|---|---|
| 0 | Normal |
| 1 | Mild decrease in uptake |
| 2 | Moderate decrease in uptake |
| 3 | Severe decrease in uptake |
| 4 | Absent uptake |
| 5 | Not Categorizable |
| X | N/A |

## AUTHENTICATION OF INTERPRETATION:

| | |
|---|---|
| ___/___/_____ | mmm/dd/yyyy |
| _____ | CRC Initials |
| ___/___/_____ | mmm/dd/yyyy |
| _____ | Reader Initials |

## WALL MOTION ASSESSMENT:

| | | Function Score Key | |
|---|---|---|---|
| 1. Anterior | | Normal | 0 |
| 2. Septal | | Abnormal | 1 |
| 3. Inferior | | Not Categorizable | 5 |
| 4. Lateral | | N/A | X |
| 5. Apex | | | |

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eAppendix4. Statistical Analysis Plan

1. Purpose

The purpose of this document is to detail the statistical analysis plan for the COMPASS study.

2. Scope

The scope of this document is limited to the analysis of the primary, secondary and exploratory endpoints as defined in the COMPASS study protocol.

**3.** Study Design and Key Measures

Data will be collected from approximately 376 patients, with a minimum of 62 cases, from up to 30 lab centers.

Subjects are included in the study if they have chest pain, anginal equivalent or dyspnea and are indicated for MPI testing because of suspected CAD. Both GES and MPI tests will be performed on all patients. The reference test used to evaluate the diagnostic performance of these two tests will be invasive coronary angiography for those patients with a positive MPI and CCTA (cardiac CT angiography) for those with a negative MPI result.

a. Sample-size Calculations

It is planned that for this study 500 subjects will be enrolled. It has been calculated that 376 subjects should have a 90% or greater power to detect superiority in the AUC of GES compared with the AUC of a random prediction of >=50% stenosis for the subjects using a two-sided test with

alpha level of 0.05. If 500 patients are enrolled, this would allow a loss of 25% due to clinical exclusions and miscellaneous data collection issues. The prevalence of patients with 50% or more stenosis is estimated to be 16%.

4. Quality Control

Samples determined to have a quality issue for either clinical or diagnostic test results will be removed from all analyses or re-run, if possible.

5. Clinical Endpoints

a. Invasive angiograms will be considered positive for obstructive disease if there is a lesion of greater than or equal to 50% stenosis in a vessel of greater than or equal to 2 mm diameter.

b. CCTA clinical reads will be considered positive for obstructive disease if there is a lesion of greater than moderate stenosis. CCTA clinical reads are qualitative assessments.

c. CCTA core lab reads will be considered positive for obstructive disease if the consensus read indicates a greater than 50% stenosis. CCTA core lab reads include two qualitative assessments and one quantitative assessment. The CCTA qualitative core lab read includes the following categories:

i. Normal: 0

ii. Minimal: <25%

iii. Mild: 25-49%

iv. Moderate: 50-69%

v. Severe: >= 70%

vi. Occluded: 100%

d. We define the CCTA core lab consensus read as the median of the three reads. For the purposes of a consensus percent stenosis, qualitative reads will be assumed to have a stenosis value at the midpoint of the category range, and the median across the three reads will be used.

e. All CCTA films with a moderate or greater stenosis based on the clinical read will be read by the CCTA core lab. A random sample of 60 films with mild/none stenosis based on the clinical read will be read by the CCTA core lab. Based on the very high reported negative predictive value of CCTA, it is anticipated that nearly all of these films will be negative based on core lab read. If this is the case, the remaining CCTA clinical films with none/mild stenosis will be considered negative, otherwise, all CCTA films will be read by the core lab. This determination will be performed prior to unblinding the GES/MPI results.

f. Prior to unblinding the GES/MPI data, a review of the CCTA core lab results will be performed: i) to determine the reproducibility of the quantitative and quantitative reads, and ii) on the subset of patient who also had invasive angiography, to determine the correspondence between core lab CCTA reads and invasive angiography reads. For the purposes of this analysis, reproducibility will be accessed based on pair-wise kappa statistics between each of the three reads per patient. Based on this

analysis, the definition of a CCTA positive read may be changed to better correspond to the invasive angiography >50% lesion threshold.

6. Primary Analysis: GES Performance

   a. Inclusion

      For the primary analysis, the values from all patients that have viable GES and a positive or negative result on angiography or CCTA will be included in the calculations. Invasive angiography results will be used preferentially over CCTA if both are available for the same patient. CCTA core lab reads will be used preferentially over CCTA clinical reads if both are available for the same patient.

   b. Methods

      i. Estimate the AUC of GES and a bootstrapped 95% CI of the AUC.

      ii. Test the hypothesis that the AUC of GES for detecting 50% stenosis is > 0.50 using a z-test. Significance will be determined based on an alpha level of .05 (two-sided).

      iii. Estimate the sensitivity, specificity, NPV, and PPV of GES. Two score thresholds (15 and 28) will be used for this assessment.

      iv. Compute the NRI (net reclassification improvement) and associated p-value of GES compared to physician pre-test probability (Pencina et al, Stat Med 2008).

      v. Compute the NRI (net reclassification improvement) and associated p-value of GES compared to Diamond-Forrester pre-test probability.

7. Secondary Analysis: GES Comparison to MPI

    a. Inclusion

        For the secondary analysis, patients will be included if they have all of the following: available MPI results, viable GES scores, and invasive angiography or CCTA results, otherwise they will be excluded. Planned analyses of MPI refer to local lab MPI results, except where noted specifically. Indeterminate MPI results will be considered positive unless the MPI is not evaluable for technical reasons.

    b. Methods

        i. Estimate the AUC of MPI and a bootstrapped 95% CI of the AUC.

        ii. Test the hypothesis that the AUC of GES for detecting 50% stenosis is greater than the AUC for MPI using the bootstrap. Significance will be determined based on an alpha level of .05 (two-sided).

        iii. Test the hypothesis that the AUC of GES for detecting 50% stenosis is non-inferior to the AUC for MPI using the bootstrap. Significance will be determined based on an alpha level of .05 (two-sided) and a non-inferiority margin of 5%.

        iv. Estimate the sensitivity, specificity, NPV, and PPV of MPI, and compare these values to GES using McNemar's test.

        v. Compute the NRI (net reclassification improvement) and associated p-value of GES compared to MPI.

8. Secondary Analysis: GES Combination with MPI

a. Inclusion

For the secondary analysis, patients will be included if they have all of the following: available MPI results, viable GES scores, and invasive angiography or CCTA results, otherwise they will be excluded. Planned analyses of MPI refer to local lab MPI results, except where noted specifically.

b. Methods

i. Develop a combined model incorporating GES and MPI for prediction of 50% stenosis.

ii. Test individual significance of GES and MPI in the context of the combined model.

iii. Estimate the AUC of GES+MPI and a bootstrapped 95% CI of the AUC.

iv. Test the hypothesis that the AUC of GES+MPI for detecting 50% stenosis is greater than the AUC for MPI using the bootstrap. Significance will be determined based on an alpha level of .05 (two-sided).

v. Test the hypothesis that the AUC of GES+MPI for detecting 50% stenosis is greater than the AUC for GES using the bootstrap. Significance will be determined based on an alpha level of .05 (two-sided).

vi. Determine optimal GES thresholds in the context of a combined GES+MPI model.

vii. Estimate the sensitivity, specificity, NPV, and PPV of GES + MPI, and compare these values to MPI alone and GES alone using McNemar's test.

viii. Compute the NRI (net reclassification improvement) and associated p-value of GES +MPI compared to MPI alone and GES alone.

Supplementary Table 1. Analysis of Clinical Factors and Medications Effect on the GES

Linear regression was used to assess the effects of clinical factors and medications with the dependent variable the gene expression portion of the GES in the COMPASS population of 431 patients.

| Clin Variable | Estimate | Std.Error | t-value | p-value |
|---|---|---|---|---|
| (Intercept) | 4.558448 | 0.355477 | 12.823 | -<2e-16 *** |
| White ethnicity | 0.015573 | 0.126825 | -0.123 | 0.902 |
| Syst BP | 0.003151 | 0.002410 | -1.308 | 0.192 |
| Dyslipidemia | 0.155434 | 0.095857 | -1.622 | 0.106 |
| BMI | 0.006015 | 0.006755 | 0.890 | 0.374 |
| SMOKE former | 0.072292 | 0.124221 | -0.582 | 0.561 |
| SMOKE never | 0.082465 | 0.114082 | 0.723 | 0.470 |
| Aspirin | 0.007805 | 0.079725 | 0.098 | 0.922 |
| Statins | 0.042664 | 0.095636 | 0.446 | 0.656 |
| Beta-blockers | 0.043843 | 0.100232 | 0.437 | 0.662 |
| ACE Inhibitors | 0.033955 | 0.088243 | -0.385 | 0.701 |

Supplemental Table 2. Complete Score Range Performance of Gene Expression Score for 50% Stenosis Case Definition

| Threshold | Sensitivity | Specificity | Negative Predictive Value | Positive Predictive Value | Number Below Threshold | Percent Below Threshold |
|---|---|---|---|---|---|---|
| 1 | 1 | 0.06 | 1 | 0.15 | 21 | 4.9 |
| 2 | 0.98 | 0.08 | 0.97 | 0.16 | 31 | 7.2 |
| 3 | 0.98 | 0.1 | 0.97 | 0.16 | 38 | 8.8 |
| 4 | 0.98 | 0.13 | 0.98 | 0.16 | 49 | 11.4 |
| 5 | 0.97 | 0.17 | 0.97 | 0.17 | 63 | 14.6 |
| 6 | 0.95 | 0.22 | 0.96 | 0.17 | 83 | 19.3 |
| 7 | 0.95 | 0.28 | 0.97 | 0.19 | 107 | 24.8 |
| 8 | 0.95 | 0.32 | 0.98 | 0.19 | 120 | 27.8 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 9 | 0.95 | 0.35 | 0.98 | 0.2 | 131 | 30.4 |
| 10 | 0.94 | 0.37 | 0.97 | 0.2 | 141 | 32.7 |
| 11 | 0.94 | 0.4 | 0.97 | 0.21 | 150 | 34.8 |
| 12 | 0.92 | 0.44 | 0.97 | 0.22 | 166 | 38.5 |
| 13 | 0.92 | 0.47 | 0.97 | 0.23 | 179 | 41.5 |
| 14 | 0.89 | 0.49 | 0.96 | 0.23 | 187 | 43.4 |
| 15 | 0.89 | 0.52 | 0.96 | 0.24 | 199 | 46.2 |
| 16 | 0.89 | 0.55 | 0.97 | 0.25 | 209 | 48.5 |
| 17 | 0.87 | 0.58 | 0.96 | 0.26 | 221 | 51.3 |
| 18 | 0.86 | 0.63 | 0.96 | 0.29 | 242 | 56.2 |
| 19 | 0.84 | 0.67 | 0.96 | 0.3 | 255 | 59.2 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 20 | 0.79 | 0.69 | 0.95 | 0.3 | 266 | 61.7 |
| 21 | 0.71 | 0.71 | 0.94 | 0.3 | 281 | 65.2 |
| 22 | 0.68 | 0.75 | 0.93 | 0.32 | 296 | 68.7 |
| 23 | 0.59 | 0.77 | 0.92 | 0.31 | 311 | 72.2 |
| 24 | 0.56 | 0.8 | 0.91 | 0.32 | 323 | 74.9 |
| 25 | 0.54 | 0.84 | 0.91 | 0.37 | 338 | 78.4 |
| 26 | 0.49 | 0.86 | 0.91 | 0.38 | 350 | 81.2 |
| 27 | 0.43 | 0.89 | 0.9 | 0.4 | 364 | 84.5 |
| 28 | 0.35 | 0.9 | 0.89 | 0.38 | 373 | 86.55 |
| 29 | 0.25 | 0.93 | 0.88 | 0.37 | 388 | 90.0 |
| 30 | 0.17 | 0.96 | 0.87 | 0.44 | 406 | 94.2 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 31 | 0.16 | 0.98 | 0.87 | 0.53 | 412 | 95.6 |
| 32 | 0.14 | 0.98 | 0.87 | 0.6 | 416 | 96.5 |
| 33 | 0.14 | 0.99 | 0.87 | 0.64 | 417 | 96.8 |
| 34 | 0.1 | 0.99 | 0.87 | 0.75 | 423 | 98.1 |
| 35 | 0.08 | 0.99 | 0.86 | 0.71 | 424 | 98.4 |
| 36 | 0.06 | 1 | 0.86 | 0.8 | 426 | 98.8 |
| 37 | 0.05 | 1 | 0.86 | 0.75 | 427 | 99.0 |
| 38 | 0.03 | 1 | 0.86 | 0.67 | 428 | 99.3 |
| 39 | 0 | 1 | 0.85 | NA | 431 | 100 |
| 40 | 0 | 1 | 0.85 | NA | 431 | 100 |

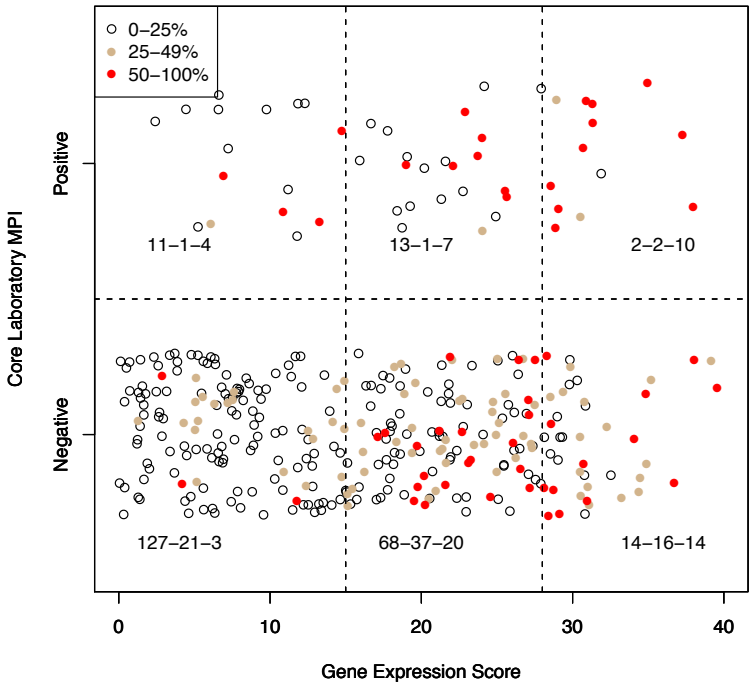
Supplemental Table 3. Patient Categorization by Maximum Percent Stenosis, Gene Expression Score, and Site-Read MPI

| Max Stenosis Category | 0-49% | 50-69% | 70-100% | All Cases | Total |
|---|---|---|---|---|---|
| Patient Set | | | | | |
| All | 368 | 35 | 28 | 63 | 431 |
| GES ≤ 15 | 192 | 4 | 3 | 7 | 199 |
| GES > 15 | 176 | 31 | 25 | 56 | 232 |
| MPI negative | | | | | |
| All | 337 | 28 | 18 | 46 | 383 |
| GES ≤ 15 | 179 | 4 | 2 | 6 | 185 |
| GES > 15 | 158 | 24 | 16 | 40 | 198 |
| MPI positive | | | | | |
| All | 31 | 7 | 10 | 17 | 48 |
| GES ≤ 15 | 13 | 0 | 1 | 1 | 14 |
| GES > 15 | 18 | 7 | 9 | 16 | 34 |

Supplemental Table 4. Patient Categorization by Maximum Percent Stenosis, Gene Expression Score, and Core Laboratory MPI

| Max Stenosis Category | 0-49% | 50-69% | 70-100% | All Cases | Total |
|---|---|---|---|---|---|
| Patient Set | | | | | |
| All Patients | 313 | 31 | 27 | 58 | 371 |
| GES ≤ 15 | 160 | 4 | 3 | 7 | 167 |
| GES > 15 | 153 | 27 | 24 | 51 | 204 |
| MPI negative | | | | | |
| All | 283 | 22 | 15 | 37 | 320 |
| GES ≤ 15 | 148 | 1 | 2 | 3 | 151 |
| GES > 15 | 135 | 21 | 13 | 34 | 169 |
| MPI positive | | | | | |
| All | 30 | 9 | 12 | 21 | 51 |
| GES ≤ 15 | 12 | 3 | 1 | 4 | 16 |
| GES > 15 | 18 | 6 | 11 | 17 | 35 |

Supplemental Figure 1.




Representation of GES and MPI Categorization as a Function of Maximum Percent Stenosis Category. Dot plot of corelab-read MPI and GES results depicting percent stenosis based on ≥25% and ≥50% stenosis by QCA and CTA. The 371 patients for whom core lab MPI results were obtainable are illustrated with the GES as a continuous variable on the abscissa and the MPI result as a categorical variable on the ordinate with random ordinate offsets for illustration purposes. Open circles are ≤25% stenosis, gray circles are 25-49%, and red circles are ≥50% stenosis. The number of patients in each stenosis category for each of the MPI and GES categories are shown on the Figure.