

ESTTA Tracking number: **ESTTA600068**

Filing date: **04/23/2014**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE TRADEMARK TRIAL AND APPEAL BOARD

Notice of Opposition

Notice is hereby given that the following party opposes registration of the indicated application.

Opposer Information

Name	CardioDx, Inc.
Granted to Date of previous extension	04/23/2014
Address	2500 Faber Place Palo Alto, CA 94303 UNITED STATES
Attorney information	Lisa Greenwald-Swire Fish & Richardson, P.C. PO Box 1022 Minneapolis, MN 55440-1022 UNITED STATES tmdoctc@fr.com, TrademarkGroup-SV@fr.com Phone:650-839-5070

Applicant Information

Application No	85794852	Publication date	12/24/2013
Opposition Filing Date	04/23/2014	Opposition Period Ends	04/23/2014
Applicant	Cardiox Corporation Suite 100 Columbus, OH 43220 UNITED STATES		

Goods/Services Affected by Opposition

Class 010. First Use: 0 First Use In Commerce: 0 All goods and services in the class are opposed, namely: medical apparatus, appliances and instruments, namely, cardiac flow detecton systems, hemodynamic monitoring systems, clinical fluorometry systems, liver function assessment systems, and organ perfusion assessment systems
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Grounds for Opposition

Priority and likelihood of confusion	Trademark Act section 2(d)
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Marks Cited by Opposer as Basis for Opposition

U.S. Registration No.	3604577	Application Date	03/28/2007
Registration Date	04/07/2009	Foreign Priority Date	NONE
Word Mark	CARDIODX		

Design Mark	CARDIODX
Description of Mark	NONE
Goods/Services	Class 010. First use: First Use: 2009/02/25 First Use In Commerce: 2009/02/25 Medical apparatus and instruments, namely, test kits comprised of apparatus for biological sample collection and storage for medical laboratory use for cardiac and vascular prognostic and diagnostic testing Class 044. First use: First Use: 2007/07/13 First Use In Commerce: 2007/07/13 Medical services, namely, cardiac and vascular, prognostic, and diagnostic testing services

U.S. Application No.	85889778	Application Date	03/28/2013
Registration Date	NONE	Foreign Priority Date	NONE
Word Mark	CARDIODX		
Design Mark	CARDIODX		
Description of Mark	NONE		
Goods/Services	Class 044. First use: First Use: 2007/07/13 First Use In Commerce: 2007/07/13 Medical services, namely, cardiac and vascular, prognostic, and diagnostic testing services		

Attachments	77143077#TMSN.jpeg(bytes) 85889778#TMSN.jpeg(bytes) 24231-0067PP1 Notice of Opposition - to File.pdf(3296197 bytes) Itemized Index of Exhibits to Opp.pdf(60256 bytes) Exh A CARDIODX reg.pdf(44783 bytes) Exh B CARDIODX app.pdf(128918 bytes) Exh C - Medicare CAP.pdf(240856 bytes) Exh D - 1.pdf(3907007 bytes)
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	Exh D - 2.pdf(3133478 bytes) Exh D - 3.pdf(4303015 bytes) Exh E - CardioDx awards.pdf(618579 bytes) Exh F - News Articles.pdf(3727106 bytes)
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Certificate of Service

The undersigned hereby certifies that a copy of this paper has been served upon all parties, at their address record by First Class Mail on this date.

Signature	/lisa greenwald-swire/
Name	Lisa Greenwald-Swire
Date	04/23/2014

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE TRADEMARK TRIAL AND APPEAL BOARD**

In the matter of Application Serial No. 85/794,852
Filed on December 5, 2012
For the mark CARDIOX
Published in the *Official Gazette* on December 24, 2013

CARDIODX, INC.,

Opposer,

v.

CARDIOX CORPORATION,

Applicant.

NOTICE OF OPPOSITION

NOTICE OF OPPOSITION

Opposer CardioDx, Inc. believes that it will be damaged by registration of the mark shown in the above-identified application and hereby opposes the same pursuant to the provisions of 15 U.S.C. § 1063.

The grounds for opposition are as follows:

THE PARTIES

1. Opposer CardioDx, Inc. (“CardioDx” or “Opposer”) is a Delaware corporation with a place of business at 2500 Faber Place, Palo Alto, CA 94303. CardioDx is a cardiovascular genomic diagnostics company focused on coronary artery disease, cardiac arrhythmia, and heart failure.

2. Upon information and belief, Applicant Cardiox Corporation (“Applicant”) is a Delaware corporation with a place of business at 4100 Horizons Drive, Suite 100, Columbus,

OH 43220 that allegedly intends to sell medical apparatuses, appliances, and instruments specific to the cardiac field.

FACTUAL BACKGROUND

Opposer's CARDIODX Mark

3. CardioDx was founded in 2004 and has been a pioneer in the field of cardiovascular genomic diagnostics. CardioDx has been offering products and services rendered under and in connection with the CARDIODX mark continuously since at least as early as 2007.

4. In addition to its common law rights in the CARDIODX trade name and trademark, CardioDx owns Registration No. 3,604,577 for the mark CARDIODX for “medical apparatus and instruments, namely, test kits comprised of apparatus for biological sample collection and storage for medical laboratory use for cardiac and vascular prognostic and diagnostic testing” in Class 10 and “medical services, namely, cardiac and vascular, prognostic, and diagnostic testing services” in Class 44, which issued on April 7, 2009 on the Supplemental Register, with a March 28, 2007 priority date. A copy of Registration No. 3,604,577 is attached at **Exhibit A**.

5. CardioDx also owns Application Serial No. 85/889,778 for the mark CARDIODX for “medical services, namely, cardiac and vascular, prognostic, and diagnostic testing services” in Class 44, which was filed on March 28, 2013. Upon demonstrating that CardioDx had acquired distinctiveness through its exclusive and continuous use of the CARDIODX mark for more than six years, on April 8, 2014 the United States Patent and Trademark Office (“PTO”) approved the CARDIODX mark for publication in the *Official Gazette* on the Principal Register. A copy of the application particulars for Serial No. 85/889,778 is attached as **Exhibit B**.

6. The CARDIODX mark has become distinctive of the products and services through CardioDx's substantially exclusive and continuous use of the mark in interstate commerce since at least as early as July 13, 2007. As a result of CardioDx's extensive and continuous promotion and sales of products and services since 2007, as well as the high level of recognition and success it has obtained, the CARDIODX mark has taken on a secondary meaning in the minds of consumers as the source of quality products and services.

7. In August 2012, CardioDx's Corus CAD product, which is branded under or in connection with its CARDIODX mark, obtained Medicare Part B coverage from the regional Medicare Administrative Contractor, or MAC, in California, making the test a covered benefit for the estimated 48 million covered lives in the United States. CardioDx performs the Corus CAD test under or in connection with its CARDIODX mark in its clinical laboratory, which has been certified by the Clinical Laboratory Improvement Amendments of 1988, or CLIA, under the regulations of the Centers for Medicare & Medicaid Services, or CMS, and also has been accredited by the College of American Pathologists, or CAP. *See Exhibit C.*

8. Advertisements and articles for CardioDx and the products and services rendered under and in connection with the CARDIODX mark have been featured in the following publications in leading industry peer reviewed journals: *American Heart Journal, American Heart Association Circulation: Cardiovascular Genetics, Critical Pathways in Cardiology, Journal of Cardiovascular Translational Research, Journal of the American College of Cardiology, Annals of Internal Medicine, BMC Medical Genomics, PLoS ONE, PLoS Currents.* *See Exhibit D.*

9. In 2010, CardioDx was honored as a winner of the *Wall Street Journal's* prestigious Technology Innovation Awards and its Corus CAD technology was named one of

Time Magazine's Top Ten Medical Breakthroughs. In 2012, CardioDx was honored as one of *FierceMedicalDevices'* Fierce 15 most promising private companies, and was also named the Gold Edison Award winner for science and medicine. See **Exhibit E**. In short, the CARDIODX products have been recognized as breakthrough technology and are receiving such attention in the cardiovascular field.

10. CardioDx and the products and services rendered under and in connection with the CARDIODX mark have been featured in unsolicited news stories, including: a *Men's Fitness* article entitled, "6 Health Tests that Could Save Your Life," and *ABC Local KGO News* television report entitled, "Bay Area company develops new heart disease test." See **Exhibit F**.

Applicant's CARDIOX Mark

11. On December 5, 2012, Applicant filed an intent-to-use application to register the mark CARDIOX in connection with "medical apparatus, appliances and instruments, namely, cardiac flow detection systems, hemodynamic monitoring systems, clinical fluorometry systems, liver function assessment systems, and organ perfusion assessment systems" in class 10 ("Applicant's Goods"), which was designated Application Serial No. 85/794,852 (the "Application").

12. Applicant filed the Application based on an alleged intent to use and has not, to CardioDx's knowledge, filed an allegation of use in connection with the Application.

13. The Application was published for opposition in the *Official Gazette* on December 24, 2013. Thereafter, CardioDx sought and obtained an initial 30 day extension of time to oppose the Application until February 22, 2014, and then a further 60 day extension of time to oppose the Application until April 23, 2014. Consequently, this Notice of Opposition is timely filed.

GROUNDNS FOR OPPOSITION

Priority and Likelihood of Confusion

14. CardioDx has been using the CARDIODX mark in commerce since at least as early as July 13, 2007, whereas Applicant's priority is based on its December 5, 2012 intent-to-use application filing date. Upon information and belief, Applicant has not yet used the proposed CARDIOX mark in commerce. Opposer's common law and statutory priority dates precede the priority date of the Application and, upon information and belief, any priority date on which Applicant may rely.

15. Applicant allegedly intends to use the mark CARDIOX, which is near-identical to CardioDx's mark in that the primary element of both marks is the word portion "CARDIO," and Applicant's mark uses the suffix "X" while Protestor's mark uses the suffix "DX." Applicant and CardioDx both use an "X" in the suffix of their word marks, and the letter "D" is the only missing suffix-element in Applicant's mark. Finally, the marks sound nearly identical, which increases the likelihood of consumer confusion.

16. Applicant allegedly intends to use the CARDIOX mark on goods that are near identical to those offered by CardioDx, that is, medical apparatus and instruments.

17. On information and belief, both CardioDx and Applicant target the exact same industries and potential customers, that is, medical professionals in the specific field of cardiac health.

18. CardioDx has continuously used its CARDIODX mark in commerce since its date of first use in commerce on July 13, 2007. This is approximately seven and half years prior to Applicant's filing of its Section 1(b) application with the PTO.

19. Consumers are likely to believe that CARDIOX medical apparatuses and instruments for cardiac health originate from CardioDx or are somehow affiliated with CardioDx and its CARDIODX mark. As such, Applicant's CARDIOX mark so resembles CardioDx's previously used and registered CARDIODX trade name and mark, as to be likely, when used in connection with Applicant's Goods, to cause confusion, to cause mistake, or to deceive. Purchasers and prospective purchasers are likely to mistakenly believe that the products Applicant allegedly intends to sell under the CARDIOX designation are produced, sponsored, endorsed, or approved by CardioDx, or are in some way affiliated, connected, or associated with CardioDx, all to the detriment of CardioDx. Registration of Applicant's mark, therefore, should be refused under 15 U.S.C. §§ 1052(d) and 1063.

20. Registration of Applicant's proposed mark CARDIOX would be a further source of damage to CardioDx because it would confer upon Applicant various statutory presumptions to which it is not entitled in view of CardioDx's prior adoption, use, and registration of its marks.

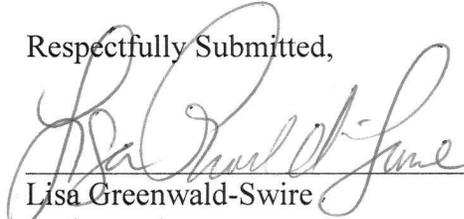
WHEREFORE, pursuant to Section 13 of the Lanham Act, 15 U.S.C. § 1063, Opposer CardioDx respectfully requests that its Notice of Opposition be sustained and that the registration of the mark shown in Application Serial No. 85/794,852 be refused.

Please direct all correspondence with respect to this Opposition to Lisa Greenwald-Swire at the address below. Please charge the Notice of Opposition fee to the Deposit Account of Fish & Richardson P.C., Account No. 06-1050.

Date

April 23, 2014

Respectfully Submitted,



Lisa Greenwald-Swire

Kathy Tsai

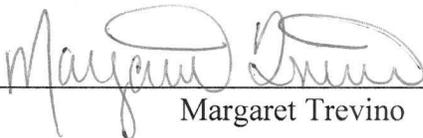
FISH & RICHARDSON P.C.
500 Arguello Street, Suite 500
Redwood City, CA 94063
Telephone: (650) 839-5070
Facsimile: (650) 839-5071

Attorneys for CardioDx, Inc.

CERTIFICATE OF SERVICE

The undersigned hereby certifies that a true and complete copy of the foregoing NOTICE OF OPPOSITION has been served this 23rd day of April, 2014, by First Class U.S. Mail, postage prepaid, upon Applicant Cardiox Corporation's attorney of record at the following address:

ROBERT J. MORGAN
PORTER, WRIGHT, MORRIS & ARTHUR, LLP
41 S HIGH ST STE 2900
COLUMBUS, OHIO 43215-6165
UNITED STATES



Margaret Trevino

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE TRADEMARK TRIAL AND APPEAL BOARD**

In the matter of Application Serial No. 85/794,852
Filed on December 5, 2012
For the mark CARDIOX
Published in the *Official Gazette* on December 24, 2013

CARDIODX, INC.,

Opposer,

v.

CARDIOX CORPORATION,

Applicant.

**ITEMIZED LIST OF EXHIBITS TO
NOTICE OF OPPOSITION**

Exhibit Letter	Short Description
Exhibit A	CARDIODX Registration Certificate
Exhibit B	CARDIODX Application Particulars
Exhibit C	Press Release re CardioDx's Medicare Coverage
Exhibit D	Advertisements and Journal Articles re CardioDx
Exhibit E	CardioDx Awards
Exhibit F	CardioDx News Articles

EXHIBIT A

Int. Cls.: 10 and 44

Prior U.S. Cls.: 26, 39, 44, 100, and 101

United States Patent and Trademark Office

Reg. No. 3,604,577

Registered Apr. 7, 2009

**TRADEMARK
SERVICE MARK
SUPPLEMENTAL REGISTER**

CARDIODX

CARDIODX, INC. (DELAWARE CORPORATION)
2500 FABER PLACE
PALO ALTO, CA 94303

FOR: MEDICAL APPARATUS AND INSTRUMENTS, NAMELY, TEST KITS COMPRISED OF APPARATUS FOR BIOLOGICAL SAMPLE COLLECTION AND STORAGE FOR MEDICAL LABORATORY USE FOR CARDIAC AND VASCULAR PROGNOSTIC AND DIAGNOSTIC TESTING, IN CLASS 10 (U.S. CLS. 26, 39 AND 44).

FIRST USE 2-25-2009; IN COMMERCE 2-25-2009.

FOR: MEDICAL SERVICES, NAMELY, CARDIAC AND VASCULAR, PROGNOSTIC, AND DIAGNOSTIC TESTING SERVICES, IN CLASS 44 (U.S. CLS. 100 AND 101).

FIRST USE 7-13-2007; IN COMMERCE 7-13-2007.

THE MARK CONSISTS OF STANDARD CHARACTERS WITHOUT CLAIM TO ANY PARTICULAR FONT, STYLE, SIZE, OR COLOR.

SER. NO. 77-143,077, FILED P.R. 3-28-2007; AM. S.R. 3-2-2009.

ALICE BENMAMAN, EXAMINING ATTORNEY

EXHIBIT B

STATUS DOCUMENTS

[Back to Search](#)

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Mark: CARDIODX

CARDIODX

US Serial Number: 85889778

Application Filing Date: Mar. 28, 2013

Register: Principal

Mark Type: Service Mark

Status: Application has been published for opposition. The opposition period begins on the date of publication.

Status Date: Apr. 08, 2014

Publication Date: Apr. 08, 2014

Mark Information

Mark Literal Elements: CARDIODX

Standard Character Claim: Yes. The mark consists of standard characters without claim to any particular font style, size, or color.

Mark Drawing Type: 4 - STANDARD CHARACTER MARK

Acquired Distinctiveness Claim: In whole

Related Properties Information

Claimed Ownership of US [3604577](#)

Registrations:

Goods and Services

Note:

The following symbols indicate that the registrant/owner has amended the goods/services:

- Brackets [...] indicate deleted goods/services;
- Double parenthesis ((...)) identify any goods/services not claimed in a Section 15 affidavit of
- Asterisks *...* identify additional (new) wording in the goods/services.

For: Medical services, namely, cardiac and vascular, prognostic, and diagnostic testing services

International Class(es): 044 - Primary Class

U.S Class(es): 100, 101

Class Status: ACTIVE

Basis: 1(a)

First Use: Jul. 13, 2007

Use in Commerce: Jul. 13, 2007

Basis Information (Case Level)

Filed Use: Yes

Currently Use: Yes

Amended Use: No

Filed ITU: No

Currently ITU: No

Amended ITU: No

Filed 44D: No

Currently 44D: No

Amended 44D: No

Filed 44E: No

Currently 44E: No

Amended 44E: No

Filed 66A: No

Currently 66A: No

Filed No Basis: No

Currently No Basis: No

Current Owner(s) Information

Owner Name: CardioDX, Inc.

Owner Address: 2500 Faber Place
Palo Alto, CALIFORNIA 94303
UNITED STATES

Legal Entity Type: CORPORATION

State or Country Where Organized: DELAWARE

Attorney/Correspondence Information

Attorney of Record

Attorney Name: Lisa Greenwald-Swire

Docket Number: 24231-000300

Attorney Primary Email tmdoctc@fr.com

Attorney Email Authorized: Yes

Address:

Correspondent

Correspondent Name/Address: LISA GREENWALD-SWIRE
 FISH & RICHARDSON P.C.
 PO BOX 1022
 MINNEAPOLIS, MINNESOTA 55440-1022
 UNITED STATES

Phone: 650-839-5070

Fax: 877-769-7945

Correspondent e-mail: tmdoctc@fr.comCorrespondent e-mail
Authorized: Yes

Domestic Representative - Not Found

Prosecution History

Date	Description	Proceeding Number
Apr. 08, 2014	OFFICIAL GAZETTE PUBLICATION CONFIRMATION E-MAILED	
Apr. 08, 2014	PUBLISHED FOR OPPOSITION	
Mar. 19, 2014	NOTIFICATION OF NOTICE OF PUBLICATION E-MAILED	
Mar. 01, 2014	LAW OFFICE PUBLICATION REVIEW COMPLETED	68171
Mar. 01, 2014	ASSIGNED TO LIE	68171
Feb. 10, 2014	APPROVED FOR PUB - PRINCIPAL REGISTER	
Jan. 18, 2014	TEAS/EMAIL CORRESPONDENCE ENTERED	88889
Jan. 17, 2014	CORRESPONDENCE RECEIVED IN LAW OFFICE	88889
Jan. 17, 2014	TEAS RESPONSE TO OFFICE ACTION RECEIVED	
Jul. 19, 2013	NOTIFICATION OF NON-FINAL ACTION E-MAILED	6325
Jul. 19, 2013	NON-FINAL ACTION E-MAILED	6325
Jul. 19, 2013	NON-FINAL ACTION WRITTEN	77782
Jul. 09, 2013	ASSIGNED TO EXAMINER	77782
Apr. 04, 2013	NEW APPLICATION OFFICE SUPPLIED DATA ENTERED IN TRAM	
Apr. 01, 2013	NEW APPLICATION ENTERED IN TRAM	

TM Staff and Location Information

TM Staff Information

TM Attorney: YONTEF, DAVID ERIC

Law Office Assigned: LAW OFFICE 118

File Location

Current Location: PUBLICATION AND ISSUE SECTION

Date in Location: Mar. 01, 2014

[Assignment Abstract Of Title Information - Click to Load](#)

[Proceedings - Click to Load](#)

EXHIBIT C



CardioDx Announces Medicare Coverage for Corus CAD Gene Expression Test for the Diagnosis of Obstructive Coronary Artery Disease

Coverage of Blood-Based Test Has Potential to Improve Quality of Care and Lower Costs of Diagnosis of Obstructive Coronary Artery Disease Beyond Traditional Methods

PALO ALTO, Calif. – August 08, 2012 – CardioDx, Inc., a pioneer in the field of cardiovascular genomic diagnostics, today announced that Palmetto GBA, a national contractor that administers Medicare benefits, has established coverage for the company's Corus[®] CAD gene expression test for the evaluation of patients presenting with typical and atypical symptoms suggestive of coronary artery disease. With this decision, the Corus CAD gene expression test is now a covered benefit for more than 40 million Medicare enrollees in the U.S.

With a simple blood draw, Corus CAD can safely, accurately and conveniently help primary care clinicians and cardiologists assess whether or not a stable non-diabetic patient's symptoms are due to obstructive coronary artery disease (CAD), enabling many patients to avoid unnecessary invasive testing and exposure to imaging-related radiation risks or imaging agent intolerance. The test has been clinically validated in multiple independent patient cohorts, including two prospective, multicenter U.S. trials, PREDICT and COMPASS. Additionally, a retrospective, multicenter chart review study and the prospective IMPACT trial at Vanderbilt University demonstrated that Corus CAD use yielded significant and clinically relevant changes in patient management decisions in both primary care and cardiology settings.

"While the Corus CAD test was recognized by TIME Magazine as a top 10 medical breakthrough in 2010, the year the PREDICT validation study was published, fulfilling Medicare reimbursement criteria is now a major step forward," said Eric Topol, M.D., Principal Investigator of the PREDICT trial, Chief Academic Officer at Scripps Health and Professor of Genomics at The Scripps Research Institute. "Utilization of this gene expression test could lead to avoidance of a large number of unnecessary cardiac catheterization procedures and scans involving radiation." Dr. Topol has no financial relationship whatsoever with CardioDx.

Studies have shown that typical and atypical presentations of stable chest pain account for up to two percent of outpatient office visits each year in the U.S., but as many as 62 percent of stable patients who undergo elective invasive angiographic procedures are found to have no obstructive coronary artery blockage, despite broad usage of prior noninvasive imaging. The authors of a 2010 *New England Journal of Medicine* study of nearly 400,000 coronary angiography patients concluded that current modalities used to identify patients for elective invasive angiography to diagnose obstructive coronary artery disease have limitations, and that better methods are needed for patient risk stratification.

"Identifying those symptomatic patients without a coronary blockage who may be able to avoid imaging or invasive testing is a significant problem for physicians, involving up to 10,000 patients daily in the U.S.," said David Levison, President and CEO of CardioDx. "By providing Medicare beneficiaries access to Corus CAD, this coverage decision enables patients to avoid unnecessary procedures and risks associated with cardiac imaging and elective invasive angiography, while helping payers address an area of significant health care spend."

About Corus CAD

With a simple blood draw, Corus CAD can help primary care clinicians and cardiologists exclude obstructive coronary artery disease as the cause of a stable non-diabetic patient's symptoms. It is the first sex-specific test for obstructive coronary artery disease, accounting for critical biological differences between men and women. The test is safe and does not expose patients to radiation risks or imaging agent intolerance. Corus CAD is intended for use in stable patients presenting with typical and atypical symptoms suggestive of obstructive coronary artery disease. Corus CAD is not intended for use in patients who are diabetic, have been diagnosed with prior myocardial infarction (MI) or have had a previous revascularization procedure, or are currently taking steroids, immunosuppressive agents or chemotherapeutic agents.

The Corus CAD test measures the RNA levels of 23 genes. Because blood cell RNA levels are altered when obstructive coronary artery disease is present, the Corus CAD score aids clinicians in assessing whether an individual patient's symptoms may be due to obstructive coronary artery disease.

Corus CAD is commercially available through an innovative patient sample kit that includes everything needed for blood collection and express delivery to the company's CLIA-certified Palo Alto, Calif. laboratory. Test results are delivered promptly to the clinician's office. Corus CAD is currently available in the United States.

Corus CAD has been recognized by *The Wall Street Journal's* Technology Innovation Awards, honored as a Gold Edison Award recipient, and named one of *TIME's* Top Ten Medical Breakthroughs.

For more information please visit <http://www.cardiodx.com/media-kit/>.

About CardioDx

CardioDx, Inc., a pioneer in the field of cardiovascular genomic diagnostics, is committed to developing clinically validated tests that empower clinicians to better tailor care to each individual patient. Strategically focused on coronary artery disease, cardiac arrhythmia and heart failure, CardioDx is poised to expand patient access and improve healthcare quality and efficiency through the commercialization of genomic technologies. For more information, please visit www.cardiodx.com.

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Media Contact:

Nicole Osmer

650.454.0504

nicole@nicleosmer.com



CardioDx Receives College of American Pathologists (CAP) Accreditation

- CAP Accreditation is Awarded to Clinical Laboratories Meeting the Highest Standards of Excellence in Quality Laboratory Practices -

PALO ALTO, Calif. – May 9, 2013 – CardioDx, Inc., a pioneer in the field of [cardiovascular genomic diagnostics](#), today announced its onsite clinical laboratory has received accreditation from the College of American Pathologists (CAP), which is awarded to facilities that meet the highest standards of excellence in quality laboratory practices. This recognition adds to the certification of the clinical laboratory previously received from the Centers for Medicare and Medicaid Services' (CMS) Clinical Laboratory Improvements Amendment (CLIA) and to the licensure issued by the New York State Department of Health. CardioDx offers [Corus[®] CAD](#), the only clinically validated [gene expression blood test](#) for evaluating patients with signs and symptoms suggestive of obstructive [coronary artery disease](#) (CAD), nationally through its accredited laboratory in Palo Alto, CA.

The CAP Laboratory Accreditation Program is an internationally recognized program and the only one of its kind to utilize teams of practicing laboratory professionals as inspectors. During the accreditation process, inspectors examine the clinical laboratory's records and quality control procedures, as well as the laboratory's equipment, facilities, safety program and overall laboratory management to ensure it meets CAP's stringent requirements. The goal of the CAP program is to improve patient safety by advancing the quality of pathology and laboratory services through education and rigorous standards, which meet or exceed regulatory requirements. CMS has granted the CAP Laboratory Accreditation Program deeming authority. The program is also recognized by the Joint Commission, and can be used to meet many state certification requirements.

"CardioDx is proud to join an outstanding group of more than 7,300 laboratories worldwide that have received CAP accreditation," said David Levison, President and CEO of CardioDx. "This important milestone reflects our commitment to providing the highest quality standards for our clinicians and their patients."

About Obstructive Coronary Artery Disease

Coronary artery disease is a very common heart condition in the United States. One in five deaths among Americans is caused by CAD.¹ CAD can cause a narrowing or blockage of the coronary arteries (vessels to the heart that supply the heart with blood, oxygen, and nutrients), reducing blood flow to the heart muscle. This narrowing or blockage in the coronary arteries is often referred to as obstructive CAD, characterized by the presence of atherosclerosis or plaque.

About Corus CAD

With a simple blood draw, Corus CAD can safely, accurately and conveniently help primary care clinicians and cardiologists assess whether or not a stable non-diabetic patient's symptoms are due to obstructive coronary artery disease, enabling many patients to avoid unnecessary invasive procedures and exposure to imaging-related radiation risks or imaging agent intolerance. The test has been clinically validated in multiple independent patient cohorts, including two prospective, multicenter U.S. studies,

PREDICT and COMPASS. Additionally, a retrospective, multicenter chart review study and the prospective IMPACT trial at Vanderbilt University demonstrated that Corus CAD use yields statistically significant and clinically relevant changes in patient management decisions in both primary care and cardiology settings. The test has been used commercially by clinicians in more than 38,000 patients and is a covered benefit for more than 40 million Medicare enrollees in the U.S. Corus CAD is the only test for CAD that is sex specific and accounts for key biological differences between men and women.

Corus CAD has also been recognized by *The Wall Street Journal's* Technology Innovation Awards, honored as a Gold Edison Award recipient, and named one of *TIME's* Top Ten Medical Breakthroughs. CardioDx was recently honored as one of *FierceMedicalDevices'* "Fierce 15" most promising privately held medical device and diagnostic companies.

The Corus CAD test is intended for use in non-diabetic stable patients who present with typical or atypical symptoms suggestive of CAD, with no known history of CAD, no prior myocardial infarction (MI) or revascularization procedure, and who are not currently taking steroids, immunosuppressive agents or chemotherapeutic agents.

About CardioDx

CardioDx, Inc., a pioneer in the field of cardiovascular genomic diagnostics, is committed to developing clinically validated tests that empower clinicians to better tailor care to each individual patient. Strategically focused on coronary artery disease, cardiac arrhythmia and heart failure, CardioDx is poised to expand patient access and improve healthcare quality and efficiency through the commercialization of genomic technologies. For more information, please visit www.cardiodx.com.

Forward-Looking Statements

This press release may contain forward-looking statements, including statements regarding the business strategy of CardioDx, the safety and efficacy, adoption rate and size of the market for Corus CAD, and beliefs regarding the need for and value of gene expression diagnostics. These statements relate to future events and involve known and unknown risks, uncertainties and other factors that could cause actual levels of activity, performance or achievement to differ materially from those expressed or implied by these forward-looking statements. These statements reflect the views of CardioDx as of the date of this press release with respect to future events and, except as required by law, it undertakes no obligation to update or revise publicly any forward-looking statements, whether as a result of new information, future events or otherwise after the date of this press release.

About the College of American Pathologists

As the leading organization for board-certified pathologists, the College of American Pathologists (CAP) serves patients, pathologists, and the public by fostering and advocating excellence in the practice of pathology and laboratory medicine worldwide. With more than 18,000 physician members, the CAP has led as the gold standard in laboratory accreditation for 50 years with more than 7,300 CAP-accredited laboratories in 50 countries. Find more information about the CAP at www.cap.org.

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For media inquiries in the U.S., please contact Wenli Chiu of Lazar Partners, +1-646-871-8492, wchiu@lazarpartners.com.

¹ Lloyd-Jones D, Adams R, Carnethon M, et al. Heart Disease and Stroke Statistics--2009 Update: A Report From the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. *Circulation*. 2009;119:480–486.

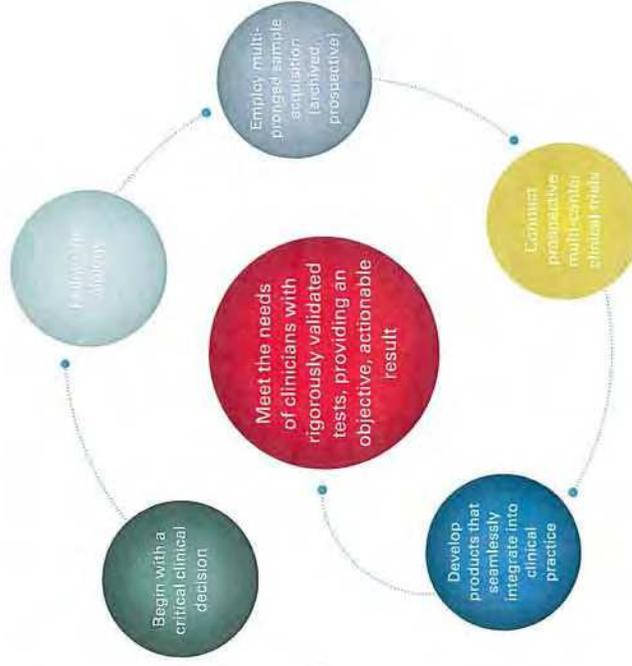
EXHIBIT D

- Enhancing patient care through actionable, objective cardiovascular genomic testing



Our Approach

We target our product development efforts where genomics can enhance patient care. By following the biology associated with cardiovascular disease, we select state-of-the-art research and development tools to deliver the genomic test that will best meet the identified clinical need.



About CardioDx

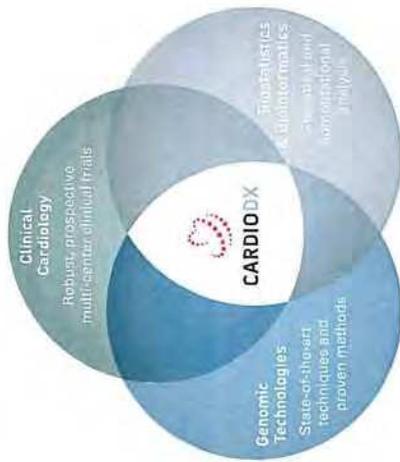
Founded in 2004, CardioDx is a cardiovascular genomic diagnostics company located in Palo Alto, CA. CardioDx develops clinically validated genomic tests to aid in assessing and tailoring care of individual patients with cardiovascular disease, including coronary artery disease (CAD), cardiac arrhythmias and heart failure.

Our genomic tests provide timely and reliable information, allowing physicians to make more informed patient care decisions.

... **Cardiology** is entering a new era of **genomic medicine**.

Our Core Competencies

At CardioDx, we fuse expertise in genomics, biostatistics, and cardiology to develop objective, reproducible tests that provide clinicians with information to enhance patient care.

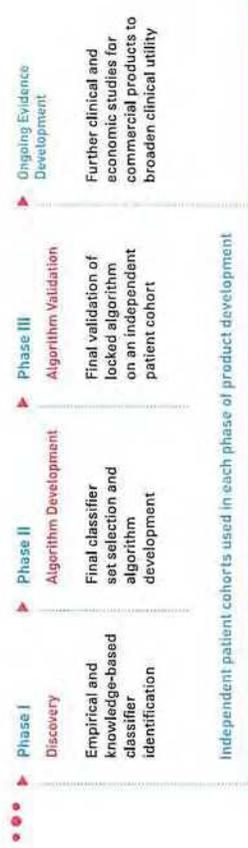


Highly Specialized Lab Processes

We have developed highly specialized methods that combine reproducible and automated laboratory processes with biocomputational analysis to deliver timely results. All these processes are performed in our CLIA-licensed CardioDx Commercial Laboratory, where we methodically employ real-time quality checks to ensure exceptional quality control.

Rigorous Development and Validation Methods

- The complex nature of cardiovascular disease and genomic diagnostics demands rigor in both clinical studies and research methodologies
- Independent patient cohorts are used in each phase of product development to ensure a robust test that delivers reliable results in real-world clinical settings



Introducing Corus™ CAD 2,3

Corus CAD is the first and only clinically validated gene expression test that objectively quantifies the likelihood of obstructive* coronary artery disease (CAD) in a stable chest pain patient. Corus CAD:

- Assesses a patient's disease at the molecular level, giving quantitative information about the likelihood that an individual patient has obstructive CAD
- Has been validated in a rigorous, multi-center trial and integrates the expression levels of 23 genes and other patient characteristics that have been demonstrated to indicate obstructive CAD
- Requires only a standard blood draw procedure and the results are promptly delivered to the physician

When used in conjunction with standard clinical assessments, Corus CAD empowers physicians to identify individual patient likelihood of having obstructive CAD.

* Obstructive CAD is defined as at least one atherosclerotic plaque causing ≥50% luminal diameter stenosis in a major coronary artery (≥1.5 mm lumen diameter) as determined by invasive quantitative coronary angiography (QCA).

CORUS™ CAD
Gene Expression Test By CardioDx

Partnership Every Step of the Way

Customers can count on a comprehensive support program with superior service every step of the way, including:

- Clinician product and procedure training
- Specialized consultations with a CardioDx cardiologist to assist in patient selection and test result interpretation
- Patient Advocates to handle insurance, billing and patient financial questions

••• CardioDx develops **state-of-the-art** genomic tests that enable physicians to enhance cardiovascular **patient care**.



References

1. CLIA #05D1083624.
2. Wingrove JA, Daniels SE, Sehnert AJ, et al. Correlation of peripheral-blood gene expression with the extent of coronary artery stenosis. *Circ Cardiovasc Genet.* 2008;1:31-38.
3. PREDICT trial. Clinical trial summary found at: www.clinicaltrials.gov, NCT00500617.

Corus™ CAD Intended Use

The Corus CAD test is a quantitative in vitro diagnostic test performed in a single laboratory, using the gene expression profile of cells found in peripheral blood specimens to be used as an aid to identify patients who are likely to have coronary artery stenosis of at least 50%. The test should be performed on patients with a history of chest pain, with suspected anginal equivalent to chest pain, or with a high risk of coronary artery disease, but with no known prior myocardial infarction or revascularization procedures. The test is not intended for patients with acute myocardial infarction, high risk unstable angina, systemic infectious or systemic inflammatory conditions, diabetes, and/or who are currently taking steroids, immunosuppressive agents, or chemotherapeutic agents.

The test is performed on a blood specimen obtained from the patient. The test incorporates the expression levels of multiple genes using an algorithm with weighted functions to generate a quantitative score. The results of the test should be used by clinicians in conjunction with other tests and clinical information in their assessment of a patient's coronary artery disease.

The Corus CAD test is for prescription use only. The test is not intended to be used to screen for stenosis among patients who are asymptomatic and not considered at high risk for coronary artery disease, to predict or detect response to therapy, or to help select the optimal therapy for patients.

CardioDx®

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service@cardiodx.com
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Reimbursement Operations

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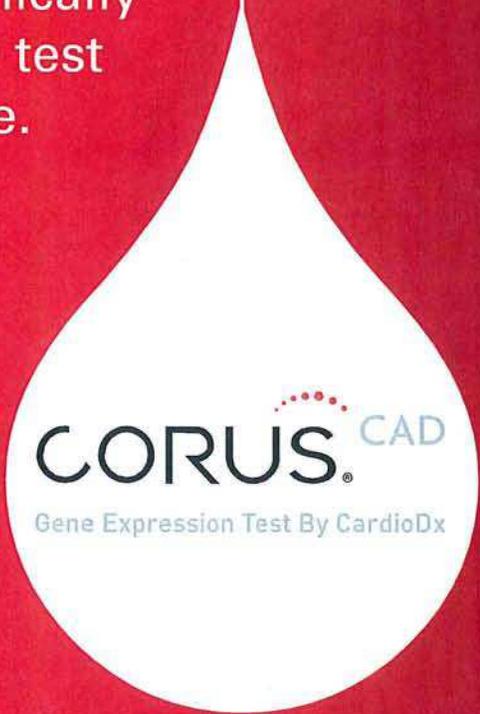
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LEN-090001 Rev.00 | Effective Date 08-Jun-2009

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**Now, there's a blood test that
can help you confidently exclude
obstructive CAD, right in your office.**

Corus[®] CAD is the only clinically
validated gene expression test
for coronary artery disease.



CORUS[®] CAD
Gene Expression Test By CardioDx



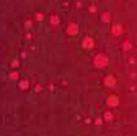
CARDIODX[®]

To learn more, visit www.cardiodx.com or call 866.941.4996

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Now, there's a blood test that can help you confidently exclude obstructive CAD, right in your office.

Corus[®] CAD is the only clinically validated gene expression test for coronary artery disease.



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COUNTER

Corus[™] CAD Clinician Summit:

Integrating genomic testing into real-world patient management

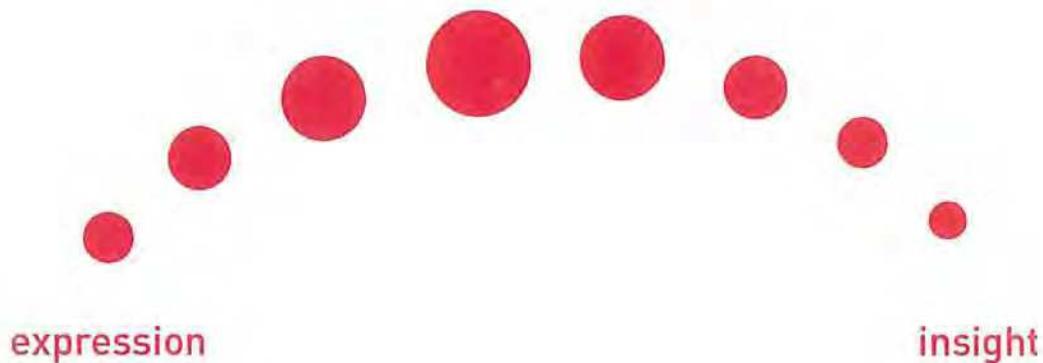
Please join your colleagues

for an evening of discussion about Corus CAD and its role in clinical practice.

- when Monday November 16, 2009
6:00 — 8:00pm
- where Maggiano's Bella Boardroom
9101 International Dr # 2400
Orlando, FL 32819-8120
- rsvp Janna Osborne
josborne@cardiodx.com
650.475.2725

Dinner and cocktails will be served.





Corus™ CAD is the first and only clinically validated gene expression test that objectively quantifies the likelihood of obstructive coronary artery disease (CAD) in a stable chest pain patient.

CardioDx® develops clinically validated genomic tests to aid in assessing and tailoring care of individuals with cardiovascular disease, including CAD, cardiac arrhythmias, and heart failure.



CORUS[®] CAD
Gene Expression Test By CardioDx

Corus[®] CAD is a convenient blood test to help your healthcare provider assess if you may have a blockage in your heart.

Now available in this office • Ask your healthcare provider if Corus CAD may be right for you



Your healthcare provider may not need to go deeper than your blood to know | what's happening.

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Is a proud supporter of the 2012 Go Red For Women Movement



**Your healthcare provider may not need to go deeper than your
blood to know what's happening.**

**To learn more about cardiovascular genomic diagnostics for
coronary artery disease, visit www.cardiodx.com or call us at
1.866.941.4996**

Join Us for an Educational Webinar on Tuesday, April 30th

Emerging Clinical Trial Data for Corus[®] CAD in the Primary Care Setting

Chief Medical Officer Mark Monane will review
the latest Corus CAD clinical data including:

- COMPASS Study
- PREDICT Study
Focus on Women
- Retrospective Chart Review
Focus on Patients Aged 65
and Older

.....

Three times are available on Tuesday, April 30th:

- 12:00 pm to 1:00 pm ET
- 12:30 pm to 1:30 pm CT
- 12:30 pm to 1:30 pm PT

To access the webinar, please use the following dial-in information and link:

Dial 1-877-668-4490 Access code: 576 706 817

Navigate to <https://www.webex.com/login/attend-a-meeting>, enter meeting ID# 576 706 817

It is recommended that you use Internet Explorer as your web browser for the webinar. If you are having technical issues, please contact Jonathan Anonuevo at janonuevo@cardiodx.com or phone 650-475-2781.

RSVP to your CardioDx Territory Manager



CARDIODX[®]

CardioDx®

CardioDx, Inc., a pioneer in the field of cardiovascular genomic diagnostics, is committed to developing clinically validated tests that empower clinicians to better tailor care to each individual patient. Strategically focused on coronary artery disease, cardiac arrhythmia and heart failure, CardioDx is poised to expand patient access and improve healthcare quality and efficiency through the commercialization of genomic technologies.

Corus® CAD Intended Use

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CARDIODX®

The American College of Cardiology ACC.12 Chicago, March 24 - 27, 2012

We invite you to attend the following presentations:

Scientific and Clinical Presentations

Saturday, March 24, 9:30 am - 12:00 pm

McCormick Place South, Hall A

Alexandra Lansky, MD (Poster Presentation)

The Diagnostic Yield of Coronary Angiography Remains Low, Despite Prior Myocardial Perfusion Imaging Testing, in Patients With Suspected Obstructive Coronary Artery Disease: Results of the PREDICT Trial

Sunday, March 25, 11:30 - 11:45 am

McCormick Place South, Room S403

Szilard Voros, MD (Oral Presentation)

A Previously Validated Peripheral Gene Expression Score is Mostly Determined by Non-Calcified Plaque and Luminal Stenosis as Assessed by Quantitative, 3-Dimensional Measurements by CT Angiography in the Multi-Center, Prospective COMPASS Study

Sunday, March 25, 11:48 am - 12:00 pm

McCormick Place North, Room N230

Robert Schwartz, MD (Oral Presentation)

A Peripheral Blood Gene Expression Score for Coronary Artery Disease in Non-Diabetic Patients Identifies Patients at Low Risk for Major Cardiovascular Events and Interventional Procedures in the Next 12 Months (PREDICT 1-Year Follow-Up)

Satellite Symposium / Educational Forum

Saturday, March 24, 8:00 - 9:00 am

McCormick Place North, N228

Geoffrey Ginsburg, MD, PhD

Translational Research Symposium:
New Breakthroughs in Cardiovascular Genomics

Monday, March 26, 1:50 - 2:00 pm

Hall A2, CV Theater, #22097

Mark Monane, MD, MS, FACP

CV Innovations Educational Forum, Personalized Medicine:
A New Gene Expression Test to Detect Coronary Artery Disease



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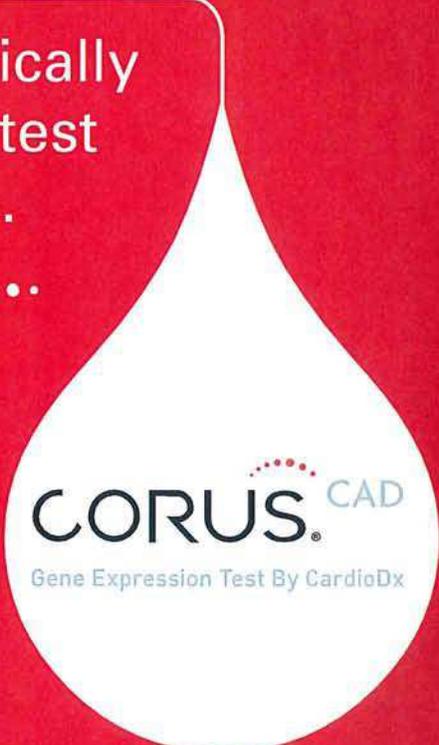
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CORUS. CAD
Gene Expression Test By CardioDx



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May 18, 2011

CardioDx to Use \$60M Funding Round to Make Clinical Utility Case for Corus CAD

CARDIODX HAS RAISED \$60 MILLION in equity financing that it will invest in expanding reimbursement for its Corus CAD genetic test for coronary artery disease.

Corus CAD is a gene expression test that cardiologists can use in addition to other medical information to assess whether non-diabetic patients' medical symptoms are due to obstructive CAD.

CardioDx runs the test, which gauges the expression of mRNA by 23 genes via real-time PCR, at its CLIA-certified laboratory in Palo Alto, Calif. The expression of each gene is measured and interpreted through the company's Corus CAD software, and the patient's genetic test results are sent in a report to the doctor.

A spokesperson for CardioDx told *PGx Reporter* that the company will use the funding to complete additional studies that demonstrate the clinical utility of Corus CAD, continue to educate primary care physicians and cardiologists as to how the test can be used in the care of their patients, and reach out to payors to help them understand the circumstances under which they should pay for the test.

Specifically, CardioDx may use the money to "collaborate on registry-type studies with the payors," the spokesperson said.

Obstructive CAD, at the minimum, can cause chest pain, but it can also lead to a heart attack or death. In its discussions with payors, CardioDx likely asserts that tools such as Corus CAD could save healthcare dollars by diagnosing such a condition early and therefore avoiding more serious complications.

In addition, published studies indicate that current methods for identifying which patients should undergo elective invasive coronary angiography to diagnose coronary artery disease do not always accurately assess a patient's risk for the condition. The gold standard for diagnosing obstructive CAD is catheter-based coronary angiography, which is performed with other methods that expose patients to radiation and contrast agents. CardioDx and the developers of Corus CAD believe that the genetic test offers advantages over other CAD diagnostics because it only requires patients to give a blood sample.

"With a simple blood draw, CardioDx's Corus CAD test provides actionable information regarding the diagnosis of cardiovascular disease that helps physicians make better decisions, helps patients avoid unnecessary procedures and radiation exposure, and helps payors address a major expense category," said Patrick Enright, managing director of Longitude Capital and CardioDx board member, in a statement.

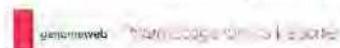
CardioDx CEO David Levison estimated that doctors have so far used Corus CAD to gauge obstructive CAD in more than 13,000 patients. The company estimates that approximately 120 health plans have reimbursed the Corus CAD test to date.

"We are working with those plans to help them better understand how Corus CAD can improve patient care and reduce unnecessary medical procedures," the CardioDx spokesperson said. "It is our goal to gain reimbursement

continued on next page



www.genomeweb.com
The GenomeWeb Intelligence Network



coverage of Corus CAD for a large majority of the insured population in the US.”

On its website, CardioDx offers to file all insurance claims and appeals on the patients' behalf to help make the economic and clinical utility case to payors. Additionally, the company has a financial assistance program for those who are deemed eligible for aid.

In April, CardioDx presented data at the American College of Cardiology annual meeting demonstrating that Corus CAD could help physicians diagnose obstructive coronary stenosis while adding independent information to coronary calcium scoring in patients undergoing CT angiography. In this study, the company reported a more than 95 percent negative predictive value for Corus CAD.

This study presented at ACC analyzed data from the multi-center, prospective Personalized Risk Evaluation and Diagnosis In the Coronary Tree, or PREDICT trial, which was originally published in October 2010 in the *Annals of Internal Medicine*. In PREDICT researchers led by CardioDx Chief Scientific Officer Steven Rosenberg investigated whether Corus CAD can help doctors more accurately assess obstructive CAD as measured by quantitative coronary angiography. The ACC study expanded on these initial findings to show that the test can gauge obstructive CAD as measured by coronary CT angiography.

Corus CAD yields a score between 0 and 40. The higher scores correspond to a higher likelihood of obstructive CAD and a higher maximum percentage of stenosis in the patient tested.

In PREDICT researchers found that the gene expression algorithm score used to diagnose obstructive CAD

improved patient reclassification as to whether or not they have the condition by 20 percent relative to Diamond-Forrester score and 16 percent relative to an expanded clinical model. Corus CAD provided a reclassification improvement of 21 percent over myocardial perfusion imaging, which according to the study authors is the most prevalent noninvasive test. The authors noted, however, that these results may have been exaggerated since the patient cohort included those referred for angiography.

“Our test provides a statistically significant but modest improvement in classification of patient CAD status compared with clinical factors or noninvasive imaging,” Rosenberg et al. concluded. “Further studies are needed to define the performance characteristics and clinical utility in populations with a lower pretest probability.”

In addition to using its newly raised funds to support reimbursement efforts for Corus CAD, CardioDx will also funnel some of the money toward developing other genomic diagnostics for cardiovascular conditions, the spokesperson said.

Founded in 2004, CardioDx specializes in developing genomic tests for coronary artery disease, cardiac arrhythmias, and heart failure. The company is currently running a prospective clinical trial, called Diagnostic Investigation of Sudden Cardiac Event Risk, or DISCERN, in which researchers are studying genomic factors involved in ventricular arrhythmia and sudden cardiac death.

Participating in the latest funding round were Longitude Capital, JP Morgan, Acadia Woods Partners, Artiman Ventures, and RU-COM's venture arm Bright Capital, as well as previous investors. The company has raised more than \$100 million in financing to date.

“The Gray Sheet”[®]

Medical Devices, Diagnostics & Instrumentation

September 7, 2009

Volume 35 Number 36 Page 19

CardioDx Launches Gene Test For Obstructive Coronary Disease

CardioDx is gradually rolling out its *Corus CAD* gene expression test, which quantifies the likelihood of obstructive coronary artery disease in patients with stable chest pain and no previous history of cardiac disease.

The roll-out began soon after completion of the Personalized Risk Evaluation and Diagnosis in the Coronary Tree (PREDICT) trial of Corus CAD. The study collected over 2,800 patient samples from 40 sites in the U.S. The company expects to release results of the study by the end of this year and to publish the data in 2010.

“It identifies individuals and provides the physician with the likelihood that they have obstructive disease and really focuses on clinically meaningful information,” CardioDx CEO David Levison told “The Gray Sheet.”

Despite all of the advances in cardiac imaging and functional testing, the clinical need for an assay that tests stable chest-pain patients for coronary disease is “enormous,” Levison says. “The noninvasive assessment of coronary disease continues to be challenging, and there is great diversity in how physicians are treating these patients,” he said.

In the PREDICT study, all of the patients had already been referred by a physician to an interventionalist for a coronary procedure such as stenting, but the subsequent angiograms showed that only 36% of the patients had obstructive coronary disease.

A major advantage of Corus CAD is that it yields a single objective score corresponding to the percentage chance that the patient has coronary artery disease. The score is derived from expression levels of 23 genes and other characteristics related to inflammation of the coronary arteries.

The test is sold as a kit to physicians’ offices. The kit includes all of the equipment needed to draw and store the blood sample, as well as a special cold shipping package to send the sample to CardioDx’s processing lab in Palo Alto, Calif. The total turnaround time from the time the blood sample is taken until the results are given to the doctor is two days.

So far, the test is available in Kentucky, Maryland, Illinois, Washington, Wisconsin, Minnesota, North Carolina, Texas and Arizona, and the firm expects to add more states in 2010.

“The reason to not roll out in all 50 states initially is that we really wanted to make sure we understood how this test was going to be integrated into clinical practice,” Levison said.

“We’re still just learning about what information physicians want, how to integrate this into their practice. It will take a little bit longer before we feel comfortable that we really understand how physicians want to use this product, and at that point we will look at expanding into more geography.”

CardioDx Credo: Help Doctors Make Decisions

“One of the tenets of our organization is that we only want to be working on diagnostics where the physician can make a specific decision after getting the results of our test,” Levison said. “We spent a lot of time upfront early in the process, as physicians, to write the product specifications before we spent a lot of money on R&D.”

The test is primarily intended for outpatient cardiologist clinics and primary care physician offices that need to be able to identify which patients do not have coronary artery disease. "We interviewed 1,100 physicians in the course of the last three and a half years, talking to them specifically about what their needs were in the clinical evaluation of coronary artery disease, and it was very clear from the physicians that they want a 'rule out' test."

"They want a test with high sensitivity and high negative-predictive value so that they can rest assured that those patients have a low likelihood of disease and they can focus on the patients with a higher risk, because they can't aggressively treat all the patients they see with stable chest pain."

Many Corus CAD buyers are small cardiology practices that do not have access to some of the imaging technology and this test helps determine if the patient should be referred for further evaluation, he said. Also, large cardiology practices are using the test for patients who are poor candidates for contrast imaging because of renal impairment or other contraindications.

– Reed Miller (re.miller@elsevier.com)

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CARDIO Dx:

BRINGING MOLECULAR DIAGNOSTICS INTO THE CARDIOVASCULAR ARENA

One of several molecular diagnostics companies founded by a well-known trio of West Coast VCs, CardioDx is fast finding itself in the spotlight.

BY MARK L. RATNER

- Buoyed by the success of Genomic Health, venture capital firms Kleiner Perkins, Mohr Davidow, and TPG have expanded their portfolio of molecular diagnostics companies, with many following similar themes.
- One company, CardioDx, has developed *Corus CAD*, a test cardiologists can use to distinguish between patients who should move on to imaging and the cath lab, and those they are comfortable managing without moving them on the diagnostic pathway.
- That's a challenging proposition, but initial clinician feedback for the product, which launched last year, appears good, especially among primary care physicians – somewhat to the surprise of the company.
- A confluence of issues around the use of diagnostic imaging could serve CardioDx well as it drives adoption of *Corus CAD*.

In the early part of the decade now ending, three West Coast venture capital firms – Kleiner Perkins Caufield & Byers (KPCB), Mohr Davidow Ventures (MDV), and TPG – had already established themselves as pioneer investors in molecular diagnostics. KPCB and TPG had invested early in **Genomic Health Inc.**, whose *Oncotype Dx* gene expression test has become the exemplar of a rationally based diagnostic test that can command a premium price. And the VCs were now turning their attention to other therapy areas around which to start new diagnostics companies.

"It looked like oncology was going to work," recalls Fred Cohen, MD, of TPG, "Brook and Michael [Brook Byers of KPCB and Michael Goldberg, now of MDV] and I were on the board of Genomic Health at that time and we said 'Ok, we don't want to start companies that would compete with Genomic Health because that's bad for business. [An earlier version of this article incorrectly stated that MDV was also an early investor in Genomic Health. Michael Goldberg had been on the board of Genomic Health since 2001 but did not join MDV until 2005.] So what other markets should we go after?'" They ended up focusing on several, among them **Tethys Bioscience Inc.** in diabetes, **XDx Inc.** in transplant rejection, and later, **CardioDx Inc.** in cardiovascular disease. (See *Exhibit 1*.)

The VCs had also formulated two rules of the road along the way for these new investments, based partly on their experience with Genomic Health. "We always start with the pharmacoeconomics," says Cohen: focusing on a decision that's expensive and poorly made. Otherwise, he says, "it's not a good place to work. Because ultimately, we believed then and we believe more firmly now, you have to bend the cost curve." Every one of their companies is designed to help doctors make better decisions, he says, adding that obviously, the more expensive the decision, and the more it looks like the flip of a coin, the better a setting it is for a new diagnostic modality.

The next rule is to determine whether it's feasible to construct a clinical trial that could change practice if it yielded the right result. XDx's *AlloMap* blood test, for example, assesses a patient's current risk of heart transplant rejection starting at 58 days, eliminating the need for a biopsy in cases where rejection can be ruled out. In a clinical trial, "I pretty quickly find out – in days – whether my blood test matches up to the biopsy," says Cohen. By contrast, he explains, a screening test for early-stage lung cancer, where the incidence is one in 10,000 per year in some populations, could require studying 100,000 people, drawing yearly blood samples from them, following them for seven years before knowing if it

worked. And then, at the end of seven years, to show that the test can direct therapy, to prove it changes outcomes would require another 12-year trial.

CARDIODX EMERGES

David Levison, a 20-year veteran in the health care industry (among other things, he'd sold Oncology Therapeutics Network to Bristol-Myers Squibb Co. and a handheld instrument company, iScribe, to PCS), was a partner at TPG in the early 2000s, and for a time served as interim CEO of XDx. After that stint, Levison went back to TPG, in 2004, where he joined Cohen and KPCB's Brook Byers to look around for other therapeutic areas where the approach to personalized medicine they'd refined would make sense.

In addition to the two fundamental tenets Cohen articulates, Levison adds a third: to be platform agnostic, to avoid the trap of holding a hammer and therefore seeing everything as a nail. By then, the process of making molecular measurements was becoming routine, and the challenge had shifted from making technology work to constructing a clinical trial that could change practice if you got the right result.

Over the next several months, the VCs considered everything from orthopedics to infertility. They kept coming back to cardiology, however. "Every time we looked either at the market size, the number of patients, or the challenges on the clinical side," Levison says, cardiology "just kept hitting us on the side of the head. We saw 30 years of incredible advances both on the drug and the device side. But we saw no corresponding development on the diagnostic side. We had a lot of new therapies, all very expensive, but no better tools for targeting them."

Of course, they also realized that tapping into even a small portion of the cardiology market could sustain a company – an approach Tethys was already adopting with its development of a test to measure the five-year risk of diabetes.

"We don't have the appetite or the resources to attack all 220,000 primary care physicians or the 60 million individuals at risk for diabetes," says Tethys' president Michael Richey. "There is a substantially large market, even at 5% market penetration in five years, that justifies the kind of

he says. "And if you get any one of those, you are likely to get one, if not the other two. So they are more interrelated than breast cancer is to, say, colon cancer."

That would give the company the opportunity to do product development on a very different time scale than many of its brethren. "It has taken as long as five years between the first and second products for other genomic diagnostics companies," Levison points out. "Our goal is to release a new or updated product every 18-24 months."

Exhibit 1

Following the Genomic Health Model: Selected Subsequent Investments by Kleiner Perkins, Mohr Davidow, and TPG

COMPANY	THERAPEUTIC AREA FOR TESTING	TYPE OF TEST	INVESTORS
CardioDx	Cardiology	Obstructive coronary artery disease risk assessment; Arrhythmia diagnosis	Kleiner Perkins Caufield & Byers (KPCB); Mohr Davidow Ventures (MDV); TPG; also Intel Capital Partners; and Pappas Ventures
Crescendo Bioscience	Rheumatology	Rheumatoid arthritis disease monitoring and joint damage assessment	KPCB; MDV
Nodality	Oncology	Acute myelogenous leukemia treatment response	KPCB; TPG; also Maverick Capital
On-Q-ity	Oncology	Breast cancer treatment response	MDV; also Atlas Venture; Bessemer Venture Partners; Physic Ventures; and Northgate Capital
Tethys	Diabetes	Risk assessment	KPCB; MDV; also aeris Capital; Intel Capital; and Wasatch Advisors
Veracyte	Oncology	Initial diagnosis of malignancy	KPCB; TPG; also Versant Ventures
XDx	Transplantation	Heart transplant rejection risk assessment	KPCB; TPG; also The Sprout Group; Burrill & Company; Duff, Ackerman & Goodrich; Intel Capital; Integral Capital Partners; and DBL Investors

Source: Elsevier's *Strategic Transactions*; company sources

investments we were making. So while we did not see ourselves as Pfizer or Merck, knocking on every physician's door, we had convinced ourselves that there is a segment of the market characterized by early adopters that would buy the message."

Levison also liked the idea that in cardiology, the disease states were interrelated. "If you have a problem with your heart you could have a plumbing problem, an electrical problem, or a pump problem,"

Backed by TPG and KPCB and with Levison as CEO, CardioDx became operational in February 2005. To date, it has raised less than \$50 million through its series C round, including an extension of that round in May 2009. (It hopes to close a series D round in the next few months.)

The founders had outlined three diagnostic programs to take forward: in heart failure; arrhythmia; and coronary disease. For the first year-and-a-half, CardioDx

identified cohorts of patients it could analyze retrospectively, collected samples (from trials run in Germany and at Duke University, and from the provider Intermountain Health Care Inc.), and generated and analyzed genetic and gene expression data in the lab, focused on these three product concepts.

As this early work progressed, a rule-out test for obstructive coronary artery disease (CAD) rose to the surface as the lead program. "We surveyed a thousand physicians before we launched our first product, to make sure we were working on things that were critical to physicians' decision-making," says Levison, who was now joined in the effort by Risa Stack, PhD, a partner at KPCB who remains on CardioDx's board. By early 2007, the company had prioritized the coronary disease program as the first to market and the arrhythmia program as the second.

In the meantime, Levison had hired Steven Rosenberg, PhD, who'd led XDX's AlloMap development, to be chief scientific officer, and brought Deborah Kilpatrick in from the Abbott Vascular Devices division of Abbott Laboratories Inc. (formerly Guidant) to head market development. "It's a fantastic team," says Louis Lange, MD, founder and former president and CEO of CV Therapeutics Inc. until its acquisition by Gilead Sciences Inc. last year. In October 2009, Lange, who recently joined the VC firm Asset Management, was named Chairman of CardioDx. And while that kind of glowing appraisal of management is to be expected from an incoming chairman of the board, others outside the company are equally generous.

FOCUS ON INFLAMMATION

When the CardioDx team started asking about the biggest challenges in diagnosing and treating coronary disease, physicians frequently lamented the lack of a test for future risk of heart attack.

Over the past decade, vulnerable or unstable plaque has been associated with heart attacks and bad outcomes. "They said, give me a test to show which patients are going to have an MI and which ones will not," Levison recalls. But he would then ask them: "If that diagnostic were available today, what would you do with that information? How would you treat this patient differently?" The dialog suggested that what physicians really needed was a way to prioritize those patients with angina symptoms who needed to be treated aggressively, in whatever manner.

When a patient with stable chest pain comes into the office, the physician needs to quickly and efficiently rule out non-cardiac causes of chest pain, such as indigestion or muscle spasm, in order to choose the most appropriate management strategy (e.g. noninvasive imaging or catheterization). To do so, and classify a patient as having a low or a high probability of having obstructive CAD, they look at family history, lifestyle, and other factors. But there is no real biochemical testing for this, except looking at levels of c-reactive protein (CRP). "The physicians were really telling us they needed a rule-out test to determine which patients with stable chest pain have a low probability of obstructive coronary artery disease, so they could focus their efforts appropriately," says Levison. "And since coronary artery disease is at some level a function of inflammation, and that inflammation is related to gene expression, it made sense to pursue a molecular test."

Corus CAD is the first gender-specific noninvasive tool for the assessment of obstructive coronary artery disease.

Atherosclerosis has a lipid component and an inflammatory component. There are many ways to measure what's going on with your lipids – HDL, LDL, triglyceride measurements, Lp(a), etc. – but when it comes to inflammation there's only levels of CRP, and that's just a lone marker for a multi-factorial process. "You have a lot of specificity around cholesterol and drugs to mediate changes to various subtypes of cholesterol, but not for inflammation, save for the fact that some drugs like *Lipitor* have anti-inflammatory properties," says Fred Cohen. "So we reasoned that you needed a better measure of the inflammatory piece."

CardioDx also realized that, while it had retrospective samples in hand, it could also design a prospective clinical trial in which clinicians could enroll a patient and have the end point the same day – a rapid product development and validation process. In June 2007, the company launched two multi-center prospective clinical trials, one in coronary disease (the PREDICT trial) and another in arrhythmia (the DISCERN trial).

PREDICT enrolled approximately 4,000 patients in 39 centers across the US where the physician had decided that a patient had a high enough risk of having obstructive CAD to send them to the cath lab for angiography. The company obtained angiography results and sent them to a quantitative coronary angiography (QCA) core lab at the Cardiovascular Research Foundation at Columbia University. An obstructive coronary lesion was defined as a greater than or equal to 50% diameter stenosis of a major epicardial vessel by QCA (which is approximately 65-70% diameter stenosis by a clinical read. In QCA, the image is put up on a computer screen, a stylus outlines the vessel walls, and the computer actually does the calculation of the percent blockage.)

The company used part of the data to continue to do gene discovery. Ultimately, it validated an algorithm consisting of a panel of 23 genes and other patient demographic characteristics, which are largely known to be associated with atherosclerosis and were proven in PREDICT to be indicative of obstructive CAD. The CardioDx gene expression *Corus CAD* test measures the expression (mRNA levels) of those genes using qRT-PCR. It is the first gender-specific noninvasive tool for the assessment of obstructive CAD. The test accounts for gender in three distinct ways: some genes are specific to gender; some are weighted differently by gender; and age is weighted differently by gender.

The investigators presented the work at the American Heart Association (AHA) meeting in November 2009 and the results are now in review for publication.

Corus CAD provides a score ranging from 0-40. A score of 15, for example, represents a 20% risk of having obstructive CAD. Put another way, of all patients in the validation study from the PREDICT trial with a score of 15 or less, only 20% had obstructive CAD. That is the approximate threshold for a low-risk patient and where physicians are comfortable managing that patient without pushing them forward to the cath lab.

PHYSICIAN ACCEPTANCE – TBD

All the patients in the PREDICT trial had already been selected to go to the cath lab for angiography. Thus, the study does not

mimic the proportion of patients that physicians will see who ultimately are found to have obstructive CAD. But it was the only feasible trial because of the need for a gold standard QCA end point. "We had this discussion with FDA," Levison says. "If I were to take a group of patients who were asymptomatic and low risk, no IRB [Investigational Review Board] in the country would let me run angiography on them. I was willing to introduce that bias to get the gold standard of angiography and QCA."

Many tests have aimed at the kind of rule-out that Corus CAD does, says Manesh Patel, MD, of the **Duke Clinical Research Institute**, including blood tests, electrocardiographic tests, non invasive CT angiography, and calcium scoring. And a blood test, be it genomic or metabolomic, that at a high sensitivity identified those that do have obstructive disease and those who do not, "then obviously it would be of use," he says.

That said, it remains to be seen whether physicians will be comfortable taking action based on a set of gene expression markers only validated, at least thus far, in a single study. And while developers of such first-level diagnostic tests understandably try to get patients that are already in the cath lab – because that is the gold standard for assessing obstructive CAD, as Levison points out – that kind of study does not necessarily mirror the general population for the test. "It's not a bad way to start, but what usually happens," Patel observes, "is the reported sensitivity and predictive values of tests are very dependent on the prevalence of the disease in the population." For the patients in the cath lab, "although the number of actual cases [of obstructive coronary disease] seems low to us, if you then take it into primary care, or into routine clinical practice where the actual rate of obstructive disease may be 5% or 10%, the test won't work the same," he says. "Calcium scores and CT angiography have run into those issues."

That skepticism doesn't surprise Lou Lange, who faced a similar issue when CV Therapeutics began developing its stress agent, *Lexiscan* (regadenoson, an adenosine receptor antagonist), which is used for stress testing of patients who can't exercise.

"When people started doing pharmacologic adjuncts to stress thallium around 1990, very few cardiologists were interested," he says. "Now, however, it's a \$350 million market." And the data for Corus CAD are definitely encouraging, he believes. "If you took the Corus CAD slide that has its sensitivity and specificity and rule-out information on it and dropped the name and put up stress thallium, everybody would say of course, that is very useful," he says. "And since this doesn't use radioactivity and is faster and cheaper, it could either replace MPI [nuclear myocardial perfusion imaging] or be used to refine the results of MPI. So the idea physicians would never use it is truly misinformed." Plus, the medical community has been using troponin levels and myoglobin levels as rule-outs for MI for 30 years.

Lange also sees a strong opportunity for Corus CAD to penetrate the market of high-end internists and cardiologists

who don't stent. "The test is really going to be interesting to people who don't want to lose their patients to referrals and interventionalists," he says. It also complements the findings of the long-term COURAGE study, which showed that stenting did not improve outcomes over optimum medical therapy, he points out. Corus CAD also dovetails nicely with the trend

toward reducing radiation exposure from diagnostic imaging – a subject under active scrutiny at FDA, which recently announced it is considering imposing new safety controls on medical imaging devices and is holding a public meeting at the end of March to collect suggestions about new safety features and training for medical radiation technology, and in Congress, which in February held a hearing in which experts urged the government to establish guidelines controlling the amounts of radiation patients receive.

Indeed, CardioDx launched Corus CAD at a time when the utility and safety of cardiovascular imaging is being questioned on several fronts. (See "Another Call for Better Risk Stratification in Cardiac Imaging," *START-UP*, March 2010.)

"We are using the cath lab for diagnostic-only purposes more frequently than it

was really designed for," says Levison. Even more important, perhaps, is the reality that the tools to assess whether to direct a patient to the cath lab – a stress echocardiogram or, in most cases, an MPI procedure – are often not good predictors of obstructive CAD. Nor are they as precise in the community-based setting – the target market for Corus CAD – as they are in academic settings, which are a more controlled environment, he says. "Only a little more than a third of the patients who had a positive MPI study [in PREDICT] also had obstructive coronary artery disease," says Levison. "So it's clear that the technologies we have in the physicians' hands today don't work as well in the community setting, which is one reason we wanted to develop a very reproducible test not subject to variability due to clinical setting."

According to CardioDx's estimates, there are well over a million MPI procedures in the US every year performed on patients that meet the intended use of Corus CAD. "As you move to the primary care setting, which typically evaluates patients without the use of MPI, the number of eligible patients gets very large," says Levison. And to be commercially viable, CardioDx only needs a small portion of that large market. It ran approximately 900 tests in the first six months, and expects to run over 5,000 in 2010. For most of 2009, the company had little clinical evidence to share with physicians because the majority of the data was submitted for presentation at the AHA meeting in November 2009. Once that oral abstract was accepted, there was an embargo on disclosing it prior to the meeting. Among the data presented at AHA was the fact that, if a patient had a positive MPI, but a low Corus CAD score, those patients were correctly reclassified as not having obstructive CAD 89% of the time.

The test is currently commercially available through direct sales in eight states and the company expects to expand its

"Primary care physicians have apparently been eager to use the test because it empowers them to make more informed decisions on their patients before any referral."

–David Levison, CEO

efforts substantially during 2010 – largely driven by reimbursement trends among payors known to cover such innovative molecular diagnostics tests. The limited launch also presumably creates buzz by focusing on key early adopters. “I would rather have fewer customers using this on a regular basis than have more people try it and forget about it,” says Levison. “We think the value of the test is if it’s used consistently within a practice and integrated well into their overall clinical strategy. So we have focused on making that happen.” It’s also easy to use. Corus CAD is pre-stocked in the physician’s office, and all the materials for it come in a shoebox-sized box. A 2ml sample of blood is drawn into a special tube that stabilizes the RNA, which is placed in a box with a FedEx label and shipped to CardioDx’s lab in Palo Alto, CA, which runs the test and sends results back. The whole process takes two to three days.

The test is priced at \$1,195, but the company has a generous financial assistance plan, and initially capped the out-of-pocket patient cost at \$75. CardioDx is taking on the financial risk until reimbursement is established, and is handling all of the billing and appeals processes, to minimize financial hurdles for patients and physicians. There is also a practical financial aspect to the strategy: An insurance company may take up to six months to pay, so the company needs to carry the cost of the test for that period. That’s a big working capital cost which is obviously a function of sales volume. “If we were running thousands of tests too quickly, our working capital needs would get too large too fast. In a challenging financing environment, I wanted to make sure I limited our capital requirements,” says Levison. Some payors are paying for the test, he says, and the company is planning to initiate payor discussions later in 2010.

Cushioning the initial cost to patients and physicians is typical for a new, premium-priced diagnostic test. “Most companies have had to do that,” notes Kristin Pothier, vice president at Health Advances, to determine whether the launch is working. “You look at penetration into your overall addressable population. It doesn’t matter who’s paying for it,” she says. “At the end of the day, as a company you have to understand how much traction the test is gaining for clinical benefit and you have to do it for a couple of years.”

PRIME TIME IN PRIMARY CARE

When CardioDx launched Corus CAD, it expected to have to first convince the cardiology community of the value of the test, and that they’d be the evangelists for it. But to Levison’s surprise, “We’ve had more significant adoption among primary care practices than we expected at this early stage,” he says. Primary care physicians have apparently been eager to use the test because it empowers them to make more informed decisions on their patients before any referral, he says. Payors would also like to maintain the primary care physician as the gatekeeper to specialty referral for as long as they can, because they know their costs go way up after that point. “One of the things we heard from the payor community, even before we launched the test, was their interest in seeing the test in the primary care community,” says Levison. “They perhaps had more insight than we did.”

Corus CAD may be of particular benefit to women because imaging modalities and chest pain type were less effective in the PREDICT trial in terms of identifying patients who had

obstructive CAD after being sent to the cath lab, Levison points out. In the study, approximately 20% of the women actually had obstructive CAD.

CardioDx is already developing next-generation products in CAD, as the arrhythmia program also progresses. For example, the initial version of Corus CAD is intended for use by non-diabetic patients (diabetics may have a different inflammatory profile because of the disease and their medications). However, the company has most of the patient samples it would need to develop and validate a diabetic version of Corus CAD. Ultimately, CardioDx’s tests are likely to encompass several genomic technology platforms – genetic, gene expression, even proteomic – depending on the biology associated with a given patient segment. (Women under 60, for example, are usually at low risk for coronary disease, primarily because estrogen is cardioprotective, and therefore have a different biological profile than men.)

CO-MARKETING LEVERAGE FOR PHARMA?

The promise of a range of new genetic, genomic, even proteomic tests in a relatively short time has piqued the interest of pharma. Not for sale as companion diagnostics, but as an opportunity for co-marketing.

The reasoning is that they could establish themselves as a higher-value representative by bringing in and being knowledgeable about a wider variety of products: there’s sufficient novelty and interest in genetics and genomics associated with coronary disease that they could start a dialog with a physician about Corus CAD and at some point change to their drug-oriented message. For example, a statin maker could segue from a discussion of a rule-out test to the medical treatments for patients found to be low-risk. CardioDx, for its part, would use that lead and close that sale. That was one reason why it was important to devise a test that was easy to use – to allow a marketing team to target GPs.

That rationale of using Corus CAD as a lead-in is only one potential example of how pharma could bolster its physician communications. Indeed, the *raison d’être* of new molecular tests is to significantly add to the information available to physicians to enable or change critical clinical decision-making. Especially at a time when the pace of new drug introductions is slowing and the opportunities to meet face-to-face with physicians therefore diminishing, as molecular diagnostics moves into new and broad markets like cardiology and metabolic disease, pharma could use this opportunity to its advantage in many settings.

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COMMENTS: Email the author: M.Ratner@Elsevier.com

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Use of the Corus® CAD Gene Expression Test for Assessment of Obstructive Coronary Artery Disease Likelihood in Symptomatic Non-Diabetic Patients

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Abstract

The determination of the underlying etiology of symptoms suggestive of obstructive coronary artery disease (CAD, $\geq 50\%$ stenosis in a major coronary artery) is a common clinical challenge in both primary care and cardiology clinics. Usual care in low to medium risk patients often involves a family history, risk factor assessment, and then stress testing with or without non-invasive imaging. If positive, this is often followed by invasive coronary angiography (ICA). Despite extensive adoption of this usual care paradigm, more than 60% of patients referred for angiography do not have obstructive CAD. In order to robustly identify those symptomatic patients without obstructive CAD, who can avoid subsequent cardiac testing and look elsewhere for the cause of their symptoms, a recently described whole blood gene expression score (GES: Corus® CAD, CardioDx, Inc., Palo Alto, CA) has been developed and validated in two multi-center trials. This paper reviews the published literature and assessments by independent parties regarding the analytical and clinical validity as well as the clinical utility of the Corus® CAD test.

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Clinical Scenarios

Coronary artery disease (CAD) and its clinical sequelae, including myocardial infarction and heart failure, are the leading causes of morbidity and mortality in the developed and developing world. Symptoms consistent with CAD are common, variable and quite diverse with significant gender-specific differences and overlap with other common conditions. Symptomatic patients are often first seen by primary care physicians who determine if a referral to a cardiology service is warranted, prior to investigation of other causes for the symptoms. In

cardiology practices, current practice guidelines suggest non-invasive imaging for medium risk patients and invasive coronary angiography for high risk patients¹. Despite the utilization of non-invasive imaging in most patients prior to ICA, the yield for obstructive CAD was <40% in a recent large national study². In addition, the prevalence of positive myocardial perfusion imaging studies, has decreased significantly over the last two decades, from 40% to 10% in a recent report³. Ruling out obstructive CAD in symptomatic women is particularly problematic as currently used diagnostic tests, such as EKG, myocardial perfusion imaging (MPI), and echocardiography, perform less well in women than in men⁴. The Agency for Healthcare Research and Quality highlighted the need for a better diagnostic test for women in a recent report by stating that physicians evaluating women at low to intermediate risk of CAD may want to rule-out disease with a non-invasive test with a high negative predictive value⁵. In addition, despite the common use of stress testing prior to coronary angiography, practice utilization varies significantly across different regions⁶.

Test Description

When a clinician suspects obstructive CAD as a cause of patient symptoms, a standard venous blood draw is performed into a PAXgeneTM RNA preservation tube (Pre-analytix, Valencia, CA). The sample and accompanying test requisition form are sent under temperature controlled conditions to the CLIA and College of American Pathologist's certified CardioDx laboratory. The sample is then accessioned, and RNA purification, cDNA synthesis, and quantitative real-time polymerase chain reaction (qRT-PCR) are performed. Test results are calculated based on the age and sex of the patient and the expression levels of the 23 genes in the Corus CAD algorithm and reported on a 1-40 scale⁷. The test algorithm differs between men and women based on age-dependent risk, specific genes, and relative gene weighting⁷. Increasing score is associated with increasing likelihood of obstructive CAD and increasing disease burden. A pre-specified threshold of ≤ 15 has been prospectively evaluated as a low risk boundary. Approximately 95% of samples received result in a valid test result and of more than 40,000 patients evaluated since launch, 47% have scores below the pre-specified threshold of ≤ 15 .

Public Health Importance/Prevalence

The evaluation of undiagnosed stable but symptomatic chest pain is associated with as many as 2% of all office visits or 2-3 million visits to primary care outpatient clinics each year in the United States⁸⁻¹⁰. Patients with symptoms suggestive of CAD undergo extensive non-invasive and invasive testing to exclude the presence of CAD as the cause and, in the process are exposed to risks of iatrogenic side effects such as those from ionizing radiation and contrast dye. The annual U.S. cost of non-invasive imaging tests used in the cardiac work-up of stable symptomatic patients is approximately \$7 billion¹¹⁻¹². Despite the significant resources expended only 10% of patients presenting to primary care with chest pain are ultimately diagnosed with stable obstructive CAD⁸.

Recommendations by independent groups. As part of the Clinical Laboratory Improvement Act (CLIA) licensure process, the analytical and clinical validation data for the Corus CAD test were independently evaluated by reviewers from the California and New York Departments of Public Health. In both cases these reviews resulted in positive recommendations and the CardioDx laboratory is licensed in all 50 states. In addition, as of April, 2013, the CardioDx laboratory is now accredited by the College of American Pathologists (CAP).

Guidelines. A recent policy statement from the American College of Cardiology and American Heart Association discussed the role of genetics and cardiovascular disease treatment and diagnosis but did not address gene expression as is measured in the Corus CAD Test¹³. An earlier scientific statement suggested:

"Gene expression profiling has potential application in clinical practice once specific molecular and clinically meaningful CVD signatures are developed" ¹⁴.

Recent Independent Review Articles. A number of recent independent review articles have described the scientific work underpinning the Corus CAD test ^{15,16}. A very recent review article in Nature Reviews Cardiology was solely focused on Corus CAD ¹⁷.

Evidence Overview

Analytical Validity

A large study utilizing more than 800 whole blood control samples was performed to assess the intra and inter-batch variability and inherent reproducibility of the Corus CAD test in the CardioDx commercial laboratory as a function of time, reagent batches, operators, and equipment ¹⁸. A total of 11 variables were assessed for their contribution to inter-batch variability, including four individual steps in the process (RNA purification, cDNA synthesis, sample addition, and qRT-PCR) across multiple operator, equipment, and reagent lots. Intra-batch variability estimated from 132 samples was 0.092 Cp units, dominated by inherent PCR stochastic variance, and represented approximately 70% of overall variance. Inter-batch variability was estimated across 895 samples over a two-year time frame; the largest sources of variances were reagent lots, and the overall variance was 0.11 Cp units. A comparison of overall process variability to biological dynamic range across 21,000 clinical samples showed that the biological variability was more than 10 times the process variability, demonstrating that the signal to noise was excellent. Overall process variance standard deviation corresponded to 1 unit on the Corus CAD 1-40 scale, corresponding to a clinically insignificant 1.7% change in obstructive CAD likelihood.

Clinical Validity

Two prospective multi-center trials evaluated the performance of the Corus CAD test across populations of different disease prevalence. The PREDICT trial evaluated test performance in a patient population (N=526) referred for invasive coronary angiography, the gold standard for obstructive disease evaluation ¹⁹. Disease prevalence was 37%, as measured by core laboratory quantitative coronary angiography, and very similar to that observed in a very large registry study ². A gender specific analysis of the PREDICT results showed the obstructive CAD prevalence in women was only 22%, indicating a need for better non-invasive diagnostic tools specifically in women ²⁰.

The COMPASS study evaluated test performance in symptomatic patients (N=431) referred for myocardial perfusion imaging, a procedure used prior to angiography ²¹. The gold standard was a combination of either invasive angiography and CT-angiography, both determined in core laboratories, so that all patients, independent of their MPI results, had gold standard data on their coronary anatomy. Obstructive CAD prevalence was only 15%, lower than seen in PREDICT. Positive MPI scans were seen in 11% of patients, very similar to that seen in a recent study reporting MPI positivity over the last two decades of 10% ³. Results of the two Corus CAD validation studies representing 58 centers in the US were very consistent.

- The primary endpoint of the area under the receiver-operating characteristics curve (AUC for ROC) analysis for discriminating patients with and without obstructive CAD (50% stenosis by quantitative angiography or core-lab CT-angiography) yielded AUCs of 0.70 and 0.79, for PREDICT and COMPASS, respectively ($p < 0.001$ in both cases).

- In both studies Corus CAD demonstrated excellent sensitivity (85 and 89%) and moderate specificity (43 and 52%), respectively, at a threshold of ≤ 15 which was derived from the PREDICT study and pre-specified for the COMPASS study

- Corus CAD showed high negative predictive values of 83 and 96%, respectively, in the PREDICT and COMPASS

studies, consistent with the differences in obstructive CAD prevalence.

- In the subset of PREDICT patients who had MPI and in the entire COMPASS study Corus CAD showed superior diagnostic performance to MPI driven by much greater sensitivity and diagnostic accuracy (ROC curve AUC). In the COMPASS study the AUCs were 0.79, 0.59, and 0.63 for Corus CAD, site-read, and core-lab read MPI, respectively.

- In both studies increasing Corus CAD score was significantly associated with increasing maximum percent stenosis.

- In both studies clinical follow-up for subsequent revascularization and major adverse cardiovascular events was performed. In PREDICT this was for 1 year post-index catheterization and showed a very significant association of Corus CAD score and the composite revascularization and event endpoint²². In the COMPASS trial 6 month follow-up also showed significantly fewer revascularization and events with low (≤ 15) Corus CAD scores²¹.

Clinical Utility

Three studies of the clinical utility of Corus CAD have been reported: a multi-center retrospective chart review in primary care, a prospective single center study of change in behavior in cardiology, and a prospective multi-center change in behavior study in primary care.

Retrospective Chart Review in Primary Care

To document the impact of Corus CAD in real-world primary care practice, a retrospective chart review study was completed in four primary care practices currently using Corus CAD, located in Arizona, Georgia, Louisiana, and North Carolina²³. A total of 317 patients who presented to four primary care physician sites with signs and symptoms suggestive of obstructive CAD and underwent Corus CAD testing from January 2011 to September 2011 were determined to be evaluable by medical records review and were included in this retrospective study. The objective of this study was to determine if there was a relationship between Corus CAD score and referral decision to the cardiologist: specifically, if patients with low Corus CAD scores were less likely to be referred to a cardiologist than patients with non-low scores.

In this study, 41% (129/317) of the Corus CAD patients had low scores (≤ 15), a rate consistent with the broader commercial population receiving the test and the COMPASS clinical trial population. Based upon physician self-reported referral rates, the expected referral rate to cardiology was 56.5%. The data show that the average referral rate to a cardiologist following Corus CAD testing was reduced to 30% ($p < 0.001$). In addition, the referral rate was just 9% (12/129) in the Corus CAD low scoring patient population. In multivariate analysis, after controlling for age, gender, type of symptoms, and practice site, patients with low Corus CAD scores had a relative reduction in referral likelihood of 73% ($p = 0.01$).

IMPACT-Cardiology

The IMPACT-Cardiology (Investigation of a Molecular Personalized Coronary Gene Expression Test on Cardiology Practice Pattern)(IMPACT-CARD) trial sought to assess the impact of Corus CAD use on clinical decision-making during the assessment of stable chest pain patients in the cardiology setting. The study included a prospective cohort of 83 patients eligible for analysis. These patients were referred to six cardiologists for evaluation of suspected CAD in the Vanderbilt University health care system and were matched by clinical factors to 83 patients in a historical cohort²⁴. The IMPACT-Cardiology protocol was designed to evaluate and compare the cardiologists' diagnostic strategies before and after receiving the Corus CAD results for their patients. Clinicians performed a pre-Corus CAD assessment of patients' CAD probability and noted their preliminary management decision (no intervention/medical management, referral for non-invasive imaging, or referral for invasive angiography). This pre-Corus CAD assessment was compared to physicians' assessment of CAD probability after

seeing the Corus CAD result (post-Corus CAD assessment) and determining a final management decision.

In this study following communication of Corus CAD results, a change in diagnostic testing (e.g. myocardial perfusion imaging, CTA and cardiac catheterization) was noted in 48 patients [58%, 95% CI (46%, 69%)]. More patients had a decreased versus increased level of testing (n=32 (39%) vs n=16 (19%), p=0.03). In particular, 91% (29 of 32) of patients with decreased testing had low Corus CAD (≤ 15), while 100% (16 of 16) of patients with increased testing had elevated Corus CAD (p<0.001). The most common change was among patients considered for referral to non-invasive imaging or invasive angiography prior to the Corus CAD test who were then referred to either no intervention or medical management after receiving a low Corus CAD score. Furthermore, none of the patients with low scores (≤ 15) saw an increase in testing.

The IMPACT-CARD trial demonstrated that among patients with a low Corus CAD score, the management decisions of cardiologists change, leading to a decrease in non-invasive cardiac imaging and invasive angiography.

IMPACT-PCP

The IMPACT-PCP (Investigation of a Molecular Personalized Coronary Gene Expression Test on Primary Care Practice Pattern) trial assessed the impact of Corus CAD use on clinical decision-making around the assessment of patients with symptoms of obstructive CAD in the primary care setting. The study included a prospective cohort of 251 patients, eligible for analysis, assessed by 8 community based practitioners at four sites. Clinicians performed a pre-Corus CAD assessment of patients' CAD probability and noted their preliminary management decisions (no intervention/medical management, referral for non-invasive imaging, or referral for invasive angiography). This pre-Corus CAD assessment was compared to the clinician's assessment of CAD probability after seeing the Corus CAD results (post-Corus CAD assessment) and determining a final management decision.

In this study, a change in diagnostic testing (e.g. myocardial perfusion imaging, CTA and cardiac catheterization) was noted in 145 patients following Corus CAD testing (58% observed vs 10% expected change, p<0.001). More patients had decreased (n=93, 37%) versus increased (n=52, 21%) intensity of testing (p<0.001). In particular, among the 127 low score Corus CAD patients (51% of study patients), 60% (76/127) had decreased testing, and only 2% (3/127) had increased testing. After more than 30 days of follow-up of 247 (98%) patients, there has been one MACE event (hemorrhagic stroke in a low score Corus CAD patient) reported.

In summary, Corus CAD was associated with a statistically significant and clinically relevant change in clinical decision-making among patients evaluated for suspected symptomatic CAD. In addition, the utilization of Corus CAD showed clinical utility above and beyond conventional decision-making by optimizing the patient's diagnostic evaluation, particularly around the reduction in the intensity of diagnostic testing among low Corus CAD patients.

Systematic Evidence Reviews

Palmetto Government Benefits Administrators (Palmetto, GBA), the CMS Medicare Administrative Contractor with oversight for Corus CAD, has published its assessment of the test. This review determined that the test meets standards for analytical and clinical validity, and clinical utility and is a reasonable and necessary Medicare benefit, effective January 1, 2012 ²⁵.

Overall Analysis of Evidence. The Evaluation of Genomic Applications in Practice and Prevention (EGAPP) working group has published a framework for evaluation of evidence of genomic testing, comprising analytical and clinical validity and clinical utility ²⁶. For Corus CAD with respect to analytic validity, an extensive study demonstrating very good score reproducibility and the ability to result >95% of samples, suggests a Level 1 evidence category in our judgement, for the performance of the test in the CardioDx clinical laboratory. For

clinical validity it is our assessment that the two prospective multi-center trials with core laboratory definition of disease status, representing almost 1000 patients, also correspond to Level 1 evidence according to EGAPP criteria ²⁶. The clinical utility data from the IMPACT and retrospective chart review studies are of relatively small size and limited follow-up suggesting a level 2-3 evidence determination.

Limitations

Although the results of the evaluation of the Corus CAD test are very promising, its results should be interpreted carefully as patients with diabetes mellitus and chronic inflammatory or autoimmune disorders were excluded from test development and validation. Furthermore, this test was derived and tested in predominantly Caucasian patient populations. Given the known variations in the prevalence of CAD in different ethnic/racial backgrounds ²⁷, results of this test in non-Caucasian populations should be interpreted with caution.

Conclusions

The Corus CAD test has been extensively evaluated since it was first derived, including with two prospective multi-center trials. Given the scope of the deleterious effects of CAD and the considerable costs involved in diagnosing obstructive CAD, a blood test that can help in this determination is certainly valuable. The Corus CAD test promises to have an important role in this regard particularly if it continues to perform this well in larger, more diverse cohorts.

Acknowledgements

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RESEARCH ARTICLE

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Development of a blood-based gene expression algorithm for assessment of obstructive coronary artery disease in non-diabetic patients

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Abstract

Background: Alterations in gene expression in peripheral blood cells have been shown to be sensitive to the presence and extent of coronary artery disease (CAD). A non-invasive blood test that could reliably assess obstructive CAD likelihood would have diagnostic utility.

Results: Microarray analysis of RNA samples from a 195 patient Duke CATHGEN registry case:control cohort yielded 2,438 genes with significant CAD association ($p < 0.05$), and identified the clinical/demographic factors with the largest effects on gene expression as age, sex, and diabetic status. RT-PCR analysis of 88 CAD classifier genes confirmed that diabetic status was the largest clinical factor affecting CAD associated gene expression changes. A second microarray cohort analysis limited to non-diabetics from the multi-center PREDICT study (198 patients; 99 case: control pairs matched for age and sex) evaluated gene expression, clinical, and cell population predictors of CAD and yielded 5,935 CAD genes ($p < 0.05$) with an intersection of 655 genes with the CATHGEN results. Biological pathway (gene ontology and literature) and statistical analyses (hierarchical clustering and logistic regression) were used in combination to select 113 genes for RT-PCR analysis including CAD classifiers, cell-type specific markers, and normalization genes. RT-PCR analysis of these 113 genes in a PREDICT cohort of 640 non-diabetic subject samples was used for algorithm development. Gene expression correlations identified clusters of CAD classifier genes which were reduced to meta-genes using LASSO. The final classifier for assessment of obstructive CAD was derived by Ridge Regression and contained sex-specific age functions and 6 meta-gene terms, comprising 23 genes. This algorithm showed a cross-validated estimated AUC = 0.77 (95% CI 0.73-0.81) in ROC analysis.

Conclusions: We have developed a whole blood classifier based on gene expression, age and sex for the assessment of obstructive CAD in non-diabetic patients from a combination of microarray and RT-PCR data derived from studies of patients clinically indicated for invasive angiography.

Clinical trial registration information: PREDICT, Personalized Risk Evaluation and Diagnosis in the Coronary Tree, <http://www.clinicaltrials.gov>, NCT00500617

Background

The promise of genomics to improve diagnosis and prognosis of significant diseases is dependent on a number of factors including appropriate use of technology, identification of clinical issues of significant unmet need, and the rigorous statistical derivation and testing of

genomic classifiers [1]. Multigene expression classifiers have been developed and have become incorporated into clinical guidelines in both breast cancer recurrence prognosis and heart transplant rejection monitoring [2,3]. A guideline for the metrics such classifiers should meet, including independent validation, and adding to current clinical factor algorithms has been described [4] and it has been suggested that peripheral blood cell gene expression may reflect pathological conditions in a

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variety of cardiovascular disease states [5]. In this work we describe the development of a validated whole blood based classifier for the assessment of obstructive CAD [6].

Mortality and morbidity from CAD and myocardial infarction (MI) are a major global health burden. Major determinants of current CAD likelihood are sex, age, and chest-pain type [7,8]. Other risk factors such as diabetes, smoking, dyslipidemia, hypertension and family history have been associated with future cardiovascular event risk [9]. In addition, atherosclerosis has a systemic inflammatory component including activation and migration of immune cells into the vessel wall [10,11]. Prior work has shown that quantitative measurements of circulating blood cell gene expression reflect the extent of CAD [12,13]. These observations likely reflect both changes in cell type distributions, which have prognostic value for cardiovascular events [14] and gene expression changes within a specific cell type or lineage.

The "gold standard" for detecting CAD is invasive coronary angiography; however, this is costly, and can pose risk to the patient. Prior to angiography, non-invasive diagnostic modalities such as myocardial perfusion imaging (MPI) and CT-angiography may be used, however these only add moderately to obstructive CAD identification [15]. We describe herein the development of an algorithm for the assessment of obstructive CAD using peripheral blood gene expression, age, and sex, which was subsequently validated in an independent cohort [6].

Methods

Patient selection and clinical methods

All patients were clinically referred for angiography and angiograms were performed based on local, institutional protocols. The first microarray cohort of 198 subjects (88 cases and 110 controls) was derived from the Duke University CATHGEN registry, a retrospective blood repository, enrolled between August 2004 and November, 2005 [16]. For CATHGEN patients, clinical angiographic interpretation defined cases as $\geq 75\%$ maximum stenosis in one major vessel or $\geq 50\%$ in two vessels and controls as $< 25\%$ stenosis in all major vessels. Clinical inclusion and exclusion criteria were described previously and included both diabetic and non-diabetic patients [13]. All CATHGEN patients gave written informed consent and the study protocol was approved by the Duke University IRB.

The second microarray cohort of 210 subjects (105 case: control pairs, matched for age and sex) and the RT-PCR algorithm development cohort (210 cases and 430 controls) were part of PREDICT, a multi-center US study of patients referred for coronary angiography (<http://www.clinicaltrials.gov>, NCT00500617). For PREDICT

patients, core laboratory QCA reads (Cardiovascular Research Foundation New York) were used for case: control classification. Cases had $\geq 50\%$ stenosis in at least one major coronary vessel and controls $< 50\%$ stenosis in all major vessels.

Subjects from PREDICT were eligible if they had a history of chest pain, suspected anginal-equivalent symptoms, or a high risk of CAD with no known prior MI, revascularization, or CAD. Detailed inclusion/exclusion criteria have been described [6]. Diabetic status was defined by clinical identification, blood glucose (non-fasting ≥ 200 or fasting ≥ 126), or hemoglobin A1c, (≥ 6.5), or diabetic medication prescription. Complete blood counts with differentials were obtained for all patients. PREDICT patients gave written informed consent, and the study protocol was approved by the Institutional Review Boards.

Blood collection, RNA purification and RT-PCR

Whole blood samples were collected in PAXgene[®] tubes prior to coronary angiography, according to the manufacturer's instructions, then frozen at -20°C . For the CATHGEN samples RNA was purified as described (PreAnalytix, Franklin Lakes, NJ), followed by quantitative analysis (Ribogreen, Molecular Probes, Eugene, OR). For the PREDICT samples an automated method using the Agencourt RNAdvance system was employed.

Correlation between gene expression and cell type distributions

Correlations with complete blood counts and database gene expression analysis (SymAtlas, <http://biogps.gnf.org>) were used to identify highly cell-type selective genes. In addition, whole blood cell fractionation by density centrifugation or through positive antibody selection followed by RT-PCR was performed on specific cell fractions (see Additional file 1).

Statistical methods

All statistical methods were performed using the R software package.

Microarray methods

Microarray samples were labeled and hybridized to 41K Human Whole Genome Arrays (Agilent, PN #G4112A) using the manufacturer's protocol. For PREDICT microarrays all matched pairs were labeled and hybridized together to minimize microarray batch effects. Microarray data sets have been deposited in GEO (GSE 20686).

Normalization

Agilent processed signal values for array normalization were scaled to a trimmed mean of 100 and then \log_2 transformed. Standard array QC metrics (percent

present, pair-wise correlation, and signal intensity) were used for quality assessment, resulting in 3 of 198 CATHGEN and 12 of 210 PREDICT samples being excluded.

Array analysis

For the CATHGEN array, logistic regression (unadjusted and sex/age adjusted) was used to assess gene expression association with case: control status. For the PREDICT array, given the paired design, conditional logistic regression was used. False discovery rates were used to account for multiple comparisons. BINGO was used to assess enrichment of gene ontology terms in the set of 655 genes [17]. A hyper-geometric test was used to identify overrepresented terms; results were corrected for multiple testing using Benjamini & Hochberg False Discovery Rate (FDR) correction.

Gene selection

Genes for RT-PCR were selected based on statistical significance, gene ontology pathway analysis, and literature support. Hierarchical clustering based on gene: gene correlations ensured that RT-PCR genes represented multiple clusters. Normalization genes were selected based on low variance, moderate to high expression, and no significant association with case: control status, sex, age, or cell counts. Cell-type genes were selected based on known literature or correlation to known cell-type specific markers.

PCR methods

Amplicon design, cDNA synthesis, and RT-PCR were performed as previously described [6,13]. All PCR reactions were run in triplicate and median values used for analysis. Clinical/demographic factors were assessed for CAD association using univariate and multivariate logistic regression. Gene expression association with CAD and other clinical/demographic factors was assessed by robust logistic regression (unadjusted and sex/age adjusted) [13].

Algorithm development and validation

Hierarchical clustering was used to group genes using a correlation cutoff. Clusters were reduced to meta-genes [18] and normalization genes based on correlation structure, known biology, and cell count correlation. In general, a meta-gene was a set of 1-4 genes from a specific cluster, chosen to best represent the cluster center using a parsimonious number of genes. Genes within meta-genes were equally weighted with one exception (Additional File 1). For meta-gene pairs with high correlation and opposite disease regulation, ratio terms (differences on the log scale) were defined. Meta-genes independently associated with outcome were selected by the LASSO method, with sex by meta-gene interactions allowed during variable selection [19].

The final algorithm was fit using Ridge regression [20], where the outcome variable was case: control status and the predictors the LASSO-selected meta-genes and sex-specific age terms. Sex was a binary predictor, and age a linear predictor with separate slopes for men, women >60, and women <60 (the slope for women age < 60 was estimated to be approximately 0 and thus was set to 0 in the final algorithm). The LASSO was fit using the glmnet package in R and ridge regression was fit using the Design package in R; in both cases the shrinkage parameter lambda was estimated using 10-fold cross validation. Model performance was estimated using leave-one-out cross-validation.

Results

A schematic of the patient, gene, and logic flow for gene discovery and algorithm development is shown in Figure 1. Baseline demographic characteristics of the CATHGEN registry and PREDICT study microarray patient cohorts are summarized in Table 1. Significant clinical and demographic factors for obstructive CAD were age, male sex, systolic blood pressure, and dyslipidemia; increased neutrophil count and decreased lymphocyte count trended toward significance. In all cases whole blood samples were obtained in PAXgene® tubes and microarray analysis performed using the Agilent 41K platform.

A total of 2,438 genes showed significant CAD association ($p < 0.05$) in the 195 subject case: control analysis from the CATHGEN cohort (Figure 1). Analysis of the effect of clinical factors on gene expression showed diabetes as the most significant ($p = 0.0006$, Additional file 2). Based on statistical significance and biological relevance, 88 genes (Additional file 2) were selected for RT-PCR analysis on these same samples. CAD-gene expression analysis in non-diabetic and diabetic subsets ($N = 124$ and 71 , respectively), showed 42 and 12 significant genes, respectively ($p < 0.05$), with no intersection (Figure 2). Further work was thus limited to non-diabetics. Microarray CAD gene discovery on 210 PREDICT non-diabetic patient samples used a paired case: control experimental design, to reduce confounding effects of age, sex and microarray batch processing. CAD analysis on the 99 case: control pairs which passed quality metrics yielded 5,935 significant genes ($p < 0.05$) with 655 genes in common with the CATHGEN results (Figure 3, Additional File 2). Gene Ontology (GO) analysis of these 655 genes identified 55 significant, overrepresented biological process terms (adjusted $p < 0.05$, Figure 4, Additional File 2), largely reflecting inflammation, immune cell differentiation, cell death and apoptosis. The molecular and cellular ontologies showed enrichment of 3 and 10 terms respectively, including caspase activity and ribosomal function.

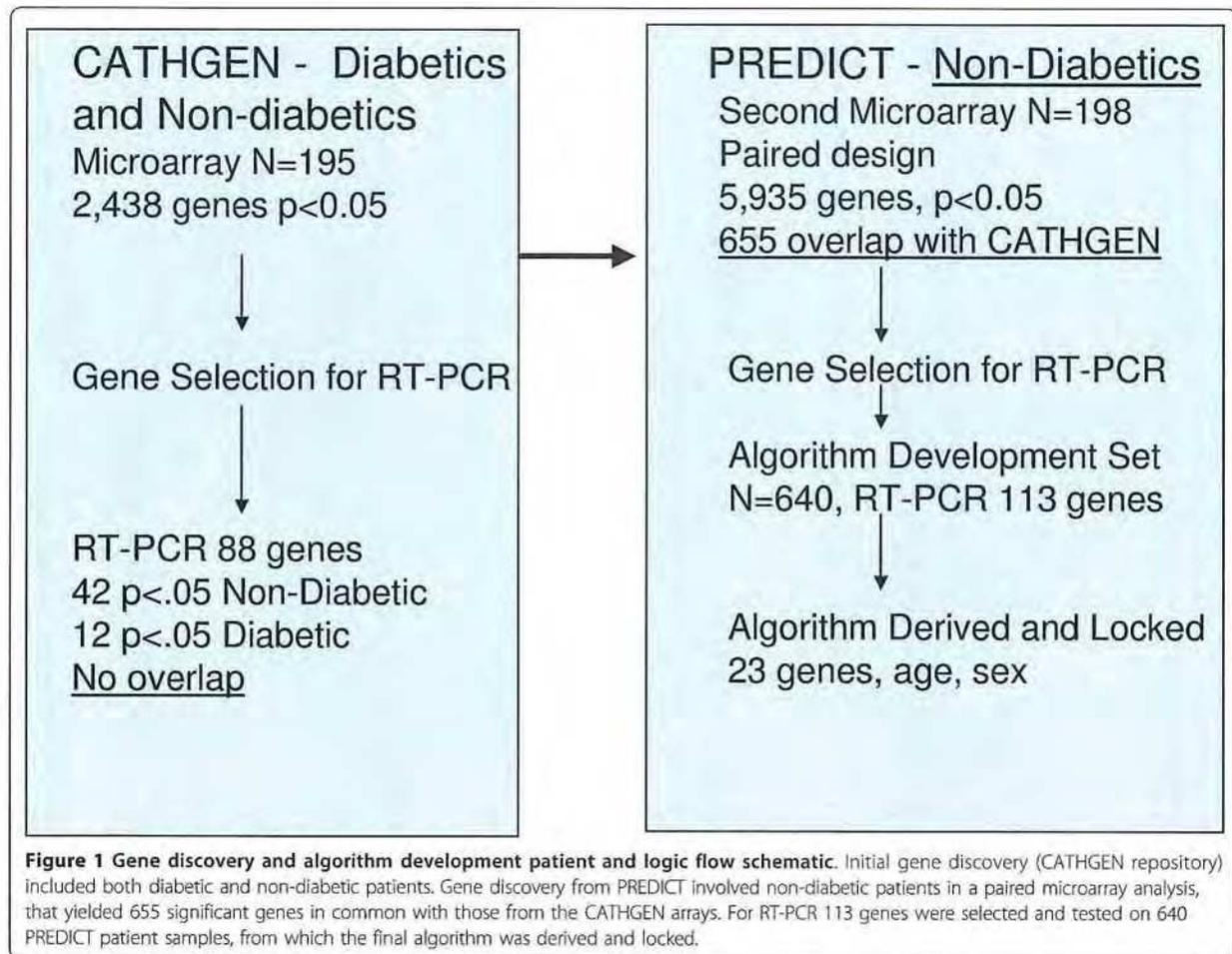
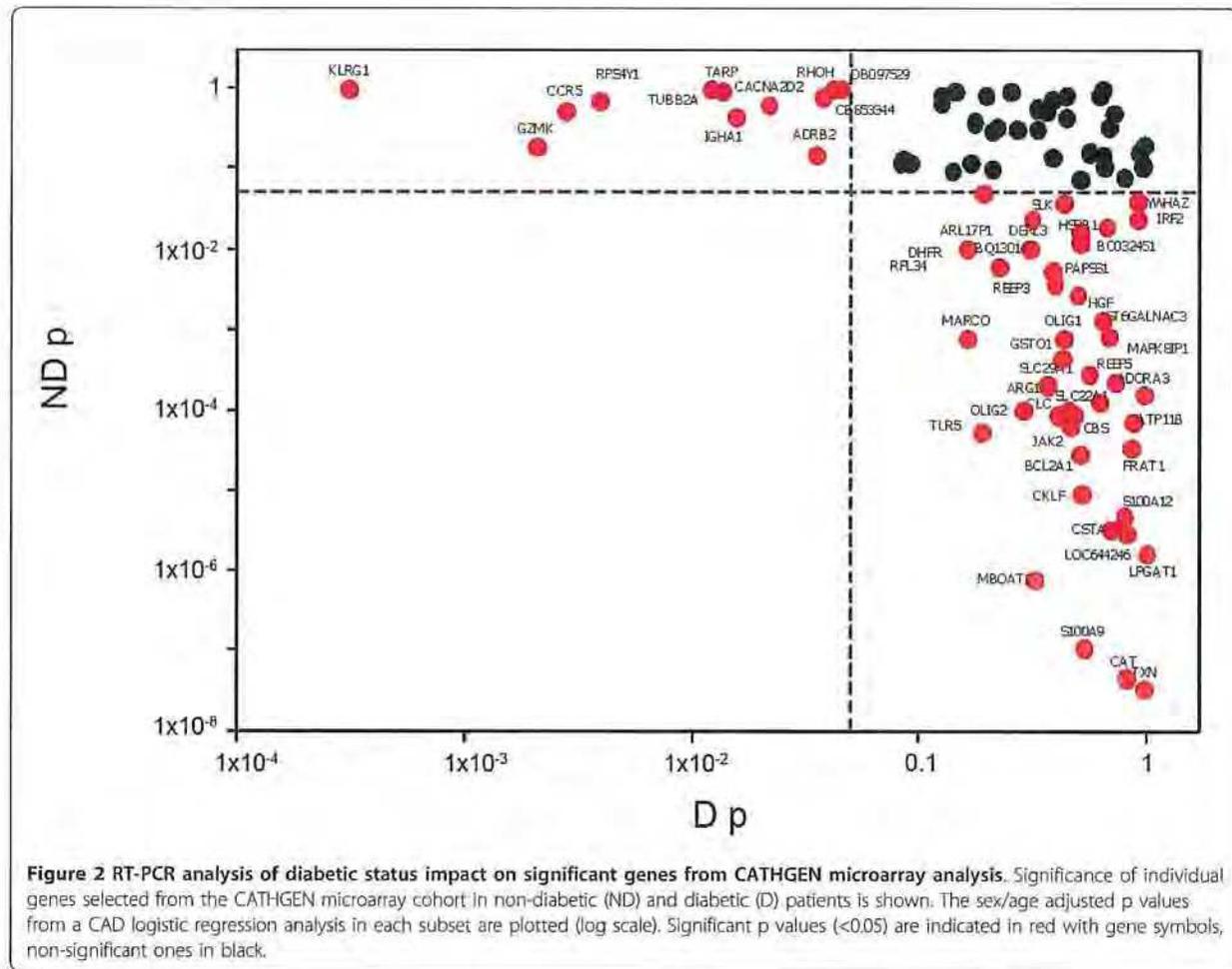


Table 1 CATHGEN and PREDICT microarray cohort clinical and demographic characteristics

Variable	CATHGEN Microarray Cohort			PREDICT Paired Microarray Cohort		
	Controls (N = 108)	Cases (N = 87)	p.value	Controls (N = 99)	Cases (N = 99)	p.value
Sex (%Male)	55 (50.9%)	58 (66.7%)	0.039	75(75.8%)	75 (75.8%)	0.868
Age (yrs)	55 ± 11	63 ± 10	<.001	55 ± 12	62 ± 11	<.001
Caucasian	56 (51.9%)	60 (69%)	0.023	85(85.9%)	92 (92.9%)	0.166
BMI	32 ± 7	30 ± 6	0.098	30 ± 7	30 ± 6	0.722
Current Smoker	41 (38%)	45 (51.7%)	0.075	14(14.1%)	25 (25.3%)	0.074
Systolic BP	144 ± 22	153 ± 25	0.007	132 ± 17	138 ± 18	0.009
Diastolic BP	83 ± 13	87 ± 15	0.077	82 ± 11	80 ± 12	0.271
Hypertension	67 (62%)	65 (74.7%)	0.084	55(55.6%)	65 (65.7%)	0.191
Dyslipidemia	55 (50.9%)	58 (66.7%)	0.039	50(50.5%)	69 (69.7%)	0.009
Neutrophil Count	3.8 ± 1.2	4 ± 1.3	0.392	3.9 ± 1.2	4.3 ± 1.5	0.037
Lymphocyte Count	1.8 ± 0.7	1.9 ± 0.7	0.87	2 ± 0.7	1.9 ± 0.6	0.239

¹Microarray cohort analyses are restricted to those whose arrays passed QC analysis (195/198 for CATHGEN and 198/210 for the PREDICT samples).



Gene selection

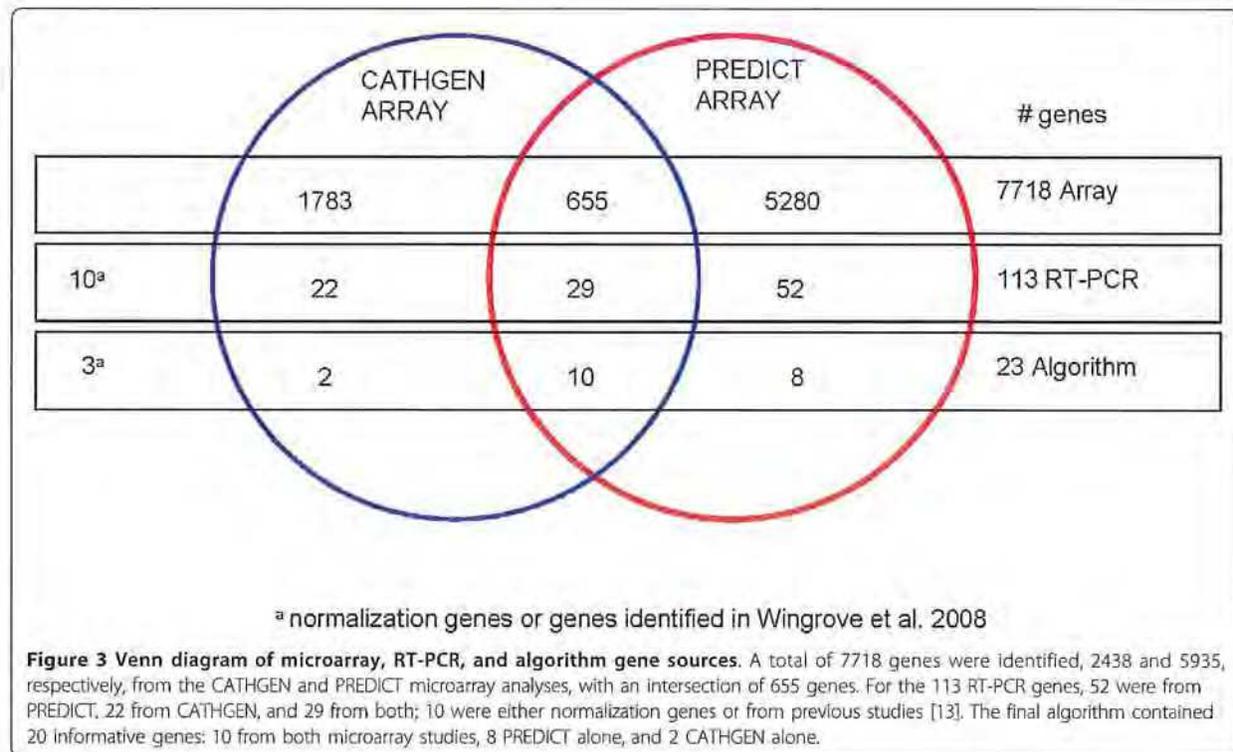
A total of 113 genes (Table 2) were selected by statistical significance, biological relevance, and prior association with CAD from RT-PCR gene expression measurements in the 640 patient PREDICT algorithm development cohort (Figure 1, Table 3). Known cell-type specific markers, those correlated with cell counts in PREDICT, and candidate normalization genes, were also represented.

Analysis of algorithm development cohort: clinical and gene expression factors

The most significant clinical/demographic factors for CAD association were age, sex, chest pain type and neutrophil count. Age and sex were independent risk factors for CAD (Table 3) and showed significant gene expression correlation. Chest pain type was also a significant independent risk factor ($p = 0.0004$), especially in men, but was gene expression independent. Neutrophil count was significantly correlated (positively or negatively) to expression of 93 of 113 RT-PCR genes, and was significantly associated with CAD in men ($p = 0.049$) but not women ($p = 0.77$).

Neutrophil-associated genes showed both up and down regulation with CAD status, whereas lymphocyte-associated genes were generally down-regulated. There was significant gender-specific regulation of neutrophil correlated genes (men 40/42 genes up-regulated, women, 41/42 down-regulated) whereas lymphocyte gene down-regulation was gender independent.

Hierarchical clustering of the 113 PCR genes resulted in 18 correlated clusters (Figure 5, Table 2), a significant fraction of which could be mapped to cell-type specific gene expression groups, with finer correlation substructure within the lymphocyte and neutrophil associated genes. There were 3 lymphocyte subgroups representing T-cells (clusters 1,2,3), B-cells (cluster 3) and NK cells (cluster 12). Three neutrophil subgroups were also identified: previously described neutrophil genes (IL8RB, S100A8, S100A12, TXN, BCL2A1; cluster 13, 16); newly identified up-regulated neutrophil genes (CLEC4E, CASP5, TNFAIP6; cluster 16) and down-regulated neutrophil genes (KCNE3, TLR4, TNFRSF10C; clusters 13, 14) [13]. Cluster 8 appears to be eosinophil specific.



The 26 genes in clusters 4-7 and 9-11 did not have clear cell-type association.

Algorithm derivation and performance

Based on gene expression correlation clustering and cell-type analyses, 15 meta-genes and 3 normalization genes were defined as inputs for model variable selection (Table 2, Figure 6). Selection by the LASSO method and further weight penalization by Ridge regression resulted in the final, locked algorithm, comprising 20 CAD-associated genes and 3 normalization genes in 6 meta-gene terms (Figure 6), where each term represents a ratio of meta-genes or meta-gene to normalization genes. The algorithm score is calculated as described (Additional file 1) and was defined as the predicted regression model value.

The estimated cross-validated algorithm AUC in ROC analysis in the PREDICT development set was 0.77 (95% CI 0.73 to 0.81) (Figure 7); prospective validation in an independent PREDICT validation set of 526 patients (192 cases, 334 controls) yielded an AUC of 0.70 (95% CI = 0.65 to 0.75) [6].

Discussion

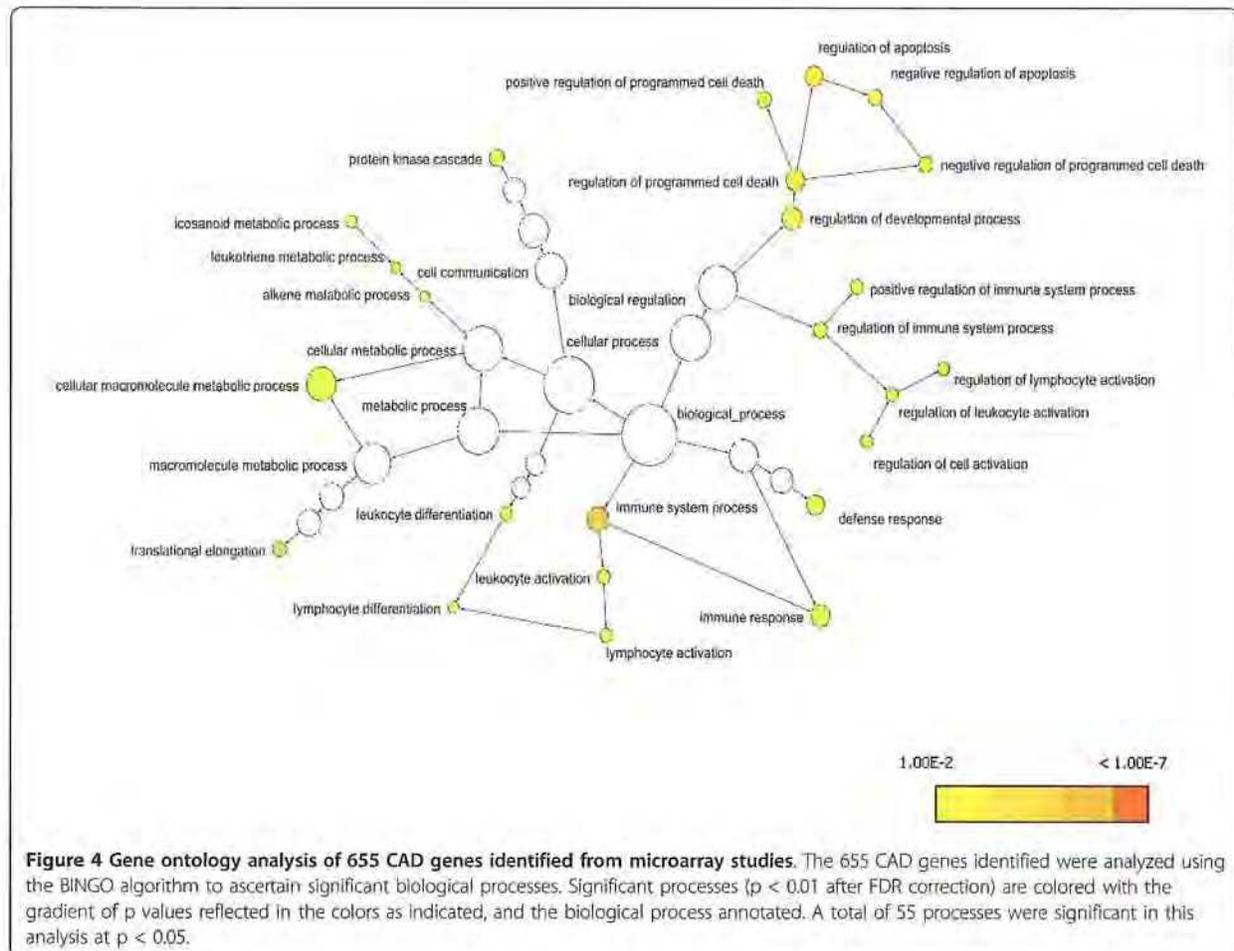
This study presents gene discovery from microarrays and development from a large RT-PCR data set of a whole blood derived RT-PCR based gene-expression algorithm for assessment of obstructive CAD likelihood

in non-diabetic patients, which was subsequently validated in an independent patient set [6].

The limitation to non-diabetic patients was due to the significant differences observed in PCR-based technical replication of the initial microarray experiment from the CATHGEN cohort, where both diabetic and non-diabetic patients were included (Figure 2). This effect could be due to differences in the pathophysiology of CAD in diabetics, as has been observed at the plaque composition level, [21] or due to diabetic medications, some of which modulate gene expression and affect cardiovascular disease [22].

A number of methodological steps deserve highlighting: first, we interrogated whole blood samples from more than 1,000 patients; second, we developed and used an automated and high reproducible RNA extraction process for the PREDICT samples; third, for the PREDICT work we also used core laboratory determined quantitative coronary angiography to define maximum percent stenosis and case: control status and fourth, we used ratios of correlated gene sets or meta-genes as building blocks for algorithm development. These methodological approaches enhanced the power of the PCR algorithm development set to investigate the relationship between CAD, clinical factors, and gene expression.

The relationships between age, sex, CAD, and gene expression are complex. Increasing age and male sex are well-known risk factors for CAD, which affect gene expression in circulating cells [23,24]. The majority of



genes measured by RT-PCR in this study correlated with lymphocyte or neutrophil fraction. Lymphocyte-associated gene expression decreases with CAD in a sex-independent fashion, consistent with decreased lymphocyte counts being correlated with increased cardiovascular risk [14]. In contrast, neutrophil-associated genes display significant sex-specific expression differences with CAD: in men 95% of the neutrophil genes were up-regulated whereas 98% were down-regulated in women, consistent with increased granulocyte counts in men being associated with higher CAD risk, with lesser effects in women [25,26].

Biological significance of algorithm terms

The use of correlated meta-genes as building blocks for the algorithm is significantly reflective of gene expression cell-type specificity. The algorithm genes are expressed selectively in multiple types of circulating cells including neutrophils, NK cells, B and T-lymphocytes, [27], supporting roles for both adaptive and innate immune responses in atherosclerosis [10].

A role for neutrophils in both the early and later stages of atherogenesis has recently been suggested, especially in connection with hyperlipidemia [28,29]. Algorithm term 1 is a ratio of neutrophil expressed meta-genes that are up and down regulated with CAD (Figure 6). This term may particularly reflect neutrophil apoptosis, as Caspase-5 is increased with CAD, whereas TNFRSF10C, an anti-apoptotic decoy receptor of TRAIL, is decreased [30]. Term 2 genes up-regulated with CAD are also expressed largely by neutrophils and likely reflect both innate immune activation, (S100A8 and S100A12), [31] and a cellular necrosis response (CLEC4E) [32]. S100A8 and S100A12 are up-regulated in chronic inflammatory conditions, including asthma, rheumatoid, and inflammatory arthritis, perhaps reflecting a more general pathophysiological signal, consistent with increased CAD in disorders such as rheumatoid arthritis [33,34].

Term 2 is highly correlated with the signature previously identified by us [13] and includes the most significant gene from that work, S100A12. This term is normalized in a sex-specific manner, perhaps reflecting

Table 2 Genes evaluated by RT-PCR in the algorithm development cohort

Gene Symbol	MicroArray Evidence ¹	Cell-Type ²	Cluster	Metagene Term	Algorithm Term ³
DDX18	3		1.1		
SSRP1	3		1.2		
CCT2	3	2	1.3		
RPL28	N	2	1.4	Norm	2b
XIST	2	1,4,5	1.5		
RASSF7	3		1.6		
PKD1	3		1.7		
AGPAT5	3	2,7	1.8		
GL5	3		1.9		
TMC8	3		1.10	1	3b,4b
RPS4Y1	2	3	1.11		
KLF12	3	4	1.12		
LCK	2,3	3,4,8	1.13		
CD3D	2,3	3,4,8	1.14	1	3b,4b
AES	3		1.15		
ZAP70	3	3,4,8	1.16		
CD81	3	7,8	1.17		
QDPR	3	2,5	1.18		
FXN	2	2	1.19		
CORO2A	3		1.20		
TCEA1	3	7	1.21		
KMO	3	5,7	2.1		
TLR7	3	5	2.2		
RHOC	3		2.3		
CX3CR1	3	6,8	2.4		
IL11RA	1,2	3,4	3.1		
IL7R	1,2,3	3,4,8	3.2	3	
FAIM3	2,3	3,4,7	3.3		
TCF7	2,3	3,4,8	3.4	3	
CD79B	2,3	7	3.5	2	4a
SPIB	2,3	2,5,7	3.6	2	4a
CD19	3	5,7	3.7		
BLK	3	5,7	3.8		
PI16	2		3.9		
LRRN3	3	3,4	3.10	4	
HNRNPF	N		4.1	Norm	5b,6b
TFCP2	N		4.2	Norm	5b,6b
ACBD5	3		4.3		
DIAPH1	3		4.4		
CD37	3	7	4.5		
PLAGL2	3	1	4.6		
SRA1	3		5.1		
CD300A	2	8	5.2		
ELMO2	3	5,8	5.3		
CD33	2	1,6	6.1		
CSPG2	1,2		6.2		
CAT	2	2,5	6.3		
NOD2	1,3	1,6	6.4		
KCNMB1	2		6.5	5	
TCF7L2	3	1,6,8	6.6	5	
PDK4	3		6.7	5	

Table 2 Genes evaluated by RT-PCR in the algorithm development cohort (Continued)

TBC1D8	3	1,5,6	6.8		
NR4A1	3	5	7.1		
CDKN1C	3	6,8	7.2		
C2	2		7.3		
CLC	2	1,2	8.1	6	
OLIG2	2		8.2		
ADORA3	2		8.3	6	
MMD	1,2,3	7	9.1		
HIST1H2AE	1,3	4,7	9.2	7	
AMFR	2		10.1		
CD34	N	2	10.2		
A_24_P128361 (AF289562)	3		11.1	8	5a
CD248	2,3	4	11.2		
KLRC4	2	4,8	12.1	9	3a
TARP	2,3	4,8	12.2		
CCRS	2	4,5	12.3		
CD8A	1	3,4,8	12.4		
SLAMF7	2	5,8	12.5	9	3a
KLRC2	2	3,4,8	12.6		
PRSS23	2	8	12.7		
NCAM1	N	8	12.8		
TNFRSF10C	3		13.1	11	1b
IL8RB	1,3	1,6,8	13.2	11	1b
TLR4	3	1,6	13.3	11	1b
NAMPT	3	1,5,6	13.4		
AQP9	3	1,6	13.5	10	2c
S100A8	1,2,3	1,5,6	13.6	12	2a
NCF4	2,3	1,6	13.7	10	2c
GLT1D1	1,2,3		13.8		
TXN	2,3	2,5	13.9		
GABARAPL1	3		13.10		
SIRPB2	1,3		13.11		
TRPM6	3		13.12		
CD93	1,2,3	1,5,6	13.13		
ASPRV1	3		13.14		
ALOX5AP	2,3	5	13.15		
BCL2A1	1,2,3	1,6,8	13.16		
F11R	3		14.1		
PTAFR	3	1,6	14.2		
H3F3B	3	7	14.3		
TYROBP	2,3	1,6,8	14.4		
NCF2	3	1,5,6	14.5		
KCNE3	2,3	1,6	14.6	11	1b
LAMP2	2,3	1	14.7		
PLAUR	3	1,6	14.8		
CD14	1	1,5,6	14.9		
HK3	1,2	1,6,8	14.10		
IL18	1		14.11		
RGS18	1,2	1,6	15.1		
BMX	2,3		16.1		
MMP9	2,3		16.2		
S100A12	1,2,3	1,5,6	16.3	12	2a

Table 2 Genes evaluated by RT-PCR in the algorithm development cohort (Continued)

CLEC4E	2,3		16.4	12	2a
CLEC4D	2,3	1,6	16.5		
CASP5	2,3		16.6	13	1a
TNFAIP6	2,3	1	16.7	13	1a
IL18RAP	1,3	3,4,8	16.8	13	1a
ARG1	2,3		17.1	14	
HP	1	1,2	17.2		
CBS	2,3		17.3	14	
AF161365	3		17.4	15	6a
ALAS2	CN		18.1		

¹Microarray Evidence: 1 = Wingrove et al, 2 = CATHGEN, 3 = PREDICT, CN = cell-type specific normalization gene, N = global normalization gene

²Cell Type: 1 = CD33+ (myeloid), 2 = CD34+(hematopoietic precursor), 3 = CD4+, (T cell) 4 = CD8+ (T cell), 5 = Dendritic, 6 = CD14+ (monocyte), 7 = CD19+ (B cell), 8 = CD56+ (natural killer cell).

³For algorithm term identification, genes in the numerators are listed as Na whereas those in the denominators are Nb.

sex-specific differences in the significance of neutrophil counts in CAD and MI [26]. In men normalization to RPL28 which is strongly expressed in lymphocytes, reflects the neutrophil to lymphocyte ratio, which is prognostic for death or MI in a CAD population [14]. In women normalization to AQP9 and NCF4, two CAD insensitive neutrophil genes, permits assessment of neutrophil up-regulation of the S100s and CLEC4E, independent of neutrophil count.

Term 3 consists of 2 NK cell receptors, SLAMF7 and KLRC4, normalized to T-cell specific genes (TMC8 and CD3D). SLAMF7 may specifically activate NK cell function, while inhibiting B and T cells [35]. KLRC4 is also likely involved in NK cell activation [36]. NK cells have been associated with atherosclerosis in both mouse

models and humans, and reduced lymphocyte counts associated with cardiac events [14,37].

Term 4 is a gene expression based measure of the B/T-cell ratio. The roles of both T and B cells in atherosclerosis development are complex; mouse models have shown B cells to be both athero-protective and more recently, atherogenic [38-40]. In this study apparent upregulation of B-cell specific genes is correlated with CAD, perhaps supporting the latter. The last two terms, based on AF289562 (AF2) and TSPAN16 are genes of unknown function that will require further work to clarify their role in atherosclerosis.

Previous work by Sinnaeve and coworkers also examined peripheral blood gene expression in a coronary disease population [12]. As noted by these authors, there is little overlap between their genes and the signatures identified in our previous study [13] or this one. A number of significant differences in the study populations (restricted age range, combining two sex specific cohorts) in their study may have contributed to this. In addition, there are differences in both RNA isolation methodology and microarray platforms. Further work is needed to resolve these issues.

Table 3 PREDICT algorithm development cohort clinical and demographic characteristics¹

Variable	Controls (N = 410)	Cases (N = 230)	p.value
Sex (%Male)	193 (47.1%)	180 (78.3%)	<.001
Age (yrs)	57 ± 12	64 ± 11	<.001
Caucasian	347 (84.6%)	210 (91.3%)	0.022
BMI	31 ± 8	30 ± 6	0.348
Current Smoker	87 (21.2%)	45 (19.6%)	0.693
Systolic BP	133 ± 18	138 ± 18	<.001
Diastolic BP	80 ± 12	80 ± 11	0.944
Hypertension	248 (60.5%)	167 (72.6%)	0.003
Dyslipidemia	225 (54.9%)	170 (73.9%)	<.001
Neutrophil Count	4 ± 1.2	4.3 ± 1.4	0.054
Lymphocyte Count	2 ± 0.6	1.9 ± 0.6	0.007
Chest Pain Category			.0004
Asymptomatic	141 (35.4%)	90 (39.6%)	
Atypical	56 (14.0%)	29 (12.8%)	
Non-Anginal	137 (34.3%)	47 (20.7%)	
Typical	65 (16.3%)	61 (26.9%)	

¹Clinical and demographic characteristics for the 640 PREDICT algorithm development cohort are shown.

Algorithm development

For algorithm development, as described above, we used an approach that minimized the effect of any single gene by using meta-genes as building blocks [18,41] Penalized stepwise logistic regression (LASSO) selected significant meta-genes from a 640 patient data set which greatly exceeded the number of candidate variables (15 meta-genes), reducing the likelihood of over-fitting. Further, in order to minimize over-weighting of individual terms, meta-gene coefficients were penalized using Ridge regression. An alternative approach would have been to use a combined two-step shrinkage method such as the elastic net [42]. Although correlations with

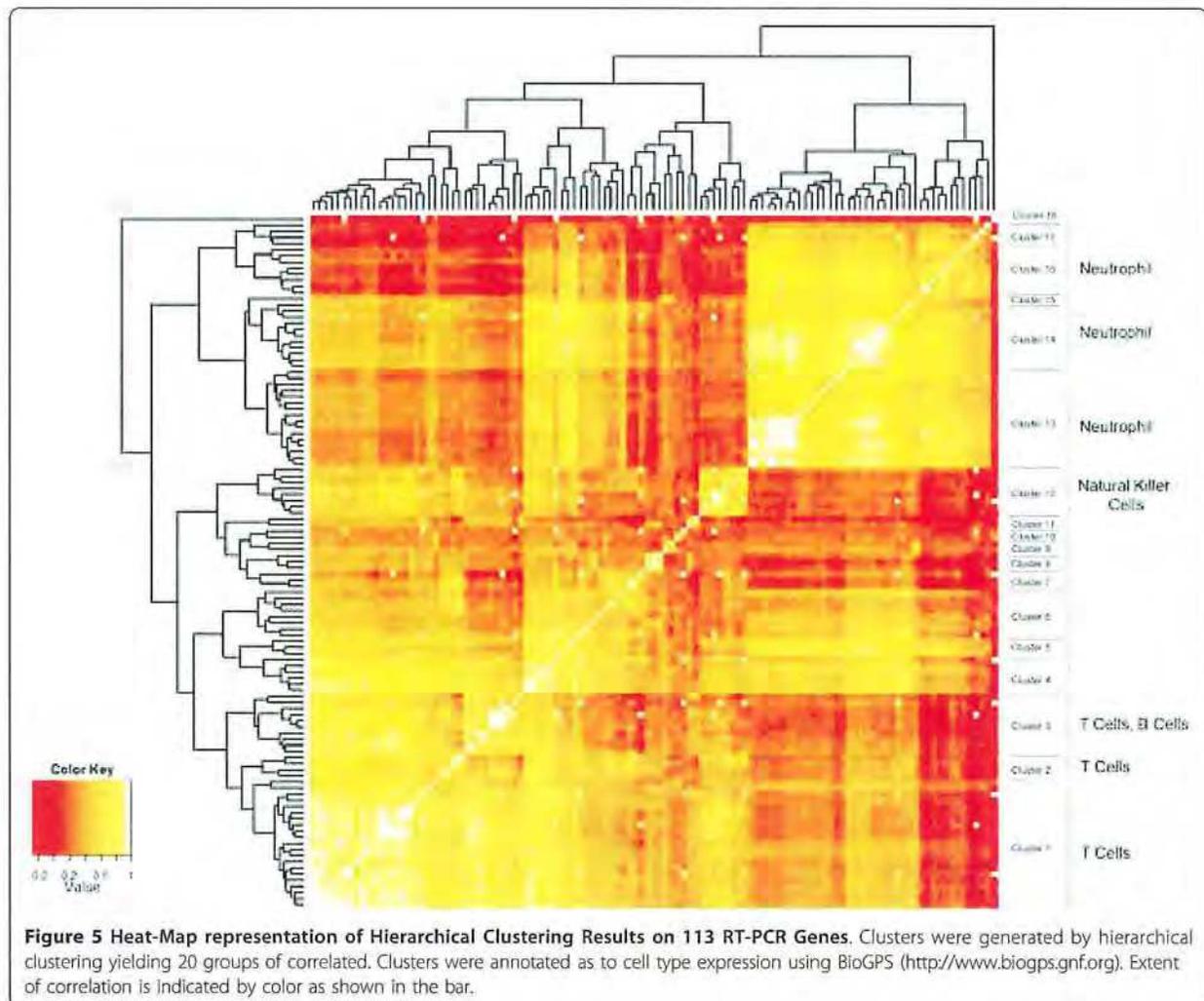


Figure 5 Heat-Map representation of Hierarchical Clustering Results on 113 RT-PCR Genes. Clusters were generated by hierarchical clustering yielding 20 groups of correlated. Clusters were annotated as to cell type expression using BioGPS (<http://www.biogps.gnf.org>). Extent of correlation is indicated by color as shown in the bar.

specific cell types was a key observation, recent methodologies described for deconvoluting gene expression data sets from complex cell mixtures might have led to improved results [43].

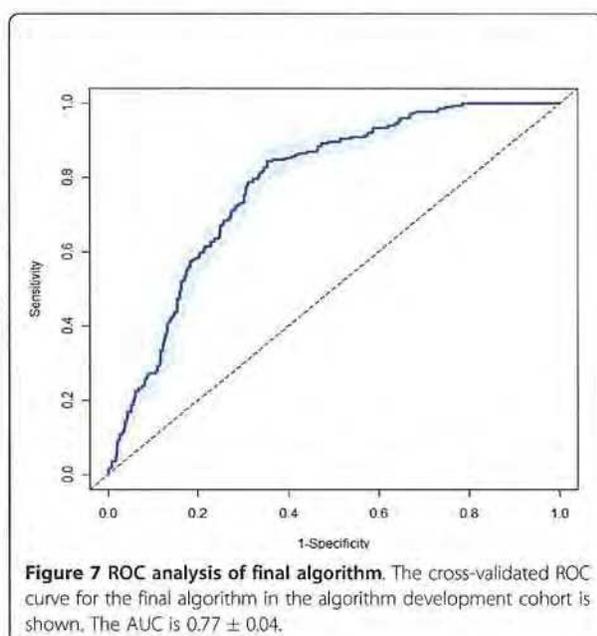
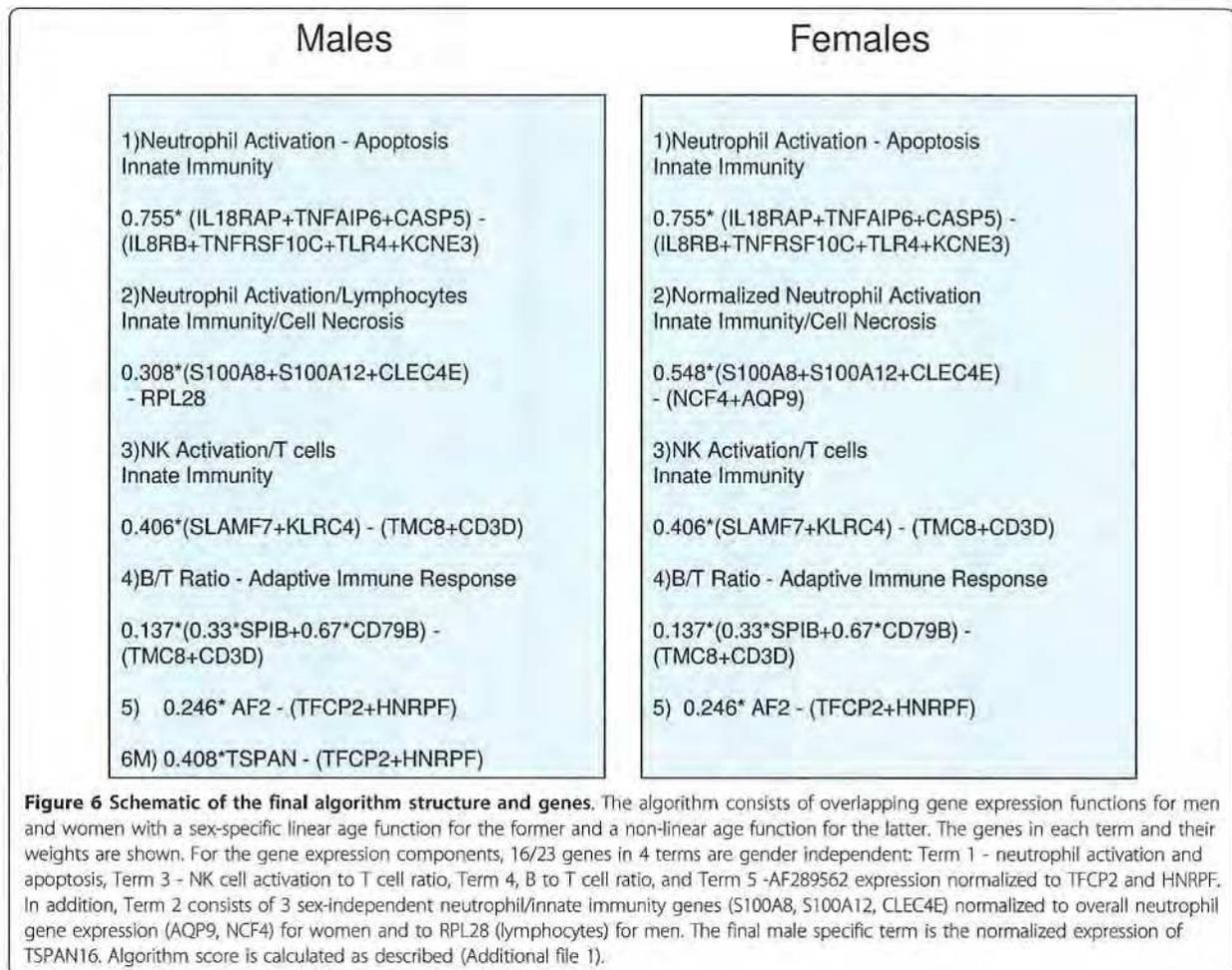
The cross-validated model AUC was 0.77 (95% CI 0.73 to 0.81), suggesting that the algorithm score was a significant CAD predictor. A decrease to an AUC of 0.70, with overlapping confidence intervals (95% CI = 0.65 to 0.75), was observed in the independent validation set [6]. This decrease may reflect an over-optimistic cross-validation estimate, as we did not re-select terms during each iteration. Ultimately, the validation results provide the most informative measure of a model's prediction accuracy.

Limitations

Although this is one of the largest studies examining gene expression in peripheral blood in CAD patients and has yielded a specific algorithm for the assessment of CAD status, it has several limitations.

From a clinical perspective, diabetics and patients with known chronic inflammatory disorders were excluded. The differences observed between diabetics and non-diabetics with CAD could be due to differences in the molecular pathophysiology of the disease, medications, or some combination of the two. In addition, although race was not an independent risk factor after adjustment for age and sex, the number of minority patients was low, so conclusions with respect to them are significantly underpowered. The use of a dichotomous angiographic endpoint does not account for variations in disease burden or external remodeling, and is not a measure of ischemia. Finally, the contribution of atherosclerosis in other vascular beds is outside the scope of this study, but may be important in asymptomatic high-risk individuals.

From a cellular and gene expression perspective, the relative ease of obtaining peripheral blood cell RNA is counterbalanced by not directly interrogating changes in the



diseased vascular wall. Another complementary approach could be to examine secreted proteins in the blood that might reflect endothelial or vascular dysfunction. Finally, given the chronic nature of atherosclerotic disease, it is likely the gene expression signature observed reflects a response to disease rather than the underlying cause.

Conclusions

Using a series of microarray and RT-PCR data sets, comprising more than 1,000 patients, we have derived an algorithm, consisting of the expression levels of 23 genes, sex, and age, which can assess the likelihood of obstructive CAD in non-diabetic patients.

Additional material

Additional file 1: Algorithm Score Calculation and Transformation. Cell fractionation and cell specific gene expression analysis.

Additional file 2: Data Tables. Table S1 - Significance of Clinical Variables in CATHGEN gene discovery cohort. Table S2 - Significance of RT-PCR results for the 88 genes tested in the CATHGEN discovery cohort.

in the non-diabetic and diabetic subsets, Table S3 - The 655 genes identified in both the CATHGEN and PREDICT discovery microarray experiments. Table S4 - The significant biological process, cellular compartment and molecular function ontologies from GO analysis of the 655 genes.

Abbreviations

CAD: coronary artery disease; MI: myocardial infarction; MPI: myocardial perfusion imaging; RT-PCR: real-time polymerase chain reaction; QCA: quantitative coronary angiography; ROC: receiver-operator characteristics; AUC: area under the curve.

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MRE, PB, JAW, SED, WGT, SR, SZ, GG, AL, WEK, RSS, and EJT contributed to the Conception, Design, and Data Analysis for this work as well as drafting and approving the final manuscript. SE, NT, RT, and JM helped critically revise the manuscript and all authors approved the final version. The remaining PREDICT investigators are listed in the Acknowledgements.

Competing interests

This work was funded by CardioDx, Inc. MRE, JAW, PB, SED, and SR are employees of CardioDx, Inc and have equity interests and/or stock options in CardioDx. WGT is a former employee and has equity or stock options in CardioDx. SR, MRE, JAW, PB and WGT have filed patent applications on behalf of CardioDx, Inc. WEK reports research support from CardioDx. EJT is supported in part by the Scripps Translational Science Institute Clinical Translational Science Award (NIHU54RR02504-01). AJL reports funding from CardioDx to complete the QCA studies reported herein. RSS, SZ, RW, JM, and NT report no conflicts of interest with respect to the contents of this manuscript.

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Clinical Implications of Referral Bias in the Diagnostic Performance of Exercise Testing for Coronary Artery Disease

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Background—Exercise testing with echocardiography or myocardial perfusion imaging is widely used to risk-stratify patients with suspected coronary artery disease. However, reports of diagnostic performance rarely adjust for referral bias, and this practice may adversely influence patient care. Therefore, we evaluated the potential impact of referral bias on diagnostic effectiveness and clinical decision-making.

Methods and Results—Searching PubMed and EMBASE (1990–2012), 2 investigators independently evaluated eligibility and abstracted data on study characteristics and referral patterns. Diagnostic performance reported in 4 previously published meta-analyses of exercise echocardiography and myocardial perfusion imaging was adjusted using pooled referral rates and Bayesian methods. Twenty-one studies reported referral patterns in 49 006 patients (mean age 60.7 years, 39.6% women, and 0.8% prior history of myocardial infarction). Catheterization referral rates after normal and abnormal exercise tests were 4.0% (95% CI, 2.9% to 5.0%) and 42.5% (36.2% to 48.9%), respectively, with odds ratio for referral after an abnormal test of 14.6 (10.7 to 19.9). After adjustment for referral, exercise echocardiography sensitivity fell from 84% (80% to 89%) to 34% (27% to 41%), and specificity rose from 77% (69% to 86%) to 99% (99% to 100%). Similarly, exercise myocardial perfusion imaging sensitivity fell from 85% (81% to 88%) to 38% (31% to 44%), and specificity rose from 69% (61% to 78%) to 99% (99% to 100%). Summary receiver operating curve analysis demonstrated only modest changes in overall discriminatory power but adjusting for referral increased positive-predictive value and reduced negative-predictive value.

Conclusions—Exercise echocardiography and myocardial perfusion imaging are considerably less sensitive and more specific for coronary artery disease after adjustment for referral. Given these findings, future work should assess the comparative ability of these and other tests to rule-in versus rule-out coronary artery disease. (*J Am Heart Assoc.* 2013;2:e000505 doi: 10.1161/JAHA.113.000505)

Key Words: coronary artery disease • diagnostic performance • echocardiography • exercise testing • myocardial perfusion imaging

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An accompanying Appendix S1 is available at <http://jaha.ahajournals.org/content/2/6/e000505/suppl/DC1>

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Exercise testing with radionuclide imaging or echocardiography is widely used to evaluate patients with suspected cardiovascular disease, and >12 million studies are performed annually in the United States.^{1,2} The American College of Cardiology/American Heart Association practice guidelines for exercise testing recommend that physicians interpret stress test results in the context of a patient's pretest risk, using posttest disease probability as well as test performance characteristics to guide clinical decision-making.³ However, an important limitation of this approach is that reports of stress test diagnostic performance may be influenced by referral bias^{4–6}—sometimes called “verification bias” or “workup bias”—and studies do not routinely adjust for this phenomenon.^{7–10} Referral bias occurs when patients with an abnormal stress test result are referred to cardiac catheterization at a higher rate than are patients with normal stress test results. While clinically appropriate, failing to adjust for this difference in referral rates when measuring test performance can significantly distort the observed diagnostic characteristics of exercise testing. This is because patients

referred for cardiac catheterization, the gold standard, may have a higher likelihood of disease than those who are not.^{11,12} An exception is the work by Gibbons and colleagues, in which a posttest referral bias correction was applied, with adjustment of sensitivity and specificity.^{3,11}

The clinical implications of referral bias for patient management and decision making have received little prior attention but may be substantial. However, for reasons that are unclear, referral bias is almost universally unaccounted for in studies of exercise testing.^{7–10} We hypothesize that this may be because prior studies of referral bias enrolled populations from single centers,^{5,11–14} thus limiting their impact due to concerns about generalizability. To address this issue, we systematically reviewed the literature on referral rates after exercise echocardiogram (ECHO) or myocardial perfusion imaging (MPI) and used these rates to adjust measures of exercise test performance. We also examined the potential impact of referral bias on posttest disease risk and clinical decision making.

Methods

Search Strategy

We searched PubMed and EMBASE from January 1990 to November 2012 for English-language articles reporting cardiac catheterization referral rates after normal or abnormal exercise MPI and ECHO. Our search terms were developed with a clinical and graduate medical education librarian (D.V.) and included the Medical Subject Headings (MeSH) *coronary disease*, *exercise test*, *myocardial perfusion imaging*, *single photon emission computed tomography*, *echocardiography*, and *humans*; keywords identifying *exercise tests*, including *stress test*, *thallium*, *sestamibi*, and *technetium*; and keywords identifying referral, including *refer* and *referr** (for “referral” and variants), *verif** (for “verification” and variants), and *select*, *selected*, and *selecti** (for “selection” and variants). We also searched the reference list of meta-analyses of exercise test performance and identified additional publications through discussion between collaborators. Our report adheres to guidelines for systematic reviews published by the Meta-analysis of Observational Studies in Epidemiology (MOOSE) group (see Appendix S1 for details).

Study Selection

Two investigators (J.L. and S.B.), working independently, in duplicate, reviewed all abstracts and identified studies that indicated or suggested that the authors reported referral rates after exercise testing. Studies then underwent full text data extraction if (1) exercise ECHO or exercise MPI was performed to detect or evaluate coronary artery disease (CAD) and (2) referral rates to cardiac catheterization were reported and

stratified by stress test result (eg, normal, abnormal). Studies were excluded if they enrolled only patients with a history of myocardial infarction (MI), percutaneous transluminal coronary angioplasty (PTCA), or coronary artery bypass grafting (CABG); they enrolled patients with unstable coronary syndromes; or the majority of patients underwent pharmacological stress testing. Studies enrolling patients with a history of MI or revascularization were included if these patients comprised <15% of the study population. This threshold was considered reasonable because it was comparable to or lower than the prevalence of MI in our reference meta-analysis studies^{9,15} and another systematic review of stress MPI.⁷

Data Extraction

The same 2 investigators performed data extraction independently, in duplicate, using a standardized protocol and reporting form. Study characteristics recorded included (1) identifying information (first author, journal, country, institution, publication year), (2) patient characteristics (mean age, percentage of male patients, percentage of patients with previous MI, PTCA, or CABG), (3) stress test characteristics (test used, type of exercise, positivity criterion, how authors defined significant coronary artery disease, eg, $\geq 50\%$ stenosis or $\geq 70\%$ stenosis), (4) referral patterns (number of patients with normal or abnormal stress test results and number of patients subsequently undergoing cardiac catheterization), and (5) diagnostic yield (number of true positives, false positives, true negatives, and false negatives). Disagreements between reviewers during the abstract screening and data extraction process were resolved through discussion.

Data Analysis

Both the proportion of patients referred for cardiac catheterization and the odds ratio for cardiac catheterization referral after a normal or abnormal stress test result were derived for each study, and 95% CIs were calculated. These estimates were pooled using the Mantel–Haenszel fixed-effects model, weighted with inverse variance, and the DerSimonian–Laird random-effects model.¹⁶ We assessed the between-study heterogeneity using the Cochran Q statistic and study consistency using the I^2 statistic, which quantifies the proportion of heterogeneity that is not due to chance. If the P -value for the Q statistic was <0.10 or the I^2 statistic exceeded 50%, a random-effects model was reported instead of a fixed-effects model. Potential sources of heterogeneity also were explored. A 2-tailed P -value of <0.05 was judged as statistically significant. Because referral rates were generally not the primary outcome in any of the included studies, the possibility of publication bias was not explored. We used the METAN command of Stata (version 12, StataCorp) to perform all meta-analyses.

Adjusting Diagnostic Test Performance for Referral

As a reference standard for exercise test performance, we compiled studies of exercise ECHO and MPI from 4 sources: the most widely cited meta-analysis of stress test performance (Fleischmann et al),^{15,17} a recent meta-analysis (Heijnenbrok-Kal et al),⁹ and 2 peer-reviewed meta-analyses from the Ontario Health Technology Assessment Series.^{18,19} Henceforth, these 4 studies will be referred to collectively as our exercise test meta-analysis studies. They contributed a total of 45 unique studies of exercise ECHO (15 studies) or MPI performance (30 studies) (see Appendix S1).

We then used Bayesian methods developed by Begg and Greenes to adjust exercise test performance reported in each of these 45 studies for referral bias.⁴ Their method assumes that referral to the gold standard test (cardiac catheterization) and disease status (the presence or absence of CAD) are conditionally independent given the exercise test result. This assumption is generally considered reasonable since the decision to perform cardiac catheterization can only be influenced by “visible” factors, such as the exercise test result and other clinical characteristics.²⁰ Diagnostic test performance with and without adjustment for the referral process can then be derived with the equation:

$$\frac{\Pr(T|D)}{\Pr(\bar{T}|D)} = \frac{\Pr(T|D, R+)\Pr(R + |\bar{T})}{\Pr(\bar{T}|D, R+)\Pr(R + |T)}$$

where R is referral, which is synonymous here with cardiac catheterization; T is exercise test result or its complement (\bar{T}), equivalent to a normal or abnormal result; D is disease status; $\Pr(T|D)$ is the sensitivity (when T is abnormal and D is disease presence) of the exercise test, *accounting* for the referral process; and $\Pr(T|D, R+)$ is the sensitivity of the exercise test, as determined in the cohort undergoing cardiac catheterization (*not accounting* for the referral process). An analogous equation can be derived for specificity by reversing disease status and test result.

Estimates of exercise test sensitivity and specificity in cohorts undergoing cardiac catheterization were derived directly from our 45 exercise test meta-analysis studies, and we adjusted these values with pooled estimates of

referral rates from our literature search. To ensure we applied referral corrections appropriately, we rated each relevant study in our exercise test meta-analyses by its likelihood of referral bias. Fleischmann et al¹⁵ also categorized studies by their likelihood of referral bias, and we limited our analyses to studies for which referral bias was rated as “likely” or “certain.” We then used a random-effects model to reestimate diagnostic performance for exercise ECHO and MPI after correction for referral bias. After performing a logit transformation, we used a Taylor series expansion and the delta method to construct 95% CIs.²¹ The overall methodological approach is summarized in Figure 1.

Summary Performance of Each Test

To evaluate the impact of referral bias on the overall diagnostic performance of exercise MPI and ECHO, we performed summary receiver operating characteristic (ROC) curve analysis.²² ROC curves illustrate the trade-off between sensitivity and specificity as the threshold for defining a diagnostic test result as abnormal is varied, and they adjust for the possibility that different studies may use different test thresholds.²³ To perform our analysis, we logistically transformed the true-positive rate (TPR; sensitivity) and false-positive rate (FPR, 1–specificity) and fit a linear regression model, with the log-odds ratio (OR; log-odds TPR–log-odds FPR) as the dependent variable and the test threshold (log-odds TPR+log-odds FPR) as the independent variable.²² The regression model included an indicator variable for referral bias correction, and its β -coefficient gives a measure of the difference in diagnostic performance after adjusting for referral. Positive coefficients indicate improved discriminatory power and negative coefficients correspond to a reduction in discriminatory power. The model's dependent variable is invariant to the referral process.²⁰

Clinical Implications and Predictive Value of Diagnostic Testing

To estimate the impact of referral bias correction on posttest risk stratification and clinical decision-making, we calculated positive predictive value and negative predictive value over a

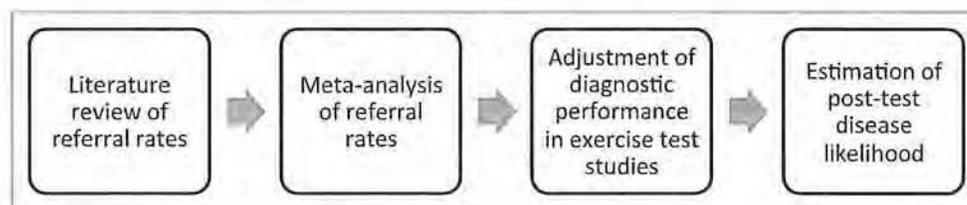


Figure 1. Flow diagram of selection process for studies included in meta-analysis.

range of pretest probabilities for CAD. We used the odds-likelihood ratio version of Bayes' theorem: posttest odds=pretest odds \times likelihood ratio; where the positive likelihood ratio (LR) is sensitivity/(1-specificity) and the negative LR is (1-sensitivity)/specificity. The delta method was used to estimate 95% CIs as previously described.

Overall Methodological Approach

In summary, we searched PubMed and EMBASE for articles reporting cardiac catheterization referral rates after normal or abnormal exercise MPI and ECHO. From studies that met inclusion criteria, we extracted data on referral rates to cardiac catheterization stratified by stress test result, along with other patient and study characteristics, and pooled these referral rates. We then identified 45 studies of exercise ECHO and MPI from previously published meta-analyses, and we used Bayesian methods to adjust exercise test performance reported in each of these 45 studies for referral bias using our pooled referral rates. We also performed summary ROC curve analysis to evaluate the impact of referral bias on the overall

diagnostic performance of exercise MPI and ECHO. Finally, to estimate the impact of referral bias correction on posttest risk stratification and clinical decision making, we calculated positive predictive value and negative predictive value over a range of pretest probabilities for CAD.

Results

Literature Search

Our literature search yielded a total of 819 citations, of which 107 were selected as being potentially relevant and obtained for further screening (Figure 2). Of these 107 studies, 17 reported referral patterns after normal or abnormal exercise tests and were included in our analysis.^{11,12,14,24-41} The characteristics of these studies and their 49 006 participants are shown in Table 1. The mean age was 60.7 years, 39.6% were women, 0.8% had a prior history of myocardial infarction (reported in 13 studies), and 0.1% had a prior history of revascularization (reported in 14 studies). In 13 studies, the form of exercise used was treadmill testing, and 3 studies

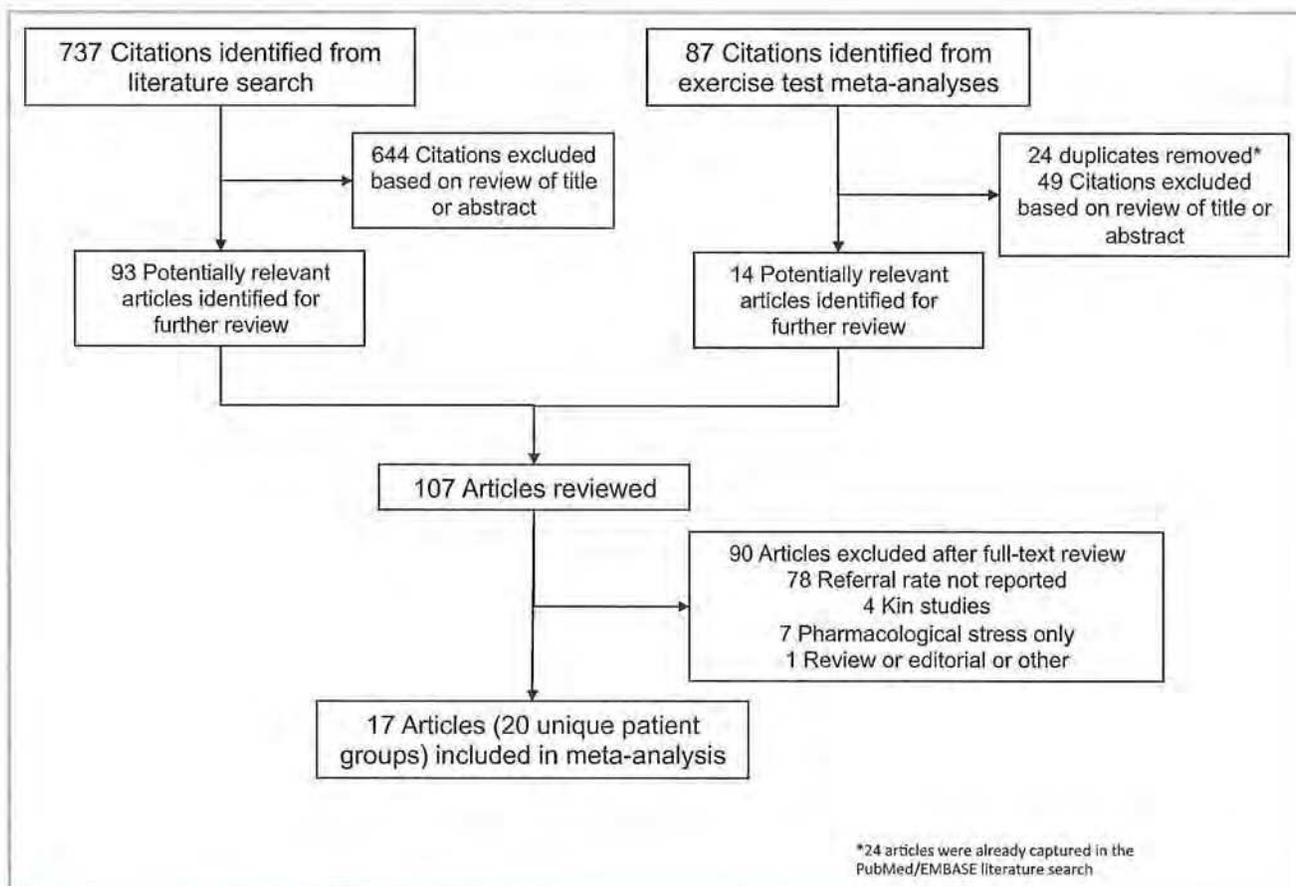


Figure 2. Overview of methodological approach.

Table 1. Characteristics of Studies Included in Meta-analysis of Referral Patterns After Normal and Abnormal Exercise Tests

Reference	Subgroup	No. of Patients	Mean Age, y	Men, %	Prior MI, %	Prior Revascularization, %*	Exercise Used	Time to Cath, d	Prevalence of CAD, % ^{†,‡}
ECHO									
Jang et al ³⁵		1287	53.2	58	0	NA	Treadmill	<30	61
Roger et al ¹²	Male only	1965	60	100	0	0	Treadmill	6	80
	Female only	1714	60	0	0	0	Treadmill	6	60
Vlachopoulos et al ³²		50	59	100	0	0	Treadmill	NA	NA
Wennike et al ³¹		200	62	46	NA	NA	Treadmill	NA	100
MPI									
Cecil et al ¹⁴		2688	NA	NA	0	0	Treadmill	<90	42
Charvat et al ³⁹		126	59.9	60	0	NA	Bicycle	NA	56
Diamond et al ³⁸		9171	NA	NA	NA	NA	Treadmill	<180	29
Hachamovitch et al ³⁷		1021	69	55	0	0	Treadmill	<60	NA
Hannoush et al ³⁶		334	56	80	14	NA	Treadmill	<90	80
Hosie et al ²⁴		80	50	55	NA	NA	Bicycle	NA	63
Kane et al ³⁴		6801	61	55.4	NA	0	Treadmill	<90	60
Koistinen et al ²⁵		136	47.6	62	0	0	Bicycle	NA	44
Lauer et al ²⁶	Male only	2351	58	100	7	0	Treadmill	<90	NA
	Female only	1318	59	0	3	0	Treadmill	<90	NA
Miller et al ¹¹		14 273	62	59	0	0	Treadmill	<90	72
Nallamothe et al ²⁷		2700	59	56	0	0	NA	<180	NA
Roeters van Lennep et al ³³	Male only	322	59.4	100	0	0	Treadmill	<90	86
	Female only	294	59.1	0	0	0	Treadmill	<90	65
Schwartz et al ²⁸		2175	NA	100	0	0	Treadmill	NA	18

CAD indicates coronary artery disease; cath, cardiac catheterization; ECHO, echocardiography; MI, myocardial infarction; MPI, myocardial perfusion imaging; NA, not available from article.

*Jang et al,³⁵ Charvat et al,³⁹ and Hannoush et al³⁶ reported prior coronary artery bypass graft surgery only and did not report prior percutaneous coronary intervention.

[†]Diamond et al³⁸ reported prevalence only in patients with normal exercise test result.

[‡]Wennike et al,³¹ Charvat et al,³⁹ Koistinen et al,²⁵ and Roeters van Lennep et al³³ reported prevalence only in patients with abnormal exercise test result.

used a bicycle; 1 study did not report the mode of exercise. Significant CAD was present in 18% to 100% of patients undergoing cardiac catheterization (reported in 13 studies). Overall, the studies were heterogeneous with respect to the population prevalence of prior coronary disease and the indications for testing and referral.

Cardiac Catheterization Referral Rates

Cardiac catheterization referral rates after normal and abnormal stress tests results from our literature review are shown in Figures 3 and 4. Because only a few studies reported referral patterns after exercise ECHO, and because we did not believe that referral patterns would differ in a clinically meaningful way between exercise ECHO and MPI, we combined all studies together. Using a random-effects model,

the pooled referral rate after a normal test result was 4.0% (95% CI, 2.9% to 5.0%), and that after an abnormal test result was 42.5% (95% CI, 36.2% to 48.9%). The pooled odds ratio for referral after an abnormal test, compared with a normal test, was 14.6 (95% CI, 10.7 to 19.9).

Diagnostic Effectiveness After Adjustment for Referral Bias

Using Bayesian methods, we adjusted the diagnostic performance reported in each of our 45 exercise test meta-analysis studies. The pooled sensitivity of exercise ECHO and MPI fell from 84% (95% CI, 80% to 89%) and 85% (95% CI, 81% to 88%) prior to adjusting for the referral process to 34% (95% CI, 27% to 41%) and 38% (95% CI, 31% to 44%) after adjustment, respectively (Table 2). The pooled specificity of exercise

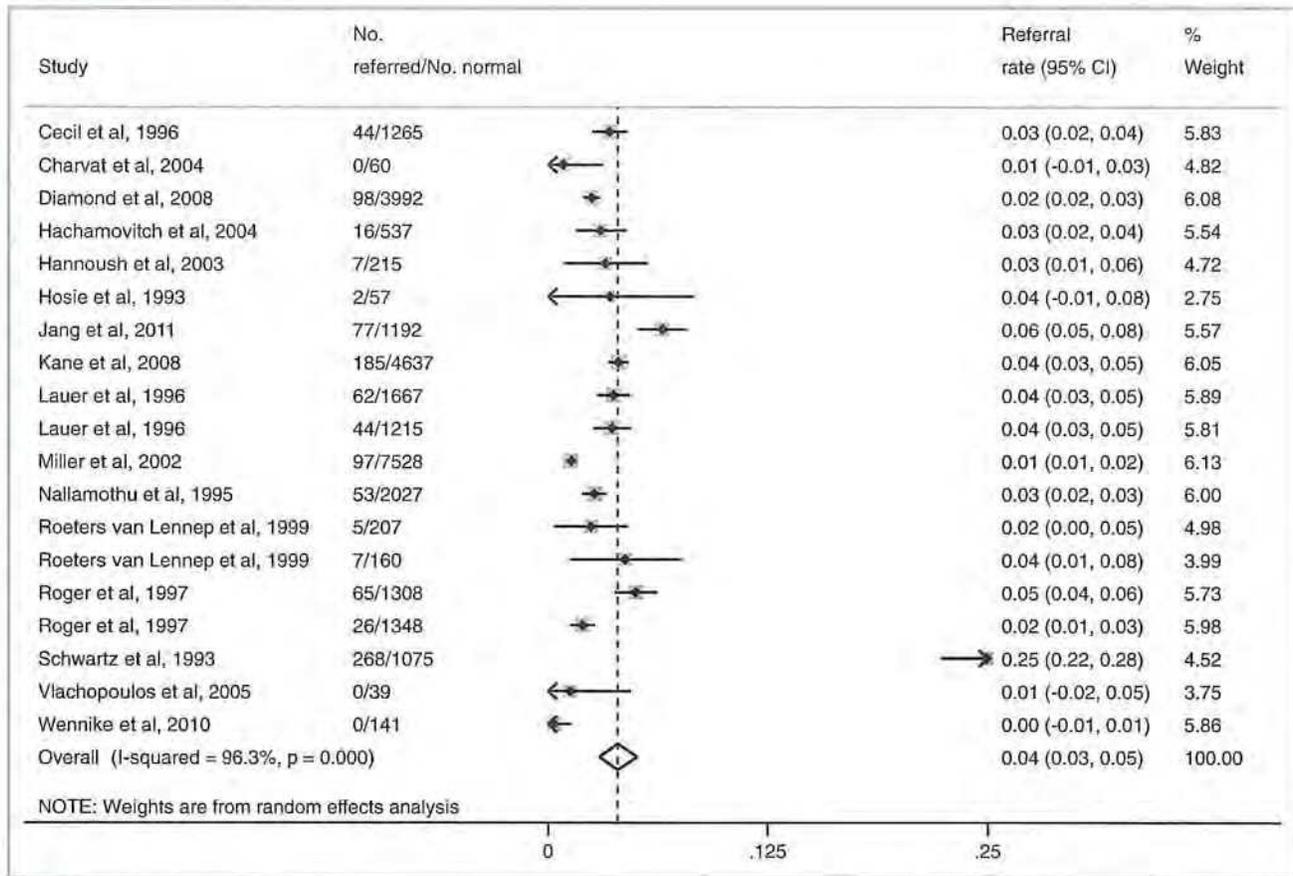


Figure 3. Cardiac catheterization referral rates after normal exercise ECHO or MPI results. Note: Lower 95% confidence intervals for some studies intersect zero. Area of each square corresponds to weight of the study in meta-analysis. CI indicates confidence interval; ECHO, echocardiography; MPI, myocardial perfusion imaging.

ECHO and MPI rose from 77% (95% CI, 69% to 86%) and 69% (95% CI, 61% to 78%) prior to adjusting for the referral process to 99% (95% CI, 99% to 100%) and 99% (95% CI, 99% to 100%) after adjustment, respectively.

Summary ROC Analysis

In a model comparing exercise ECHO without correction for referral bias to exercise ECHO with correction for referral bias, there was a trend toward a decrease in discriminatory power (parameter estimate for reduction -1.8; 95% CI, -3.6 to 0.1). No decrease in discriminatory power was found for exercise MPI (parameter estimate for reduction -0.2; 95% CI, -1.3 to 0.9).

Clinical Decision Making and Posttest Risk Stratification

Figure 5 shows how the posttest risk of CAD varies by pretest risk, with or without correction for referral bias. For both exercise ECHO and MPI, adjusting for referral resulted in an increase in the posttest risk of disease after either a normal or

abnormal test result. Posttest disease risk was comparatively higher after a normal test result primarily because referral bias adjustment significantly reduced sensitivity (thereby reducing negative predictive value). Similarly, posttest disease risk was comparatively higher after an abnormal test result primarily because referral bias adjustment significantly increased specificity (thereby increasing positive predictive value). The value of the test, in terms of guiding clinical decision making, was therefore comparatively higher after an abnormal test result than a normal test result. This is also demonstrated by the distance between the adjusted curves in Figure 5 and the 45-degree line, as the latter represents no incremental information from diagnostic testing (because pretest disease risk equals posttest disease risk along this line). Appropriately, this distance is greater after an abnormal test result than a normal test result.

Sensitivity Analyses

We evaluated the overall robustness of our results by reanalyzing our data using the upper and lower CI bounds for pooled referral rates after normal and abnormal tests,

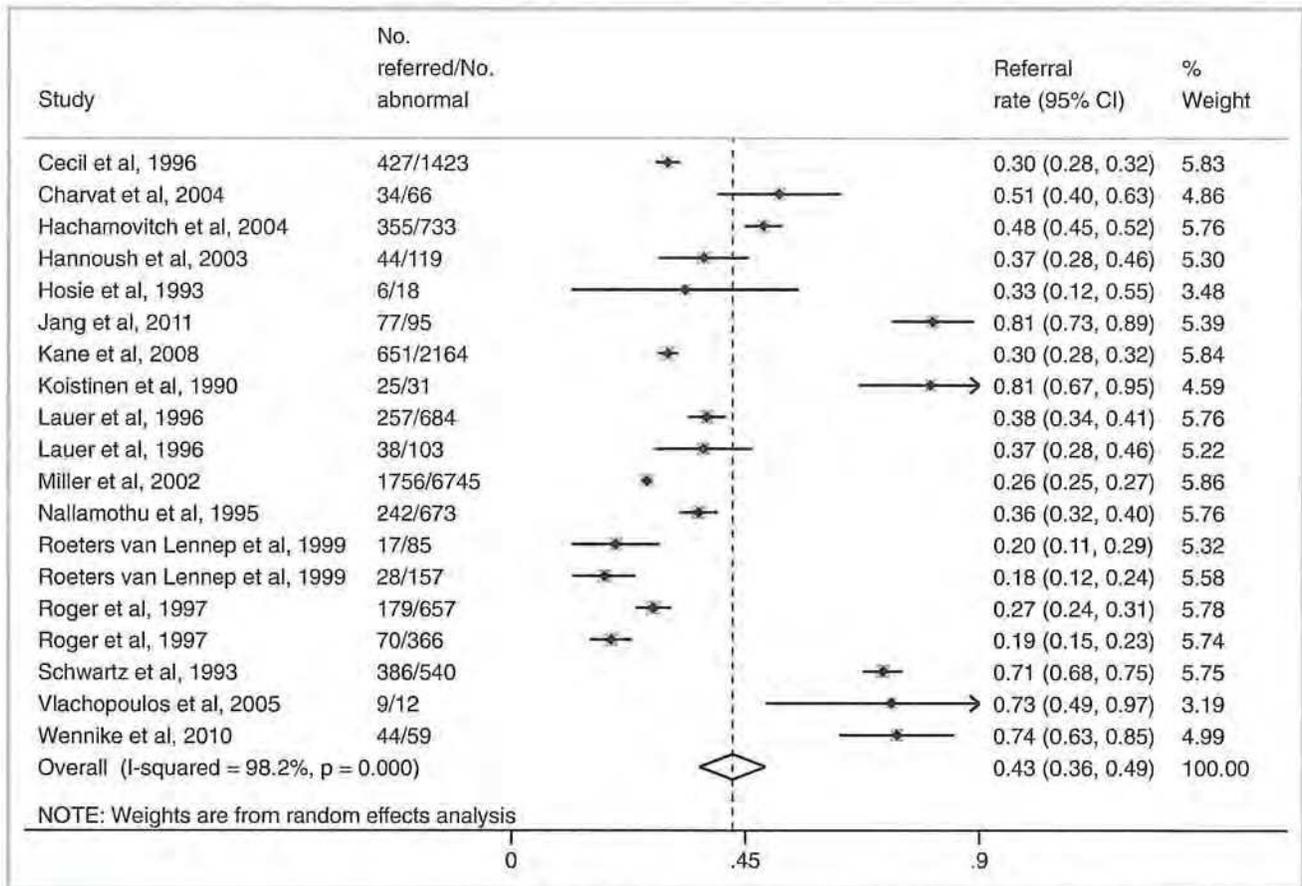


Figure 4. Cardiac catheterization referral rates after abnormal exercise ECHO or MPI results. CI indicates confidence interval; ECHO, echocardiography; MPI, myocardial perfusion imaging; No., number.

respectively. Selecting these values would be expected to attenuate the impact of referral bias by minimizing the relative difference in referral rates. In this analysis, the sensitivity of exercise ECHO and MPI fell to 44% (95% CI, 35% to 52%) and 48% (95% CI, 40% to 56%) and the specificity rose to 99% (95% CI, 98% to 100%) and 98% (95% CI, 97% to 98%), respectively.

We also excluded all studies of referral in which any participants were reported as having a history of MI or revascularization.^{26,36} In this analysis, the referral rates after normal and abnormal exercise tests were 4.1% (95% CI, 2.8% to 5.3%) and 43.7% (36.5% to 50.9%), respectively, with odds

ratio for referral after an abnormal test of 14.3 (9.8 to 20.7). After adjusting for referral, the sensitivity of exercise ECHO and MPI fell to 34% (95% CI, 26% to 41%) and 38% (95% CI, 31% to 44%) and the specificity rose to 99% (95% CI, 99% to 100%) and 99% (95% CI, 99% to 100%), respectively.

Discussion

By systematically reviewing cardiac catheterization referral rates and aggregating them to adjust pooled estimates of exercise test performance, we found that adjusting for referral

Table 2. Diagnostic Effectiveness of Exercise ECHO and MPI With and Without Adjustment for Referral

	ECHO		MPI	
	Sensitivity, % (95% CI)	Specificity, % (95% CI)	Sensitivity, % (95% CI)	Specificity, % (95% CI)
Unadjusted*	84 (80 to 89)	77 (69 to 86)	85 (81 to 88)	69 (61 to 78)
Adjusted†	34 (27 to 41)	99 (99 to 100)	38 (31 to 44)	99 (99 to 100)

ECHO indicates echocardiography; MPI, myocardial perfusion imaging.

*Diagnostic effectiveness based on random-effects meta-analysis of sensitivity and specificity reported in 15 studies of exercise ECHO and 30 studies of exercise MPI (45 studies in total).

†Adjusted for referral rates to cardiac catheterization after abnormal or normal exercise test result.

CAD is considered for stress testing in an evaluation for physiologically significant CAD. If a physician preferentially aims to rule-out CAD in this patient, given the impact of referral bias on the diagnostic accuracy of stress imaging, alternative noninvasive technologies with higher sensitivity, such as coronary computed tomography angiography or other novel technologies,⁴⁵ may be more appropriate tests to use first. Another scenario is that a patient with an intermediate likelihood of CAD is similarly considered for stress testing in an evaluation for physiologically significant CAD. If a physician preferentially aims to rule-in CAD in this patient, stress imaging may be considered as the more appropriate test to use first because it is highly specific after accounting for the referral process.

We recently published the results of a multicenter, blinded trial that enrolled 537 patients with suspected CAD who were referred for stress MPI and underwent a blood-based GES.⁴⁶ To attenuate the impact of referral bias, we attempted to determine coronary anatomy in all patients using cardiac catheterization, when clinically appropriate, or coronary computed tomography angiography. Approximately 83% of eligible patients underwent at least 1 of these 2 tests, and site-read and core-lab MPI sensitivity was 27% and 36%; specificity was 92% and 90%, respectively. Though coronary computed tomography angiography is an imperfect substitute for cardiac catheterization, these results support our findings about the impact of referral bias.

Though we report significantly lower sensitivity and higher specificity than prior meta-analyses of stress testing, the prognostic value of exercise ECHO or MPI—in terms of adverse cardiovascular events—is also cited as a component of its diagnostic utility. However, while the prognostic value of a negative stress test is favorable,⁴⁷ a recent study suggests that this may be partly driven by enrollment of patients at lower risk for CAD.⁴⁸ In a study of 39 515 patients undergoing stress-rest MPI between 1991 and 2009, Rozanski and colleagues reported a significant progressive decline in the prevalence of abnormal (from 40.9% in 1991 to 8.7% in 2009) and ischemic (from 29.6% in 1991 to 5.0% in 2009) studies. These authors concluded that more cost-effective strategies for evaluating low-risk patients are needed.

Our study has several important limitations. Our adjusted values of corrected sensitivity and specificity are analytic estimates only,⁴⁹ the study populations were heterogeneous with respect to important clinical characteristics, and the validity of our results depends on how accurately referral rates from the literature review reflect those of patients comprising our exercise test meta-analysis studies. While several studies reported fairly similar referral rates, it is important to note that referral practices vary by site and provider. In addition, the diagnostic performance of exercise ECHO and MPI also varies by site, and physicians interpreting

these studies may operate at different points on the ROC curve. Results obtained in nonacademic settings may also differ. Our summary ROC curve analysis partially accounted for these possibilities by evaluating overall diagnostic performance.

Another limitation is that our analysis also did not account for other important clinical characteristics that may affect diagnostic test performance and clinical decision making, including clinical factors such as patient-level risk and disease severity and nonclinical factors such as patient preferences and liability concerns. For example, patients with abnormal test results who are referred to cardiac catheterization may have more severe symptoms or a greater risk burden than patients with similar test results who are not referred. This practice would tend to attenuate the impact of referral bias on diagnostic test performance. Furthermore, the Fleischmann et al meta-analysis may not be reflective of contemporary practice, and some authors disagree with its findings. Similarly, the Ontario Health Technology Assessment series is not a widely recognized reference.

An additional limitation of our work is that cardiac catheterization is a poor gold standard for exercise testing. Recent studies have examined the importance of functional characteristics of the coronary arteries and microcirculation, rather than just coronary anatomy, and our understanding of these phenomena is growing.^{50,51} However, a more accurate understanding of diagnostic performance may further improve risk factor modification in this cohort.

Conclusions

Based on pooled results from several studies and clinical sites, exercise ECHO and MPI are less sensitive and more specific for coronary artery disease after adjusting for the referral process. Accounting for this adjustment may influence how clinicians use these tests to rule-in versus rule-out disease, and more sensitive noninvasive methods for diagnosing coronary artery disease may improve patient care.

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Disclosures

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Strategy

Completing the picture

By Aaron Bouchie
Senior Writer

By teaming up to promote each other's tests for coronary artery disease, **CardioDx Inc.** and **General Electric Co.'s GE Healthcare** unit believe they can provide more complete answers to physicians and patients than is possible with either test alone.

If they're right, CardioDx should benefit from GE Healthcare's marketing muscle, as well as imaging solutions for instances when its molecular diagnostics do not give clear answers. Meanwhile, GE Healthcare gets access to emerging technologies and could see increased use of its tests if earlier use of molecular diagnostics yields additional imaging.

No single test can diagnose coronary artery disease (CAD), and there are various tests that physicians can use to inform their decision. Diagnosing CAD begins with the ascertainment of risk factors like cholesterol and triglycerides, as well as co-morbidities like smoking, diabetes and hypertension.

Physicians then perform a number of tests, beginning with noninvasive tests like electrocardiograms and stress tests, followed by invasive procedures like angiography and transesophageal echocardiograms.

But a study of 398,978 patients undergoing angiography published in March in the *New England Journal of Medicine* showed that only 37.6% had obstructive CAD and 39.2% had no CAD, even though most had a positive result from noninvasive testing prior to catheterization. The authors concluded that better strategies for CAD risk stratification are needed.

"The primary reason we partnered with CardioDx is that

in certain care areas and disease states, we believe there will be an increase in opportunities to bring together disparate areas of diagnostic information that exist today," Michael Jones, EVP of business development for GE Healthcare, told BioCentury.

"Diagnostics are done very much in a siloed approach. Especially when there is a difficult decision to be made, we want to give physicians the tools to make a more definitive clinical decision," Jones added. "This is about the integration of information, not the integration of technologies."

While the partners are still finalizing details of their relationship, the deal will likely include CardioDx's Corus CAD, a CLIA-certified blood-based test launched last year that measures expression levels of 23 genes to predict the likelihood that a patient has obstructive CAD.

The test provides a score of 0-40, which correlates with likelihood of obstructive CAD. For example, a score of 7 correlates to a 13% likelihood, a score of 27 is roughly a 50% likelihood, and a maximum score of 40 equates to about a 75% chance of obstructive CAD.

In the validation phase of CardioDx's PREDICT study in 596 non-diabetic patients with stable chest pain and no previously diagnosed myocardial infarction (MI) or revascularization, Corus CAD had 85% sensitivity and 83% negative predictive value.

Corus CAD is most valuable to physicians to rule out obstructive CAD, rather than to diagnose it, according to CardioDx President and CEO David Levison.

Jones suggested a likely scenario is that physicians could use

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"This is about the integration of information, not the integration of technologies."

Michael Jones, GE Healthcare

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Corus CAD early on to try to rule out the need to subject a patient to an expensive, invasive angiograph or a stress test.

Corus CAD costs \$1,195, while a myocardial perfusion imaging (MPI) stress test costs about \$600 and angiography costs \$3,000-\$5,000, according to CardioDx.

GE Healthcare sells computed tomography (CT) machines for performing angiographies, as well as radiopharmaceutical imaging agents for use in diagnosing CAD.

Jones acknowledged that use of Corus CAD will likely reduce the use of imaging in some patients who might have otherwise been tested with GE Healthcare's products. But he added that Corus CAD could result in earlier use of imaging, for example if a patient who does not exhibit traditional risk factors is identified as high risk by the molecular diagnostic.

"So there is an element of justifying those imaging modalities," Jones said.

Levison noted that another test helps when an initial test is inconclusive.

"No single diagnostic can answer all of the questions a physician might have. So an imaging test might come back equivocal, and in those cases the Corus CAD test could give an incremental benefit. And vice versa: our test might not give a definitive answer and push a physician to give an imaging test," he said.

Details of the deal remain to be worked out. The companies will likely co-market and co-promote each other's products but continue to book sales of their own products. They also may co-develop other tests in CardioDx's pipeline.

The PREDICT trial is still enrolling patients who are undergoing invasive angiography to obtain samples for future product development. The company also is enrolling patients who are undergoing MPI in the COMPASS trial, with data expected in early 2011.

CardioDx also began the DISCERN trial about two years ago to identify and validate markers for distinguishing patients at increased risk for arrhythmias. Levison said he is not sure when data will be released. A third test, to identify patients at increased risk for heart failure, is further behind in development.

Concurrent with the deal, the GE Healthymagination Fund invested \$5 million in CardioDx as part of a series D round. Levison said the company has also raised \$5 million from an undisclosed VC, and hopes to raise at least \$10 million more before closing the round in the next few months.

This is the first investment by the GE fund, which has \$250 million to invest in diagnostics, health IT and tools companies. Jones, who also is director of the fund, said it is looking to invest in technologies that can help improve healthcare quality while lowering costs.

COMPANIES AND INSTITUTIONS MENTIONED

CardioDx Inc., Palo Alto, Calif.

General Electric Co. (NYSE:GE), Fairfield, Conn.

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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**

Gregory S. Thomas, Szilard Voros, John A. McPherson, Alexandra J. Lansky, Mary E. Winn, Timothy M. Bateman, Michael R. Elashoff, Hsiao D. Lieu, Andrea M. Johnson, Susan E. Daniels, Joseph A. Ladapo, Charles E. Phelps, Pamela S. Douglas and Steven Rosenberg

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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) trial⁸ suggesting a more conservative approach make less invasive and non-radiation-based diagnostic alternatives desirable.

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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 year¹¹ 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 $\leq 35\%$, 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

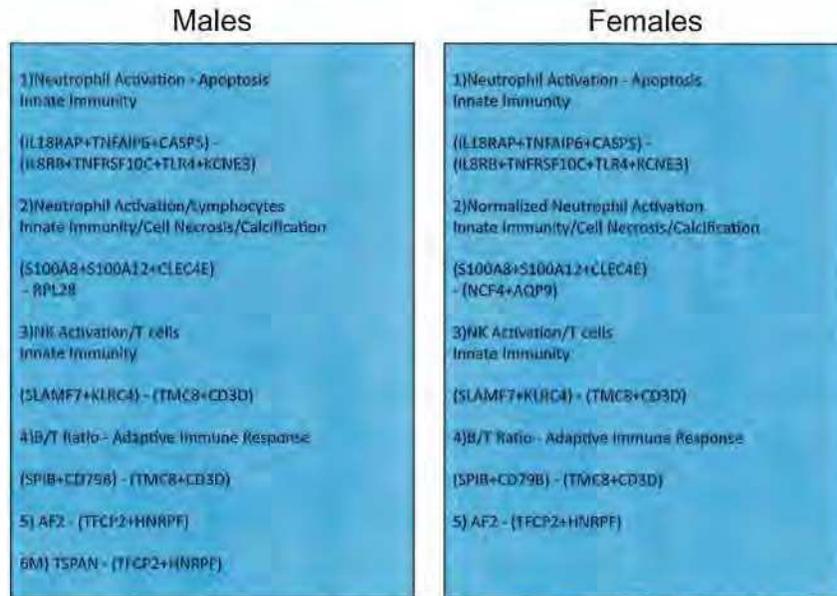


Figure 1. Schematic of gene expression score algorithm. The algorithm consists of overlapping gene expression functions for men and women with sex-specific coronary artery disease (CAD) age dependencies. The algorithm gene expression terms and their biological or cellular pathways are shown. The genes 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 \times ($N_{up} - N_{down}$) - 0.308 \times sex \times ($SCA_1 - Norm_1$) - 0.548 \times (1 - sex) \times ($SCA_1 - Neut$) - 0.406 \times ($NK_{up} - T_{cell}$) - 0.137 \times ($B_{cell} - T_{cell}$) - 0.482 \times sex \times ($TSPAN$) - 0.246 ($AF2 - Norm_2$). For men (SEX = 1) and women (sex = 0), intercept = 2.672 + 0.0449 \times Age and 1.821 + 0.123 \times (Age - 60), respectively, with only positive values allowed for women; $N_{up} = 1/3 \times (CASP5 + IL18RAP + TNFAIP6)$, $N_{down} = 0.25 \times (IL8RB + TNFRSF10C + TLR4 + KCNE3)$; $SCA_1 = 1/3 \times (S100A12 + S100A8 + CLEC4E)$; $Norm_1 = RPL28$; $Neut = 0.5 \times (AQP9 + NCF4)$; $NK_{up} = 0.5 \times (SLAMF7 + KLRC4)$; $T_{cell} = 0.5 \times (CD3D + TMC8)$; $B_{cell} = 2/3 \times CD79B + 1/3 \times SPIB$; $TSPAN = 1$ if ($AF2 - Norm_2$) > 6.27 otherwise 0; and $Norm_2 = 0.5 \times (HNRPF + TFCP2)$. 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 classification¹⁴ 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 $\geq 50\%$ 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 $\geq 25\%$ 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 (30)	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 $\geq 50\%$ maximum stenosis used as the case threshold.

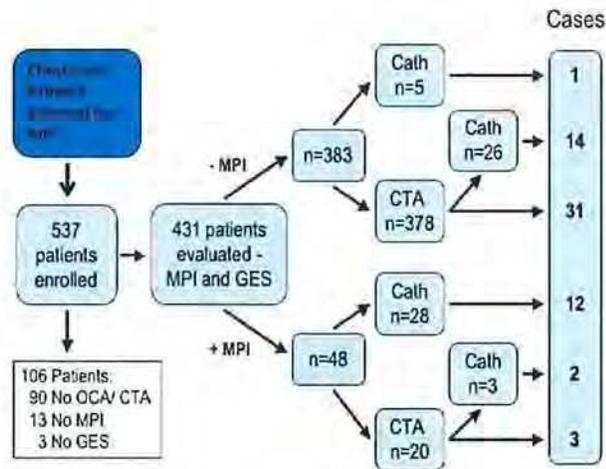


Figure 2. Study design and patient flow diagram. Nondiabetic patients without known coronary artery disease referred for myocardial perfusion imaging (MPI) were consented and had blood drawn for gene expression score (GES) before MPI. Positive MPI results were referred for invasive coronary angiography (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.

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 SDMTTools packages).²⁰ Unless otherwise specified, univariate comparisons used *t* tests for continuous variables and χ^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

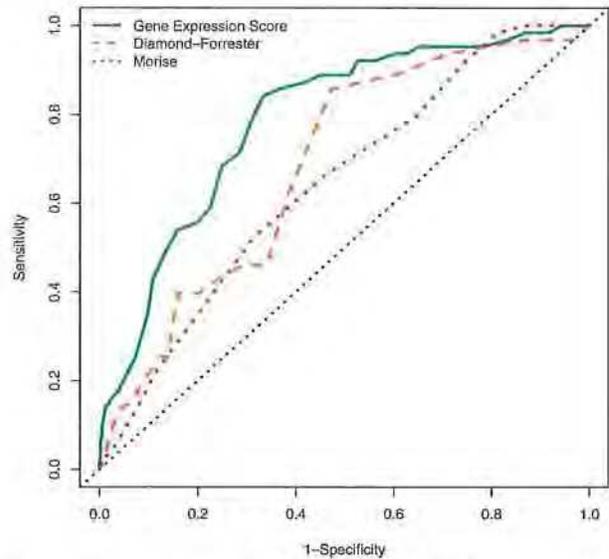


Figure 3. Receiver-operating characteristics (ROC) analysis of gene expression score (GES) and clinical factors. ROC curves for a case definition of $\geq 50\%$ maximum stenosis by either quantitative coronary angiography (QCA) or computed tomographic angiography (CTA) are shown: GES (green solid line), 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.

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% ($\kappa=0.72$), 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 $\geq 25\%$ and $\geq 50\%$ 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 ($\geq 50\%$ 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 $\geq 70\%$ 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 (Δ AUC=0.19; NRI=26%; both $P<0.001$) and by ROC for core-laboratory MPI (Δ AUC=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 $\geq 50\%$), 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

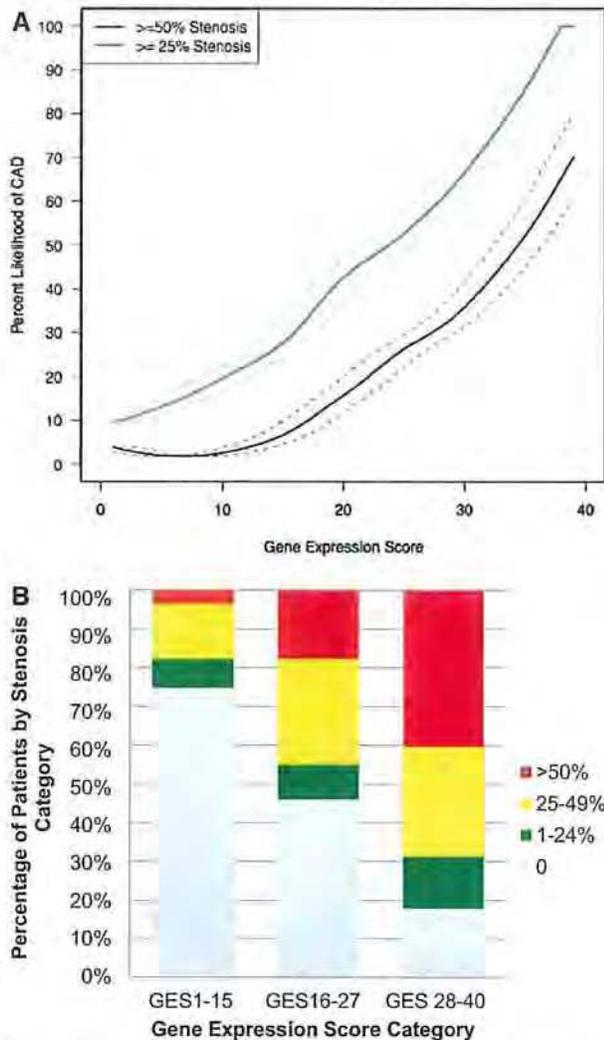


Figure 4. **A**, Likelihood of coronary artery disease (CAD) and obstructive CAD as a continuous function of gene expression score (GES). The percent likelihoods of $\geq 25\%$ stenosis (mild and obstructive CAD) and $\geq 50\%$ stenosis (obstructive CAD) are indicated by the green and red lines, respectively, 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 $\geq 50\%$ stenosis are shown in pre-specified 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.

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; $P < 0.001$)

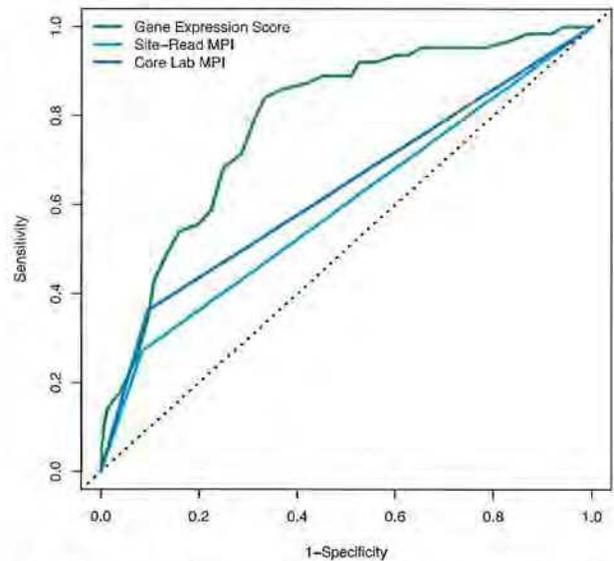


Figure 5. Receiver operating characteristics (ROC) analysis of gene expression score (GES) and myocardial perfusion imaging (MPI). ROC curves for a case definition of $\geq 50\%$ maximum stenosis by either quantitative coronary angiography or computed tomographic angiography are shown: GES (green solid 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.

in this independent, community-based, lower-risk population and was superior to clinical estimates by Diamond–Forrester and Morise scores ($\Delta AUC = 0.10$; $P = 0.003$; and $\Delta AUC = 0.12$; $P = 0.002$), 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 ($\Delta AUC = 0.19$ 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 ($\Delta\text{AUC}=0.16$; $P<0.001$), 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 MPIAUC 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 ≤ 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 ± 10 years) was lower and the frequency of exercise versus pharmacological testing (78%) was greater than those observed in another outpatient-only trial (65 ± 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

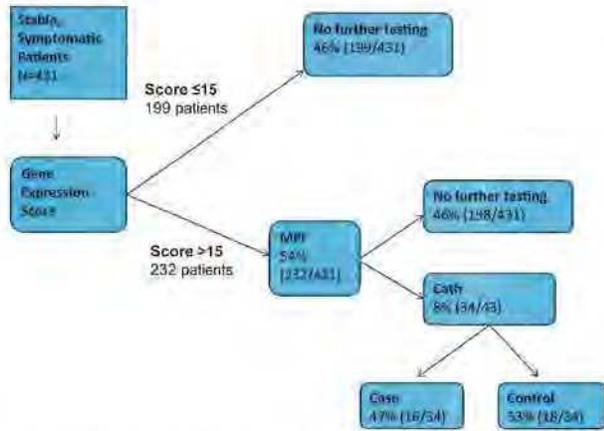


Figure 6. Clinical algorithm with sequential use of gene expression score (GES) and myocardial perfusion imaging (MPI). Based on the data in this study, the model shown is suggested. For patients with GES ≤15, no further follow-up is proposed given the high sensitivity and negative predictive value at 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 non-ischemic 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.

The Clinical Utility of Gene Expression Testing on the Diagnostic Evaluation of Patients Presenting to the Cardiologist With Symptoms of Suspected Obstructive Coronary Artery Disease: Results From the IMPACT (Investigation of a Molecular Personalized Coronary Gene Expression Test on Cardiology Practice Pattern) Trial

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Abstract: Accurate, noninvasive evaluation for obstructive coronary artery disease (CAD) remains challenging and inefficient. In this study, 171 patients presenting with stable chest pain and related symptoms without a history of CAD were referred to 6 cardiologists for evaluation. In the prospective cohort of 88 patients, the cardiologist's diagnostic strategy was evaluated before and after gene expression score (GES) testing. The GES is a validated, quantitative blood-based diagnostic test measuring peripheral blood cell expression levels of 23 genes to determine the likelihood of obstructive CAD (at least 1 vessel with $\geq 50\%$ angiographic coronary artery stenosis). The objective of the study was to measure the effect of the GES on diagnostic testing using a pre/post study design. There were 83 prospective patients evaluable for study analysis, which included 57 (69%) women, mean age 53 ± 11 years, and mean GES 12.5 ± 9 . Presenting symptoms were classified as typical angina, atypical angina, and noncardiac chest pain in 33%, 60%, and 7% of patients ($n = 27, 50$, and 6), respectively. After GES, changes in diagnostic testing occurred in 58% of patients ($n = 48, P < 0.001$). Of note, 91% (29/32) of patients with decreased testing had low GES (≤ 15), whereas 100% (16/16) of patients with increased testing had elevated GES ($P < 0.001$). A historical cohort of 83 patients, matched to the prospective cohort by clinical factors, had higher diagnostic test use compared with the post-GES prospective cohort ($P < 0.001$). In summary, the GES showed clinical utility in the evaluation of patients with suspected obstructive CAD presenting to the cardiologist's office.

Key Words: coronary disease, diagnosis, test, gene expression, clinical utility

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The evaluation of patients with chest pain and related symptoms to determine the likelihood of coronary artery disease (CAD) is a common clinical scenario, with approximately 10,000 patients presenting every day to US physician offices.¹ Yet despite its

prevalence, the diagnosis of CAD is highly variable and poses an enormous burden on the healthcare system.^{2,3} Although referrals to the cardiologist may be due to typical symptoms of angina, patients also often present with atypical symptoms that have unclear etiology: these atypical symptoms may be related to cardiac causes or noncardiac causes such as gastroesophageal reflux, anxiety, and fibromyalgia. However, after a thorough and potentially expensive work-up, only 10% to 30% of chest pain cases evaluated by physicians result in a diagnosis of obstructive CAD.^{4–6}

In current practice, physicians typically integrate available clinical data (eg, clinical history and physical examination) with the results of noninvasive test(s) (eg, exercise electrocardiographic testing, stress echocardiography, myocardial perfusion imaging [MPI], and coronary computed tomographic angiography [CCTA]) to determine the likelihood of obstructive CAD. The Diamond–Forrester classification may be used to help estimate the probability of CAD based on symptoms, age, and gender.^{7,8} Nevertheless, in a recently published registry of over 14,000 patients, the pretest probability of CAD in patients referred for advanced cardiovascular imaging based on clinical factors overestimated the actual presence of disease by 57% in patients with typical angina and 32% in patients with atypical angina.⁹ The lack of clear guidance on the use of noninvasive imaging may lead to inappropriate use of these modalities, potentially including both overutilization and underutilization of testing.^{10–13} Additional risks include radiation exposure (MPI and CCTA) and contrast-induced anaphylaxis or acute kidney injury (CCTA).^{14–16}

Better methods are needed to more accurately assess the likelihood of obstructive CAD in patients in a cardiologist office–based setting, especially among patients with low pretest probability of disease such as women and patients with atypical symptoms on presentation. The development of a gene expression score (GES) based on peripheral blood cell expression levels of 23 genes and 6 terms has been previously described.¹⁷ In the Personalized Risk Evaluation and Diagnosis in the Coronary Tree (PREDICT) and Cardiovascular Outcomes for People using Anticoagulation Strategies (COMPASS) validation studies, a GES of ≤ 15 had a negative predictive value of 83% and 96%, respectively, for obstructive CAD in evaluating patients referred for further invasive and noninvasive cardiovascular testing.^{18,19} We hypothesized that GES results would lead to a change in the cardiologist's diagnostic strategy and referral to cardiac testing among stable patients presenting in the ambulatory setting with signs and symptoms suggestive of obstructive CAD.

METHODS

We examined the clinical impact of the GES through the enrollment of patients referred to cardiology at the Vanderbilt

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University Medical Center (Nashville, TN) from December 2010 through December 2011. We recruited a representative sample of 10% of these cardiologists, all of whom were trained on the use and interpretation of the GES. Our study centers included 6 cardiologists with a referral network of 30 primary care physicians from 15 outlying clinics within a 100 mile catchment radius.

The study was a 2-arm study with a prospective cohort and a matched historical cohort. Patients were eligible if they met the GES' intended use, had symptoms of chest pain, or anginal equivalent symptoms, and were referred to a cardiologist for evaluation. The GES test is intended for patients with a history of chest pain, with suspected anginal equivalent to chest pain, or with a high risk of CAD, but with no known prior myocardial infarction or revascularization procedures. The GES test is not intended for use in patients with acute myocardial infarction, high-risk unstable angina, systemic infectious or systemic inflammatory conditions, diabetes, or who are currently taking steroids, immunosuppressive agents, or chemotherapeutic agents. The hospital institutional review board approved the study, and all patients in the prospective cohort gave written informed consent. An institutional review board waiver for informed consent was granted for patients in the historical cohort. Prespecified data were obtained by research study coordinators using standardized data collection methods and included demographics, clinical risk factors, symptoms, medical history, medications, prior testing results, and GES results. Data were verified by independent study monitors.

The GES (Corus CAD, CardioDx, Inc., Palo Alto, CA) is a Medicare approved, validated, quantitative diagnostic test that measures expression levels of 23 genes in 6 terms by quantitative RT-PCR from a peripheral blood sample to determine the likelihood of a patient having obstructive CAD (at least 1 coronary artery with $\geq 50\%$ angiographic stenosis as determined by quantitative coronary angiography).^{18,19} Our algorithm contains 2 types of terms: sex-specific age functions of obstructive CAD likelihood and gene expression terms that reflect changes in gene expression within a cell type, changes in cell type proportions, or a combination of both. The sex-specific differences in cardiovascular risk and presentation are well known and largely reflect reduced risk in premenopausal women. The algorithm genes are expressed selectively in multiple types of circulating cells including neutrophils (such as caspase-5 and S100A12), natural killer cells (SLAMF7 and KLRC4), and B and T-lymphocytes, supporting roles for both adaptive and innate immune responses in atherosclerosis.

In the prospective cohort, the cardiologist initially decided the subject's pretest probability for CAD based on risk factors, quality of anginal symptoms, and results of prior electrocardiogram stress testing if performed. Based on the cardiologist's clinical judgment, pretest probability of obstructive CAD was classified as low ($<20\%$), intermediate ($20\%–50\%$), or high ($>50\%$). The probability was determined and assessed by 2 methods: 1) a set of questions to derive a Diamond-Forrester pretest probability, and 2) the physician's self-assessed pretest probability for the patient having obstructive CAD. Symptoms suggestive of CAD were classified as typical angina, atypical angina, or noncardiac pain on the basis of the presence of one or more of the following typical symptoms: substernal chest discomfort, aggravation with exertion, and alleviation with rest as well as atypical symptoms such as heartburn, palpitations, malaise, and fatigue.²

The initial physician questionnaire captured the cardiologist's preliminary clinical impression and decision (preliminary decision) on the further evaluation and management of the subject. Patient blood samples were collected in the outpatient clinic setting into a vial of RNA preservation fluid (PAXgene RNA Blood Tubes used according to the manufacturer's instructions [PreAnalytix, Valencia, CA]) and shipped to the Clinical Laboratory Improvements Amendments

(CLIA)-certified laboratory (CardioDx, Inc., Palo Alto, CA, CLIA # 05D1083624), which reported the GES (range 1–40) to the physician within 2–3 days, on average. After the cardiologist received the GES, he decided on the appropriate evaluation and management of the subject (final decision) using the GES in conjunction with the information that was previously available.

The historical cohort was defined by 1-for-1 matching with prospective patients. The matching criteria were age (± 5 years), gender, clinical risk factors, and evaluation in the previous 3–30 months. Data related to the baseline clinical characteristics of these historical subjects, their initial triage, and the cardiac diagnostic tests performed were extracted from their medical records. Through the evaluation of the testing pattern of the historical controls as compared with the prospective cohort, we attempted to address the potential for observation and surveillance biases as part of the protocol design.

The primary objective of the study was to assess whether the use of the GES altered the cardiologist's evaluation and clinical management of the patient, as defined by a change of management pattern between the preliminary versus final decision. A change in management between the preliminary and final decision was prospectively defined as either a downgrade or upgrade in the intensity of diagnostic plan, as divided into the following categories [in hierarchical order]: 1) no further cardiac testing or medical therapy for angina or noncardiac chest pain, 2) stress testing (with/without imaging) or computed tomography coronary angiography, or 3) invasive coronary angiography (ICA). Secondary analyses assessed the patterns of change (decrease or increase in diagnostic intensity) as well as diagnostic yield on ICA as a function of GES score (defined prospectively as low GES ≤ 15 or elevated GES > 15). Major adverse cardiac event data were recorded.

In order to further evaluate the effects of change in diagnostic testing as well as the effect of these changes on patient outcomes, we conducted a follow-up phone call at 180 ± 15 days for each prospectively enrolled subject to assess the initial diagnostic plan, such as referral to any subspecialists, cardiac diagnostic tests performed, cardiac procedures performed, and results of these cardiac tests and procedures. For the historical control, an electronic medical record search was performed at 180 ± 15 days from the date of the original presentation of the historical subject to the cardiologist for further evidence of anginal symptoms or diagnostic evaluation of chest pain.

All analyses were performed using R, version 2.13 (including rms and reshape2 packages).²⁰ Standard statistical methods were used for all comparisons, with an α level of 0.05.²¹ The proportion of patients whose preliminary and final treatment decision differed was compared using a 1-sided binomial test to 15%, which is the minimum amount believed to be clinically relevant. Pre- and post-GES treatment plans were tested for independence using McNemar's test, and the trend toward increased or decreased intensity of diagnostic test prescribing was evaluated using a sign test.

RESULTS

After applying exclusion criteria for known CAD and diabetes, 88 patients were enrolled in the prospective cohort, of whom 83 patients were eligible for primary endpoint analysis, and 83 matched patients were enrolled in the historical cohort (Fig. 1). The demographics of the prospective cohort (Table 1) included 57 (69%) women, mean age 53.3 ± 11 years, mean body mass index 29.5 ± 6 , 52 (63%) patients with low GES, and mean GES 12.5 ± 9 . Presenting symptoms were evaluated as typical angina, atypical angina, and noncardiac chest pain in 33%, 60%, and 7% of patients ($n = 27, 50$, and 6), respectively. Hypertension and dyslipidemia were present in 55% and 48%, respectively. There were few differences in demographics between the prospective and historical cohort patients, with

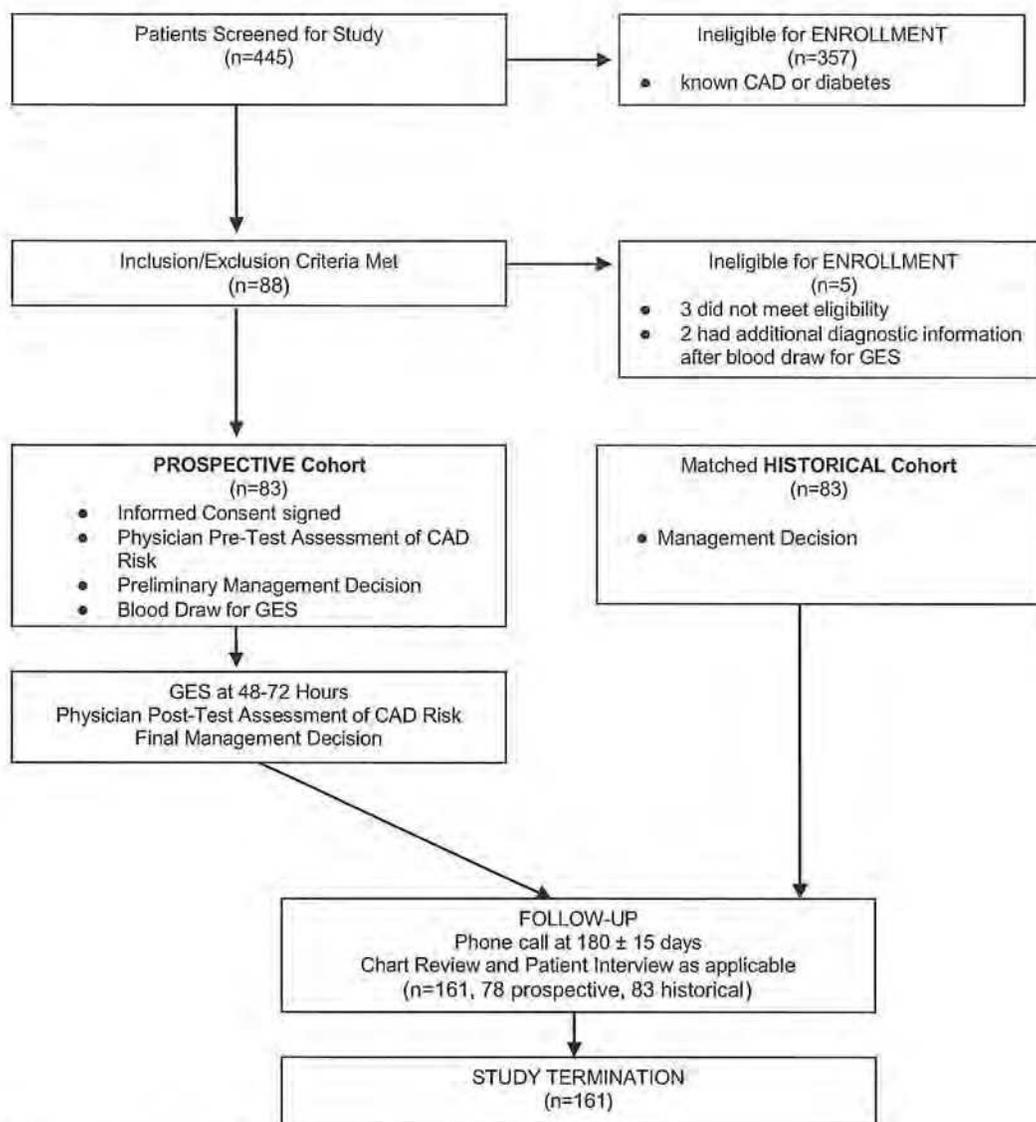


FIGURE 1. Patient screening, enrollment, and follow-up flowchart.

more hypertension and more β -blocker use among patients in the historical cohort.

In reference to the primary outcome, changes in the recommended diagnostic testing plan (eg, MPI, CCTA, and ICA) after GES testing occurred in 58% of patients ($n = 48$; 95% confidence interval, 46%–69%) ($P < 0.001$) (Table 2). In reference to the secondary outcomes, more patients had a decreased (39%, $n = 32$) versus increased (19%, $n = 16$) intensity of testing ($P = 0.03$). In particular, 91% (29/32) of patients with decreased testing had low GES, whereas 100% (16/16) of patients with increased testing had elevated GES ($P < 0.001$). In addition, there was a statistically significant reduction in additional diagnostic testing after GES evaluation, with 50 (60%) patients pre-GES and 26 (31%) patients post-GES scheduled to receive additional testing ($P < 0.001$). Similarly, when comparing the final decisions of the prospective patients with their matched historical controls (Fig. 2), we found a 71% reduction in overall diagnostic testing ($P < 0.001$) among prospective patients. No major adverse cardiovascular events were

observed for any of the 161 patients in both cohorts (97% follow-up) at 30-day and at 6 months follow-up.

We further examined the diagnostic test use patterns around ICA. A total of 30 (14 prospective cohort and 16 historical cohort) patients underwent ICA. In the prospective cohort, the triage decision around the need for ICA, as informed by GES, was associated with a trend toward improvement in the diagnostic yield at ICA ($P = 0.24$). Pre-GES, 7 patients were assigned to ICA, 5 underwent ICA, and 20% (1/5) had at least 1 lesion with $>50\%$ stenosis. Post-GES, 15 patients were assigned to ICA, 11 patients underwent ICA, and 45% (5/11) had at least 1 lesion with $>50\%$ stenosis. An additional 3 patients, not assigned to ICA with low GES, underwent testing due to either a subsequent positive stress test or clinical factors: 2 patients had normal arterics, and 1 patient had a lesion with $<50\%$ stenosis. Overall then in the prospective cohort, 11 of 14 patients (79%) had angiographic CAD, and 5 of 14 (36%) had obstructive CAD. Of the 16 patients in the historical cohort who underwent ICA, only 4 of 12 (25%) of patients had angiographic CAD, and 0% (0/16) of patients had obstructive CAD.

TABLE 1. Clinical Characteristics of Prospective and Historical Cohort Patients

Clinical Factors	Prospective	Historical	P
	n = 83	n = 83	
Female gender*	57 (69%)	57 (69%)	1.0
Age (yrs)*	53.3±11	53±12	0.86
Ethnicity, White not Hispanic	79 (95%)	76 (83%)	0.53
Chest pain			
Symptomatic—typical angina*	27 (33%)	27 (33%)	1.0
Symptomatic—atypical angina*	50 (60%)	50 (60%)	1.0
Nonanginal*	6 (7%)	6 (7%)	1.0
Hypertension	46 (55%)	60 (72%)	0.04
Dyslipidemia	40 (48%)	52 (63%)	0.09
Body mass index	29.5±6	31.1±6.4	0.10
Systolic blood pressure (mm Hg)	128±18	133±20	0.09
Medication			
β-blockers	19 (23%)	52 (63%)	<0.001
Antihyperlipidemics	33 (40%)	43 (52%)	0.16
Gene expression score	12.5±9	NA	NA

*Patients were matched based upon these characteristics.

All P values are 2-sided. Proportions were compared using a χ^2 test. Continuous values were compared using an unpaired t test. NA, not available.

DISCUSSION

Our study suggests the feasibility of use of the GES and its potential clinical utility in a low-risk population referred to the cardiologist for evaluation for suspected CAD. The GES was associated with improved diagnostic testing strategies as well as improved diagnostic yield of ICA-referred patients. Specifically, we noted a change in the diagnostic work-up in 58% of patients, a highly clinically relevant and statistically significant rate of change. We observed a directional change in testing, which was congruent with the role of the GES to exclude patients without CAD and to further risk-stratify patients: patients with low GES were more likely to have a decrease in the diagnostic testing intensity, whereas patients with elevated GES were more likely to have an increase in diagnostic testing intensity.

We note 3 areas of added interest in these findings. First, this study did not address overutilization or underutilization of testing *per se*, but the GES did appear to optimize the diagnostic plan among real world patients referred to a cardiologist for evaluation of chest pain. The GES was incorporated into the cardiologist's office setting and improved upon usual care around the assessment of clinical factors and the need for cardiac imaging. The GES provides a personalized tool for evaluating the likelihood of CAD: the test, specific to age and gender, quantitatively measures gene expression signals in

a given patient. Second, the prospective/retrospective study design allowed the physician to act as his or her own control in the comparison with the 2 separate patient groups, adding further validity to the findings of change in the diagnostic plan as a result of GES testing. Third, use of this office-based tool was not associated with untoward outcomes in the care of GES tested patients at 6 month follow-up, as no major cardiac adverse events were observed.

The observed 58% change rate in the diagnostic plan suggests the potential of GES testing to impact the efficiency of the CAD evaluation, through more appropriate risk stratification of patients and test utilization as well as potentially less exposure to ionizing radiation from noninvasive testing. For comparison, in a recent study, the incorporation of an NT-proBNP level in a diagnostic prediction model for acute heart failure resulted in reclassification of 44% of patients to either low- or high-probability categories.²² Other molecular diagnostic tests to improve physician diagnostic strategies have noted similar success, most commonly in the oncology setting. In a study focusing on optimizing breast cancer treatment, results from a 21-gene recurrence score assay led medical oncologists to change treatment recommendation in 31% of patients.²³ In a study evaluating diagnosis and management of patients with metastatic cancer of unknown origin, physicians changed the primary working diagnosis for 50% of patients (95% confidence interval, 43%–58%) after results from a 2000-gene-expression-profiling test.²⁴

We observed bidirectionality in the changes in diagnostic testing intensity; with 91% (29/32) low GES patients undergoing decreased intensity and 100% (16/16) elevated GES patients undergoing increased intensity. The COMPASS study demonstrated a 96% negative predictive value for low GES patients undergoing evaluation for obstructive CAD as well as a lack of untoward outcomes among this group.¹⁹ Thus, the work-up of low GES patients may be subsequently focused on non-cardiac causes of the patient's presenting symptoms. In this study, there was a statistically significant reduction in additional diagnostic cardiac testing after GES evaluation, with 50 patients pre-GES reduced to 26 patients post-GES scheduled to receive additional testing ($P<0.001$). The work-up of patients with symptoms of obstructive CAD who had elevated GES is more complex, and the GES clinical algorithm suggests referring these patients for more advanced cardiac imaging.

There were several limitations to the study design and conduct. The test is not intended for use in patients with diabetes mellitus or known CAD. Clinical outcomes were measured at 180 days, and it is possible that adverse cardiac events may have occurred at a later time after the diagnostic evaluation. The sample size was modest, with 171 patients evaluated using the matched control analysis and 83 patients in the prospective pre/post GES cohort. Thus, we believe that these study results demonstrate the feasibility of use of the GES in the diagnostic evaluation of stable patients with suspected CAD. In addition, study patients were gathered from a subset of the cardiology practice sites, representing a small proportion of all screened patients. However, most patients initially evaluated by the cardiologists in the

TABLE 2. The Relationship Between Preliminary vs. Final Testing Decisions in the Prospective Cohort (n = 83)

		Post-GES			
		None/Meds	Stress/CTA	ICA	Total
Pre-GES	None/Meds	25 (19, 6*)	6 (0, 6)	2 (0, 2)	33 (19, 14)
	Stress/CTA	30 (28, 2)	5 (2, 3)	8 (0, 8)	43 (30, 13)
	Angiography	2 (1, 1)	0 (0, 0)	5 (2, 3)	7 (3, 4)
	Total	57 (48, 9)	11 (2, 9)	15 (2, 13)	83 (52, 31)

*Format: Total (low, elevated Corus CAD score).
CTA indicates computed tomography angiography.

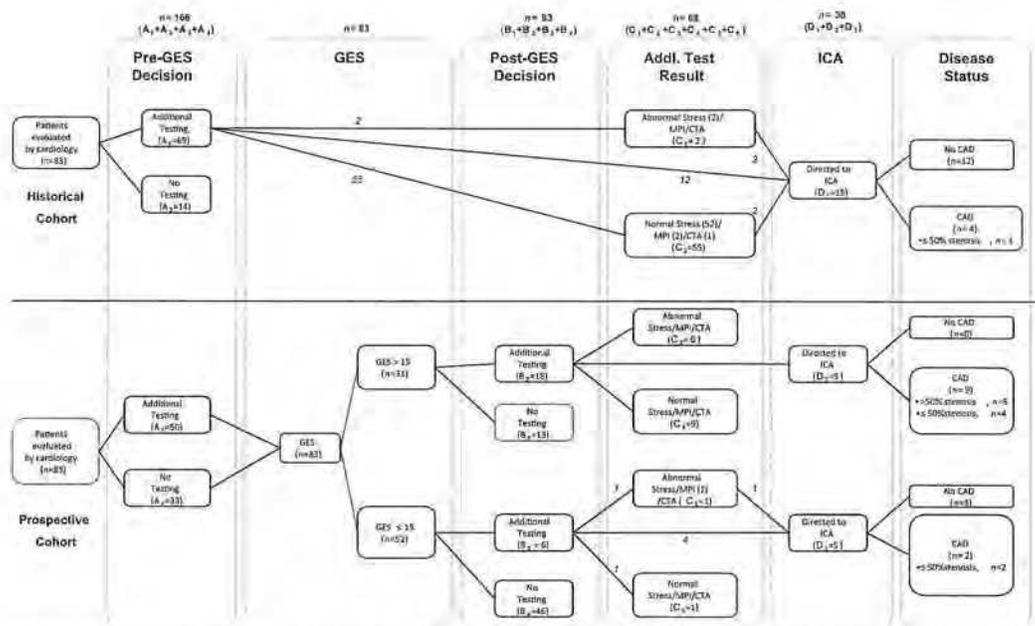


FIGURE 2. Historical controls vs. prospective cohort final patient management flow. Categories of disease status: no CAD (0% stenosis), CAD ($\leq 50\%$ stenosis), or CAD ($> 50\%$ stenosis). CTA indicates computed tomography angiography.

study already had established CAD or a diagnosis of diabetes, for which the test is contraindicated: we believe the study population is reflective of the patients meeting the GES test indications that are seen by the larger group of 50 cardiologists. Furthermore, we note the potential for selection bias toward the inclusion of low-risk patients in this study because usual care at this practice site is to admit or refer high-risk patients to urgent care. Of note, a high proportion of enrolled patients were premenopausal females with atypical symptoms. Thus, the results of the present study may not apply to higher-risk patients. Although this is generally a low-risk population, many of these patients are nonetheless referred for cardiac testing, resulting in an elevated false-positive risk, radiation exposure, and possible invasive coronary angiography.^{25–29}

CONCLUSIONS

In the PREDICT and COMPASS trials, the noninvasive, blood-based gene expression test was shown to have clinical validity in the estimation of obstructive CAD likelihood. Our study demonstrated the clinical utility of the GES, as cardiologists feasibly incorporated the GES into their decision-making process by modifying their subsequent diagnostic tests based upon GES. The use of the GES test in the diagnostic plan may thus influence the clinical management of patients through risk stratification of patients, by decreasing the intensity of testing in low GES patients as well as increasing such testing in elevated GES patients. Given current initiatives focusing on the eliminating inefficiencies in clinical care and quality of care, we believe that the GES may represent a valuable tool for the cardiology practice, especially among patients with low pretest probability of disease such as women and patients with atypical symptoms on presentation.

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Whole Blood Gene Expression Testing for Coronary Artery Disease in Nondiabetic Patients: Major Adverse Cardiovascular Events and Interventions in the PREDICT Trial

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Abstract The majority of first-time angiography patients are without obstructive coronary artery disease (CAD). A blood gene expression score (GES) for obstructive CAD likelihood was validated in the PREDICT study, but its relation to major adverse cardiovascular events (MACE) and revascularization was not assessed. Patients ($N=1,160$) were followed up for MACE and revascularization 1 year

post-index angiography and GES, with 1,116 completing follow-up. The 30-day event rate was 23% and a further 2.2% at 12 months. The GES was associated with MACE/revascularizations ($p<0.001$) and added to clinical risk scores. Patients with GES >15 trended towards increased >30 days MACE/revascularization likelihood (odds ratio=2.59, 95% confidence interval=0.89–9.14, $p=0.082$). MACE incidence

Clinical Trial Information: PREDICT (<http://www.clinicaltrials.gov>), NCT 00500617

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overall was 1.5% (17 of 1,116) and 3 of 17 patients had $GES \leq 15$. For the total low GES group ($N=396$), negative predictive value was 90% for MACE/revascularization and >99% for MACE alone, identifying a group of patients without obstructive CAD and highly unlikely to suffer MACE within 12 months.

Keywords Coronary artery disease · Peripheral blood gene expression · Genomics · Angiography · Coronary interventions · MACE

Abbreviations

GES	Gene expression score
CAD	Coronary artery disease
MACE	Major adverse cardiovascular events
NPV	Negative predictive value
MI	Myocardial infarction
QCA	Quantitative coronary angiography
D-F	Diamond–Forrester score
PCI	Percutaneous coronary intervention
CABG	Coronary artery bypass graft
TIA	Transient ischemia attack

Introduction

Chronic coronary artery disease (CAD) and adverse cardiovascular events are the largest sources of morbidity and mortality in the developed world and are diagnosed in more than 500,000 new patients annually in the USA [1]. Obstructive CAD diagnosis is challenging as patient presentation may often be variable and atypical symptoms are common [2]. Clinical evaluation of suspected CAD often includes stress testing followed by noninvasive imaging (stress echocardiography or nuclear perfusion) and, if indicated, invasive coronary angiography. Recent studies have highlighted the relatively high radiation exposure burden in the standard CAD workup [3, 4] and have indicated that, for patients without a prior CAD diagnosis, <40% have obstructive CAD when referred for coronary angiography [5]. In addition, the COURAGE trial suggested that optimal medical therapy was noninferior to percutaneous coronary intervention (PCI) for hard cardiovascular endpoints in patient populations with stable angina and CAD [6]. Thus, noninvasive genomic-based methods for CAD diagnosis may have significant clinical utility and lead to lower diagnostic costs in these patient populations.

We described differential blood cell gene expression levels in patients with CAD [7] and, more recently, the development and clinical validation in the PREDICT study of a gene expression score (GES) comprised of the expression levels of 23 genes, age, and sex [8, 9]. In this angiographic population of nondiabetic patients, approximately 80% were

symptomatic and the quantitative coronary angiography (QCA)-defined obstructive CAD prevalence was 36%; the GES negative predictive value (NPV) was 83% at a score threshold of 15, with 33% of patients below this threshold. Furthermore, the GES correlated with QCA-determined maximum percent stenosis. To evaluate the outcomes of these GES patients, we monitored 1,160 PREDICT patients for major adverse cardiovascular events (MACE) and interventional procedures for 12 months from index catheterization.

Methods

General Study Design and Study Population

Subjects were enrolled in PREDICT, a 39-center prospective study, between July 2007 and April 2009 (<http://www.clinicaltrials.gov>, NCT 00500617). The study complied with the Declaration of Helsinki, was approved by institutional review boards at all centers, and all patients gave written informed consent. Subjects referred for diagnostic coronary angiography were eligible with a history of chest pain, suspected angina equivalent symptoms, or a high risk of CAD and no known prior myocardial infarction (MI), revascularization, or obstructive CAD. Subjects were ineligible if at catheterization they had acute MI, high-risk unstable angina, severe noncoronary heart disease (congestive heart failure, cardiomyopathy, or valve disease), systemic infectious or inflammatory conditions, or were taking immunosuppressive or chemotherapeutic agents. Detailed eligibility criteria have been described [9].

From 1,354 enrolled nondiabetic subjects who met the inclusion criteria, 5 had angiographic images unsuitable for QCA and 6 had unusable blood samples. The remaining 1,343 were divided into independent algorithm development and validation cohorts sequentially based on enrollment [9]; of these, 1,166 patients had valid GES, 640 in algorithm development and 526 in validation [9]. These were evaluated for events, with six subjects from algorithm development not meeting the clinical inclusion criteria upon further evaluation.

Clinical Evaluation and Quantitative Coronary Angiography

Prespecified clinical data, including demographics, medications, clinical history, and presentation, were obtained by research study coordinators using standardized data collection methods and verified by independent study monitors.

Coronary angiograms were analyzed by computer-assisted QCA. Specifically, clinically indicated coronary angiograms performed according to site protocols were digitized, deidentified, and analyzed with a validated quantitative protocol at the Cardiovascular Research Foundation, New York, NY, USA [10]. All lesions >10% diameter

stenosis (DS) in vessels with diameter >1.5 mm were visually identified, and the minimal lumen diameter (MLD), reference lumen diameter (RLD=average diameter of normal segments proximal and distal of lesion), and %DS ($\%DS = (1 - MLD/RLD) \times 100$) were calculated.

The Diamond–Forrester (D-F) risk score, comprised of age, sex, and chest pain type, was prospectively chosen to evaluate the value of the GES with clinical factors [11]. D-F classifications of chest pain type (typical angina, atypical angina, and nonanginal chest pain) were assessed using subject interviews [11] and D-F scores assigned [12].

Obstructive CAD and Disease Group Definitions

Obstructive CAD ($N=422$) was defined prospectively as ≥ 1 atherosclerotic plaque in a major coronary artery (≥ 1.5 mm lumen diameter) causing $\geq 50\%$ luminal DS by QCA; non-obstructive CAD ($N=744$) had no lesions $>50\%$.

Clinical Procedure and Event Determination

Clinical interventions were defined as any PCI or coronary artery bypass graft (CABG). Clinical events were defined as stroke/transient ischemia attack (TIA), MI, or death. Index coronary angiography was defined as the date of planned coronary catheterization, irrespective of intervention. Coronary procedures or events occurring within 30 days of index angiography were considered baseline endpoints associated with this procedure. In addition, specifically identified staged procedures up to 45 days post-index angiography were also considered baseline endpoints. Analysis of all procedures and events was performed for the 1,160 subjects over the entire follow-up period, as well as selective analysis for patients with procedures and events beyond the 30-day threshold.

All coronary procedures and events were monitored against medical records for accuracy and were supported by medical records documenting the specific event or diagnosis and/or by supporting evidence, e.g., myocardial enzyme elevation or infarct on head computed tomography (CT). Discrepancies were resolved by direct investigator query. All other events such as aortic aneurysm repair, congestive heart failure exacerbation, and cardiac arrhythmias were reviewed and eliminated due to noncardiac origin or lack of direct association with acute coronary atherosclerosis etiology. The definitions of the MACE components, MI, stroke/TIA, and all-cause mortality are detailed in the Supplementary Methods.

GES Measurements

GES measurements were performed in the CardioDx clinical reference laboratory (Palo Alto, CA, USA) using the

Corus™ CAD process [9]. Briefly, RNA was purified using an automated bead-based method from PAXgene® RNA preservation tubes (PreAnalytiX, Valencia, CA, USA). Subsequent cDNA synthesis and reverse transcription polymerase chain reaction were then carried out [9]. The GES were reported on a 1–40 scale.

Statistical Analysis

The primary endpoint for the study was whether the GES as a continuous variable was significantly related to the combination of procedures and MACE at 30 days and 12 months following index angiography. Subjects were censored if no event occurred prior to them being lost to follow-up. Only the first endpoint of a given type (procedure or event) was counted in the analysis. Secondary analyses included the relationship of the GES to MACE across the entire follow-up period and to the combination of revascularizations and MACE occurring >30 post-index catheterization.

For categorical analyses, the GES were divided into three ranges: 1–15 ($<20\%$ likelihood), 16–27 ($\geq 20\%$ – $<50\%$ likelihood), and 28–40 ($\geq 50\%$ likelihood) [9]. Logistic regression was used to test the relation between the GES (continuous) and events/procedures; for comparison to clinical factor scores, multivariate logistic regression was used. Odds ratios (OR), associated 95% confidence intervals (95% CI), and p values were also estimated by logistic regression. A prespecified GES threshold of ≤ 15 was used to estimate test performance (sensitivity, specificity, NPV, and positive predictive value [PPV]), as well as for categorical GES OR analyses. The Cochran–Armitage trend test was used to test the relation between GES categories and events/procedures. Clinical factors were compared at baseline using either a two-sample t test (continuous measures) or Fisher's exact test (binary measures). All analyses were performed in R version 2.11 [13].

Results

From 1,166 sequential PREDICT patients comprising the algorithm development and clinical validation cohorts with QCA and GES [9], 1,160 were eligible for follow-up and 1,116 (96%) were followed up for 1 year after index angiography. Clinical and angiographic characteristics of this entire cohort and the clinical validation subset ($N=526$) are shown in Table 1. The entire cohort was 58% male with an average age of 60. Factors which were significantly ($p < 0.001$) associated with angiographically defined obstructive CAD at baseline included male sex, age, systolic blood pressure (SBP), dyslipidemia, smoking status, chest pain

Table 1 Clinical and demographic characteristics of PREDICT patient cohorts

Set parameter	Complete cohort	Clinical validation subset
<i>N</i>	1,160	526
Male sex	668 (57.6%)	299 (56.8%)
Age	59.9±11.8 (25.5 to 90.9)	60.3±11.6 (25.6 to 90.9)
SBP	134.9±18.3 (88 to 213)	135.2±18.4 (90 to 213)
Dyslipidemia	734 (63.3%)	341 (64.8%)
Smoker	412 (35.5%)	186 (35.4%)
Symptomatic	762 (65.7%)	359 (68.3%)
BMI	30.8±6.8 (13.8 to 69.4)	30.7±6.5 (13.8 to 61.7)
Aspirin use	768 (66.5%)	363 (69.1%)
Statin use	580 (50.2%)	265 (50.5%)
Beta-blocker use	425 (36.8%)	212 (40.4%)
QCAMaxStenosis ^a	38.2±32.3 (0 to 100)	38.9±32.1 (0 to 100)
QCANumLesions ^b	1.8±2.3 (0 to 12)	1.9±2.4 (0 to 10)
QCAObsDisease ^c	420 (36.2%)	192 (36.5%)
One-year follow-up	1,115 (96.1%)	507 (96.4%)

^a Maximum percent stenosis determined by core laboratory QCA

^b Number of ≥30% stenotic lesions by QCA

^c Patients with ≥50% stenosis in a major coronary artery by QCA

symptoms, higher body mass index (BMI), aspirin, statin, and beta-blocker use (Table 1). Only 36% of patients had obstructive CAD (≥50% maximum percent stenosis) at index angiography.

Fig. 1 Schematic of patient flow and endpoint summary. A total of 1,166 patients from the algorithm development and validation cohorts were followed up. There were 6 late clinical exclusions, resulting in a final cohort of 1,160 of whom follow-up data was available for 1,143 (96%). A total of 267 had interventional procedures or events associated with their index angiographic procedure (within 30 days). The remaining 850 patients had a total of 25 endpoints (14 interventional procedures and 11 adverse events) in the subsequent follow-up period, for a total of 292 endpoints (25%) over 1 year

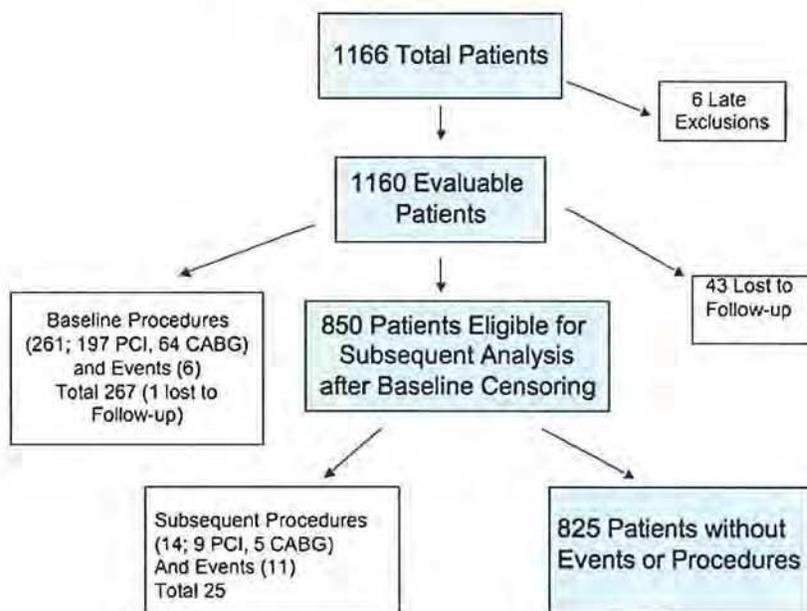


Table 2 Summary of procedures and events at 1-year follow-up

Parameter	
<i>N</i> at index angiogram	1,160
Baseline procedures	
PCI	203 (17.5%)
CABG	70 (6%)
Total procedures ^a	267 (23%)
Baseline events	6 (0.5%)
All baseline endpoints	267 (23%)
<i>N</i> with follow-up	1,116 (96%)
Follow-up procedures	14 (1.2%)
Follow-up events	11 (0.9%)
Total follow-up endpoints	25 (2.2%)
All procedures	286 (24.7%)
All events	17 (1.5%)
All endpoints ^b	292 (25.2%)

^a Some patients had more than one procedure and four patients had events after baseline procedures

^b The total baseline number of patients is used as the denominator for all calculations as baseline endpoints greatly dominate total endpoints. Some patients had more than one endpoint

The patient study flow is shown schematically in Fig. 1. A total of 267 patients (23%) had endpoints within 30 days of index procedure with the vast majority being PCI or CABG. After censoring these patients, there were only 25 additional patients (3%) with procedures or events in the next year out of the remaining 850, yielding an overall endpoint rate of 25% for all patients in the entire period. For MACE alone, the rate was 1.5% for 12 months. Events and procedures are summarized in Table 2.

GES Analysis

The GES, comprised of the peripheral blood cell expression levels of 23 genes and sex-specific age dependencies of CAD likelihood, was associated with the composite primary endpoint of MACE and procedures over 1 year by logistic regression ($p < 0.001$) and added to clinical factors, as quantified by D-F or Framingham risk scores (Supplementary Table 1). GES category also correlated with the likelihood of the combined procedures and MACE primary endpoint over this period as shown in Fig. 2a.

Previous analysis of obstructive CAD in the PREDICT clinical validation study identified a low likelihood ($< 20\%$) group with $\text{GES} \leq 15$ [9]. Using this threshold for the primary composite endpoint at 12 months follow-up, the sensitivity and specificity were 86% and 41%, respectively, corresponding to the NPV of 90% and PPV of 33%, with 396 patients (35%) in this group (Table 3). The OR for those with nonlow scores (> 15) versus low scores (≤ 15) for the 30-day and 12-month endpoints were 4.3 (95% CI, 3.0–6.4) and 4.3 (95% CI, 3.0–6.3), respectively, both $p < 0.001$ (Table 3).

There were 17 patients with MACE, of which 15 occurred more than 30 days after index angiogram; 4 of these patients had early revascularization. The clinical, angiographic, and MACE characteristics for all patient events are summarized in Table 4. The GES at index procedure was above 15 in 14 of 17 of these patients (Table 4, Fig. 2b). Thus, at most, 3 patients of 1,160 (0.3%) had both a low GES and an adverse event in the following year, yielding an NPV for events alone of 99.2%, although this did not reach statistical significance (OR=2.41, 95% CI=0.74–10.4, $p = 0.16$). There were a total of eight patients with late revascularizations whose characteristics are summarized in Table 5, with seven of eight having GES above 15. Patients with either late revascularizations or MACE more than 30 days post-index catheterization trended towards higher GES (OR=2.59, 95% CI, 0.89–9.14, $p = 0.082$) (Table 3); the relationship between the GES and these late revascularizations and events are illustrated in Fig. 2b.

Discussion

This study extended our previous validation of a blood-based GES for obstructive CAD in nondiabetic patients from an angiographic endpoint to revascularizations and MACE. We followed up and identified revascularizations and adverse events in 1,160 patients from the PREDICT trial, including the previously defined validation cohort of 526 patients for 12 months from index procedure. As expected, revascularization (PCI and CABG) were closely associated with maximum percent stenosis and angiographically determined

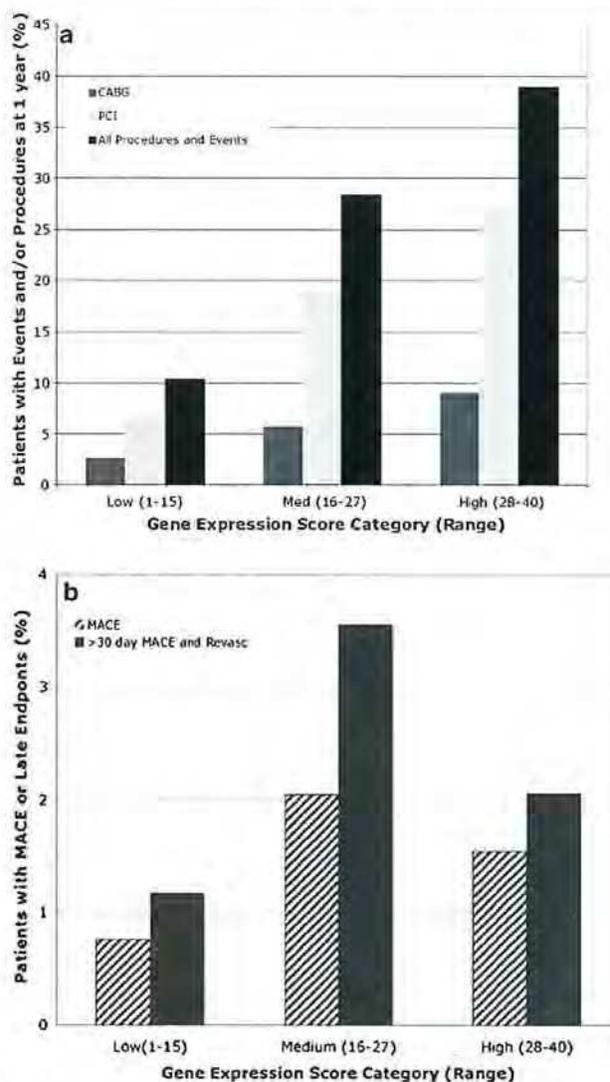


Fig. 2 a Dependence of event and interventional procedure likelihood on GES in 1 year. The percentage of patients who had interventional procedures or events within 1 year of the index catheterization are shown stratified by GES. GES are divided into low (1–15), medium (16–27), and high (28–40) categories as described in the text. Results are shown for the entire cohort of 1,160 patients. b Dependence of MACE likelihood on GES in 1 year. The percentage of patients who had MACE within 1 year of index catheterization are shown stratified by GES (striped bars). The percentage of patients with revascularization or MACE >30 days post-index catheterization are shown stratified by GES (solid bars). Scores are divided as in a. There were 3, 9, and 5 events for MACE alone (striped bars) and 4, 11, and 4 revascularizations and MACE (solid bars) in the low, medium, and high GES categories

disease burden, with the exception of chronic total occlusions which had a reduced intervention rate.

Our previous analysis showed that, in the validation set of 526 patients, using obstructive CAD as the endpoint, 33% of patients had $\text{GES} \leq 15$ with an NPV of 83%. For actual clinical endpoints up to 1 year, the NPV for all procedures and MACE was 90% at this threshold in the

Table 3 Dependence of combined procedure and MACE risk on GES

Duration and endpoints	NPV ^a (%)	PPV (%)	Sensitivity (%)	Specificity (%)	OR ^b	95% CI	P value
12-month procedures and MACE ^c	90	33	86	41	4.32	3.02–6.25	<0.001
12-month MACE	99	1.8	82	34	2.41	0.74–10.5	0.16 ^d
≤30-day procedures and MACE ^c	91	33	87	40	4.31	3.00–6.38	<0.001
>30-day procedures and MACE	99	3.0	79	41	2.59	0.89–9.14	0.082 ^e

^aNPV, PPV, sensitivity, and specificity were calculated at a threshold of 15

^bOR were calculated with a GES threshold of 15

^cProcedures (PCI or CABG) and MACE (MI, stroke/TIA, death) within 12 months of the index angiography

^dNot significant

^eProcedures and MACE occurring within 30 days of the index angiography

entire cohort. For those patients with GES ≤15 (396 of 1,160), representing 35% of total enrollment, only 41 of 1,160 (3.5%) had procedures or events. In these patients, the majority of endpoints (28 of 41) were PCI which has not been shown to improve long-term outcomes over optimal medical therapy in the COURAGE population [6].

It has been demonstrated that the fraction of obstructive CAD at cardiac catheterization in US patients without known CAD is 35–40% [5, 9]. In the entire cohort in this study, the yield of obstructive CAD was 36.2% and the

fraction of patients with interventions was 23.7%. If one did not send patients with low GES for catheterization, the yield of patients with obstructive CAD and interventions would be increased to 48.2% and 31%, respectively.

We previously observed that increasing GES correlated with maximum percent stenosis. In the current analysis, the composite endpoint likelihood also monotonically increased with GES from approximately 10% for low scores to >35% with high scores (28–40) with an OR of >4 (Fig. 2a). For high scores, this was likely an underestimate as >80% of

Table 4 Clinical characteristics of patients with subsequent events

Patient ID	Sex	Age (years)	QCACase: Control ^a	QCAMax Stenosis	ClinMax Stenosis	QCANum Lesions30 ^b	GES	Event	Days post index
C002:00400185	Male	83	Control	0	15	0	31	Stroke or TIA	328
C003:00400346	Female	58	Case	70	0	1	10	MI ^c	121
C004:00400011	Female	73	Control	39	50	1	17	MI	259
C005:00400009	Male	50	Case	100	100	1	18	CABG, MI, PCI	10
C014:00400055	Male	60	Case	76	90	5	29	Stroke or TIA	566 ^d
C015:00400040	Male	51	Case	57	40	3	24	Stroke or TIA	>180 ^d
C015:00400058	Female	46	Control	0	0	0	2	MI ^c	339
C015:00400064	Male	66	Control	15	80	0	25	Stroke or TIA	321
C015:00400092	Male	49	Control	19	30	0	25	MI	>180 ^f
C015:00400193	Female	66	Case	78	70	6	16	MI	129
C051:00400030	Male	63	Case	75	90	6	26	MI	1
C058:00400054	Male	69	Control	33	40	2	25	Death	>180 ^f
C063:00400007	Female	76	Case	80	90	5	27	MI	177 ^d
C068:00400065	Male	86	Case	81	95	1	37	Stroke or TIA	235 ^d
C073:00400040	Male	73	Control	44	65	1	30	Stroke or TIA	172 ^d
C073:00400065	Male	60	Case	63	40	3	14	MI	224
C079:00400014	Male	78	Case	100	50	7	39	Death	306

^aProspectively defined as ≥50% maximum stenosis

^bNumber of lesions >30% stenosis by QCA

^cDiscrepancy between clinical and core laboratory QCA reads; QCA confirmed on subsequent independent review

^dThese patients had a revascularization associated with their index catheterization

^eLikely vasospastic MI given underlying clinical condition and chart review

^fEvent reported at 1 year follow-up without specific date

Table 5 Clinical characteristics of patients with late revascularizations

Patient ID	Sex	Age (years)	QCA Case: Control ^a	QCAMax Stenosis	ClinMax Stenosis	QCANum Lesions ³⁰ ^b	GES	Procedure ^c	Days post index
C015:00400017	Male	54.4	Case	60.37	70	3	26	PCI	341
C054:00400009	Female	70.3	Case	100	80	3	25	CABG	75
C015:00400060	Male	55.5	Control	36.43	100	3	23	PCI	118
C055:00400036	Female	68.3	Control	24.2	40	0	20	PCI	345
C068:00400058	Female	55.2	Case	100	99	8	3	PCI	347
C001:00400105	Male	73.7	Case	60.7	90	2	32	PCI	70
C015:00400177	Male	64.5	Control	43.19	50	2	26	PCI	246
C068:00400087	Male	68.1	Case	100	100	5	37	CABG	84

^a Prospectively defined as $\geq 50\%$ maximum stenosis

^b Number of lesions $> 30\%$ stenosis by QCA

^c Either PCI or CABG occurring without prior intervention associated with index catheterization

patients with chronic total occlusions, who were electively not intervened on, had high scores. A large recent study of patients referred for CT angiography has also shown that overall mortality risk correlated with the extent of maximum percent stenosis [14].

For the small number of patients who had events, $> 80\%$ (14 of 17) had GES above the threshold of 15 (Tables 3 and 4), although this did not reach statistical significance. Retrospective analysis for the three patients with events and low GES showed one patient had no CAD angiographically with a GES of 2 and likely suffered a vasospastic MI. A second patient had no CAD by clinical angiogram, but subsequent QCA showed a 70% lesion. The third patient had a score of 14, close to the threshold, and an MI 7 months from index procedure. Thus, based upon clinical workup, 16 of 17 patients with events had scores above the threshold. Similarly, for late revascularizations, seven of eight had scores above 15.

A description of the genes which comprise the GES are shown in Table 6, along with the associated biological

functions, where known. The predominant features of these gene terms are the innate immune response, as judged by increased expression of activation genes in both neutrophils and natural killer (NK) cells, as well as an increase in proapoptotic genes (terms 1–3). In addition, term 2, and specifically S100A12, has been shown to promote coronary artery calcification in a transgenic model [15]. In addition, the somewhat counterintuitive B cell to T cell ratio comprises term 4. Although it was originally thought that B cells were atheroprotective and T cells atherogenic, recent work in mouse models has suggested a more complex picture with atherogenic B cell subsets [16] and a potential atheroprotective role for regulatory T cells [17, 18].

Given that the GES was derived to discriminate obstructive CAD, why might it have prognostic value? First, the GES is proportional to maximum percent stenosis by angiography and a recent large CT angiography study has shown that event likelihood increases with the extent of disease, even for nonobstructive disease [19]. Second, specific terms in the GES algorithm

Table 6 GES components and putative biological roles

Term	Genes	Functions
1	IL18RAP+TNFAIP6+CASP5 IL8RB+KCNE3+TLR4+TNFRSF10C	Innate immunity, apoptosis Neutrophil activation
2	S100A8+S100A12+CLEC4E RPL28 (men), NCF4+AQP9 (women)	Neutrophil activation and necrosis Calcification Neutrophil/lymphocyte ratio (men) Normalized neutrophil activation (women)
3	SLAMF7+KLRC4 TMC8+CD3D	Innate immunity, NK cell activation Normalized to T lymphocytes
4	SPIB+CD79B TMC8+CD3D	B/T cell ratio Lymphocyte subtype
5+6	AF289562+TSPAN16 (men) TFCP2+HNRPF	Unknown function genes

reflect cell type-specific gene expression ratios, which in the case of the neutrophil to lymphocyte ratio has been shown to have prognostic significance in a large catheterization laboratory population [20]. In addition, a very recent large study has shown that neutrophil counts alone are associated with subsequent MI and mortality [21]. Third, circulating levels of the protein products of genes which are present in the GES, such as S100A8 and S100A12, have been shown to be associated with cardiovascular events [22, 23]. Finally, the observed GES proportionality to disease burden is most likely a reflection of the dysregulation of gene expression in the circulating cells in response to both the extent and inflammatory activity of atherosclerotic plaque, perhaps reflecting plaque composition.

This study had several limitations. First, the population was nondiabetic and largely symptomatic with high-risk unstable angina and low-risk asymptomatic patients excluded. Second, the follow-up period was limited and the number of events subsequent to the index catheterization small. Thus, any conclusions about the PPV of the GES for prognosis will require larger cohorts, more extended follow-up, and a higher absolute number of cumulative events. Given the observed OR for MACE in this study, we estimate that a study of 2,300 patients with 2-year follow-up would have 80% power to detect a significant relationship of the GES to MACE. The PROMISE study (<http://www.clinicaltrials.gov>, NCT 01174550) might be an appropriate setting to further test this hypothesis. Third, we did not have lesion-specific information to determine if revascularizations or events were due to baseline-identified lesions or disease progression. Fourth, since this was an angiographic population, it had more disease than an intended use population before referral, which may affect the results. Fifth, with respect to the GES analysis, the combined cohort may have been biased by inclusion of the algorithm development set. This seems unlikely to be a very significant factor as procedures and events were not used to derive the algorithm, and the validation subset analyses showed results indistinguishable from the entire population. Finally, while the GES added significantly to Framingham with respect to the primary composite endpoint, it did not add significantly to MACE prediction alone, although that comparison was underpowered due to the low event rate.

In summary, this study examined the relationship between a peripheral blood GES measured at index angiography and revascularization and MACE at up to 12 months. Independent of the GES, more than 75% of patients had neither a procedure nor MACE in the next year. For those with low GES, representing 35% of patients, 90% were in this category. Thus, low GES appeared to identify a population at low risk for both obstructive CAD and subsequent procedures or events. While these results were encouraging

for a clinical correlation with the initial angiographic validation, studies in larger populations with longer-term follow-up would be needed to further support this hypothesis.

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Conflict of Interest This work was funded by CardioDx, Inc.; MRE, BB, and SR are employees and have equity interests and/or stock options in CardioDx, Inc. HDL is a consultant employee at CardioDx, Inc. WEK, RSS, SV, and SGE report research support, JM reports minor consulting income, and AL reports funding for QCA studies all from CardioDx, Inc. EJT is supported in part by the Scripps Translational Science Institute Clinical Translational Science Award from the National Institutes of Health (NIHU54RR02504-01). RW reports no conflicts of interest with respect to this manuscript.

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A gender-specific blood-based gene expression score for assessing obstructive coronary artery disease in nondiabetic patients: Results of the Personalized Risk Evaluation and Diagnosis in the Coronary Tree (PREDICT) Trial

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Background Currently available noninvasive tests to risk stratify patients for obstructive coronary disease result in many unnecessary cardiac catheterizations, especially in women. We sought to compare the diagnostic accuracy of presenting symptoms, noninvasive test results, and a gene expression score (GES) in identifying obstructive coronary artery disease (CAD) according to gender, using quantitative coronary angiography as the criterion standard.

Methods The PREDICT trial is a prospective multicenter observational study designed to develop and validate gene expression algorithms to assess obstructive CAD, defined as at least one $\geq 50\%$ diameter stenosis measured by quantitative coronary angiography. Patients referred for diagnostic cardiac catheterization with suspected but previously unknown CAD were enrolled. Noninvasive myocardial perfusion imaging (MPI) was available in 60% of patients. The GES, comprising gender-specific age functions and 6 gene expression terms containing 23 genes, was performed for all patients.

Results A total of 1,160 consecutive patients (57.6% men and 42.4% women) were enrolled in PREDICT. The prevalence of obstructive CAD was 46.7% in men and 22.0% in women. Chest pain symptoms were a discriminator of obstructive CAD in men ($P < .001$) but not in women. The positive predictive value of MPI was significantly higher in men (45%) than in women (22%). An abnormal site-read MPI was not significantly associated with obstructive or severity of CAD. The GES was significantly associated with a 2-fold increase in the odds of obstructive CAD for every 10-point increment in the GES and had a significant association with all measures of severity and burden of CAD. By multivariable analysis, GES was an independent predictor of obstructive CAD in the overall population (odds ratio [OR] 2.53, $P = .001$) and in the male (OR 1.99, $P = .001$) and female (OR 3.45, $P = .001$) subgroups separately, whereas MPI was not.

Conclusions Commonly used diagnostic approaches including symptom evaluation and MPI performed less well in women than in men for identifying significant CAD. In contrast, gender-specific GES performed similarly in women and men. Gene expression score offers a reliable diagnostic approach for the assessment of nondiabetic patients and, in particular, women with suspected obstructive CAD. (*Am Heart J* 2012;0:1-7.)

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Noninvasive diagnostic testing for detecting coronary artery disease (CAD) remains a challenge in clinical practice because of variable site-dependent diagnostic accuracy; high false-negative rates, particularly in women¹; cost; and associated adverse effects such as radiation exposure, exercise intolerance, and interreader variability.² This has resulted in overutilization of invasive diagnostic catheterization in approximately 50% to 60% of referred cases.³ Therefore, a gender-specific blood test designed to reliably and noninvasively identify patients with CAD would have diagnostic utility to improve

clinical decision making and minimize unnecessary cardiac catheterizations.

The PREDICT prospective, multicenter, observational study was designed to develop and validate a gene expression score (GES).⁴ The GES comprises age, sex, and expression levels of 23 genes and is validated to assess the likelihood of obstructive CAD in a population of nondiabetic patients with suspected obstructive CAD. We report the gender-specific results of clinical factors, noninvasive imaging, and GES in the PREDICT trial.

Materials and methods

Patient selection and definitions

The PREDICT trial is a prospective multicenter observational study designed to develop and validate a gene expression algorithm to assess the likelihood of obstructive CAD.⁴ Subjects were eligible for enrollment in PREDICT if they were older than 20 years; had a history of chest pain, anginal symptoms suggesting myocardial ischemia, unstable angina, or asymptomatic with a high risk of CAD with no known prior CAD. Patients with known CAD, New York Heart Association class III or IV, left ventricular ejection fraction < 35%, severe valve regurgitation or stenosis, systemic infection, known rheumatologic, autoimmune or hematologic conditions, and organ transplant; and were requiring immunosuppression, chemotherapy, or transfusion within the preceding 2 months were excluded. In addition, diabetic patients were excluded because of their distinct gene expression signature relative to CAD classification.⁴ *Angina status* was defined based on the Diamond-Forrester (D-F) classification⁵ as follows: *typical angina*, requiring all 3 features: (1) substernal chest discomfort with a characteristic quality and duration that was (2) provoked by exertion or emotional stress and (3) relieved by rest or nitroglycerin; *atypical angina*, required 2 of the typical anginal characteristics; and *nonangina*, required 1 or none of the typical anginal characteristics. Subjects without chest pain were classified as *asymptomatic*.

Complete blood counts with differential were obtained for all patients, and serum biomarkers (cholesterol and high-sensitivity C-reactive protein [hsCRP]) were obtained for most patients. Whole blood samples were collected on all patients and processed as previously described.⁴ Noninvasive stress testing including exercise or pharmacologic myocardial perfusion imaging (MPI) was categorized as positive, negative, or indeterminate based on the site-reported clinical interpretation. All patients gave written informed consent, and the study protocol was approved by the institutional review board at 39 participating US centers. To maintain a balanced population, no single clinical study center was permitted to enroll more than 20% of the entire study population.

Gene expression score

The GES was derived by dividing the study population into a development cohort and validated with a validation cohort. The clinical characteristics of the 2 cohorts were similar.⁴ The development and validation of the GES have been described in detail.⁴ In brief, the algorithm uses gender-specific age terms and 6 gene expression terms, each composed of several genes,

for a total of 23 genes. Some of the gene expression terms are specific for men, some are specific for women, and some apply to both genders. The output of the test is a score based on a 1- to 40-point scale, where increasing scores indicate increasing likelihood of obstructive CAD. All gene expression tests were performed in the CardioDX CLIA-approved reference laboratory at 2500 Faber Place, Palo Alto, CA, using the Corus CAD protocols.⁴

Quantitative angiography and case/control definitions

All angiograms were reviewed by an independent angiographic core laboratory for quantitative angiography (QCA) using standardized methodology (Medis, Leiden, the Netherlands). All major epicardial vessels and side branches (> 2 mm in diameter) were systematically screened for the presence of CAD. All lesions with a visual diameter stenosis of $\geq 20\%$ were identified and underwent QCA. Cases were defined by a $\geq 50\%$ stenosis by QCA in at least 1 epicardial vessel or major side branch (≥ 2.0 mm) and controls with < 50% stenosis in all epicardial vessels and major side branches. In addition to obstructive disease, 3 measures of disease severity were calculated based on the QCA results, including (1) maximum percent stenosis, (2) the number of lesions with stenosis of $\geq 20\%$, and (3) the total plaque volume (product of lesion length and cross section area, summed over all lesions). The sponsor of the PREDICT trial was CardioDX.

Statistical methods

A series of regression models were fit with GES and clinical risk factors as predictor variables of CAD disease severity (> 50% disease severity) as the response. Likelihood ratio χ^2 statistics were then used to assess the additive contribution of the GES vs the clinical risk factors alone. Analysis of MPI used the same methods as for the GES. The response variables were angiographic maximum percent diameter stenosis, total plaque volume, number of lesions (> 30% stenosis), and obstructive disease (stenosis $\geq 50\%$). For the continuous measures of disease severity (maximum percent stenosis, total plaque volume), linear regression was used with GES and clinical risk factors as independent variables. For clinical risk factors, the Framingham risk score was used (includes sex, age, sex by age interaction, blood pressure, lipids, and smoking status) in combination with D-F symptom characteristics. For the ordinal measure of disease severity (number of lesions), Poisson regression was used with GES and clinical factors as independent variables. Finally, for the binary outcome of obstructive disease, logistic regression was used with GES and clinical factors as independent variables. The above regression analyses were conducted in the overall validation and development cohort and in the male and female subgroups. Multivariable logistic regression analysis was performed to assess the predictors of obstructive CAD (dependent variable) in the overall population and in men and women subgroups separately. Independent variables included in the model were as follows: age, age squared, sex, typical angina, hyperlipidemia, hypertension, age/sex interaction, MPI (positive/negative), and GES. For univariate analyses, categorical variables were compared by χ^2 test and continuous variables by t test. All statistical methods were performed using the R software package, version 2.11.0 (including the MASS and rms packages).⁶

Table 1. Baseline demographic and clinical characteristics by gender

	Total		P [†]
	Women	Men	
n [‡]	492	668	–
Age (y), mean (quartiles)	60 (52, 69)	59 (51, 68)	.122
Body mass index (kg/m ²), mean (quartiles)	31 (26, 35)	31 (26, 34)	.306
Race			.332
White (%)	87.8	88.9	.332
Black (%)	6.7	4.8	
All other (%)	5.5	6.3	
Hypertension (%)	66.7	64.2	.423
Smoker (%)	17.3	20.5	.191
Dyslipidemia (%)	63.4	63.2	.982
Family history of CAD (%)	3.5	2.8	.673
Peripheral vascular disease (%)	3.3	3.3	.898
Statin therapy (%)	35.8	38.9	.301
Aspirin therapy (%)	58.1	65.9	.009
β-Blockers (%)	40.4	32.5	.006
Laboratory measures			
Total cholesterol (mg/dL), mean (quartiles)	192 (169, 214)	181 (151, 205)	<.001
LDL cholesterol (mg/dL), mean (quartiles)	117 (93, 135)	115 (86, 135)	.611
HDL cholesterol (mg/dL), mean (quartiles)	57 (47, 65)	45 (36, 51)	<.001
hsCRP, mean (quartiles)	4.6 (1.2, 5.0)	2.8 (0.7, 3.0)	.017
WBC count, mean (quartiles)	6.7 (5.5, 7.7)	6.7 (5.6, 7.7)	.494
D-F chest pain presentation (%)			<.001
Asymptomatic	27.2	39.5	
Atypical	16.3	14.4	
Nonanginal	40.0	25.6	
Typical	15.9	19.6	
% Obstructive disease	22.0	46.7	<.001
Maximum % stenosis, mean (quartiles)	27.2 (0, 43.0)	46.2 (20.2, 73.4)	<.001
No. of obstructive lesions, mean (quartiles)	0.44 (0, 0)	1.2 (0, 2)	<.001
No. of lesions, mean (quartiles)	1.6 (1, 2)	3.2 (1, 5)	<.001
Total plaque volume (mm ³), mean (quartiles)	32.2 (0, 40.6)	79.6 (10, 128)	<.001

LDL, low-density lipoprotein; HDL, high-density lipoprotein; WBC, white blood cell count.³

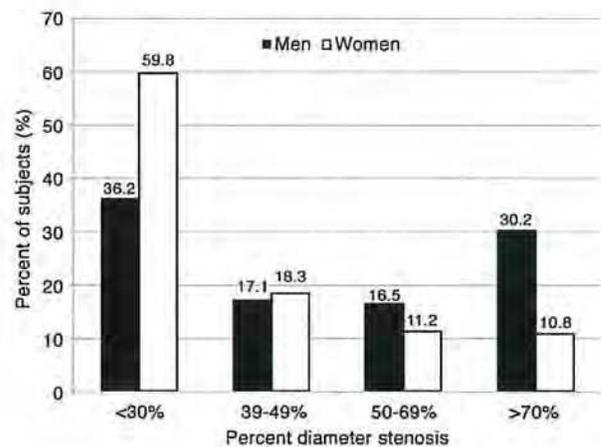
[†]P values were calculated as described in "Materials and methods" and are unadjusted for multiple comparison.

[‡]n = number of patients.

The authors are solely responsible for the design and conduct of this study, all study analyses, the drafting and editing of the paper, and its final contents.

Results

The development and validation cohorts from the PREDICT trial comprised 1,166 consecutive nondiabetic

Figure 1

Extent and severity of CAD by sex as defined by quantitative coronary angiography. The maximum percent stenosis is depicted categorically as < 30%, 30% to 49%, 50% to 69%, and 70% in a sex-stratified representation.

patients. Coronary angiograms of suitable quality for quantitative analysis were available in 1,160 of the 1,166 patients in these cohorts. Baseline demographics demonstrated that men and women had similar risk factors including age, body mass index, race distribution, and incidence of hypertension and dyslipidemia (Table 1). Women were less likely to be on aspirin therapy but more likely to be on β-blockers than men. Women had significantly higher hsCRP, high-density lipoprotein and total cholesterol, but low-density lipoprotein cholesterol was similar between sexes. Women had less extensive CAD (mean number of lesions $\geq 20\%$, $P < .001$), less severe CAD (maximum stenosis, $P < .001$), and less plaque volume (32 mm^3 vs 80 mm^3 , $P < .001$) compared with men (Table 1). Obstructive CAD (stenosis $\geq 50\%$ by QCA) was present in 36.2% of the population, 46.7% in men and 22.0% in women (Figure 1). Overall, 26.1% of patients had no evidence of CAD (stenosis < 10% by QCA); this was more common in women (17.7% in men and 37.6% in women).

Clinical correlates of obstructive CAD

For both men and women, the presence and severity of disease were characterized by older age, hypertension, and dyslipidemia. Diamond-Forrester symptom characteristics were significantly associated with all measures of CAD severity in men but not in women (Table 2). In men, typical chest pain symptoms were associated with a 2-fold increase in the rate of obstructive CAD ($P = .002$) and significantly greater number of lesions, maximum percent diameter stenosis, and plaque burden ($P < .001$).

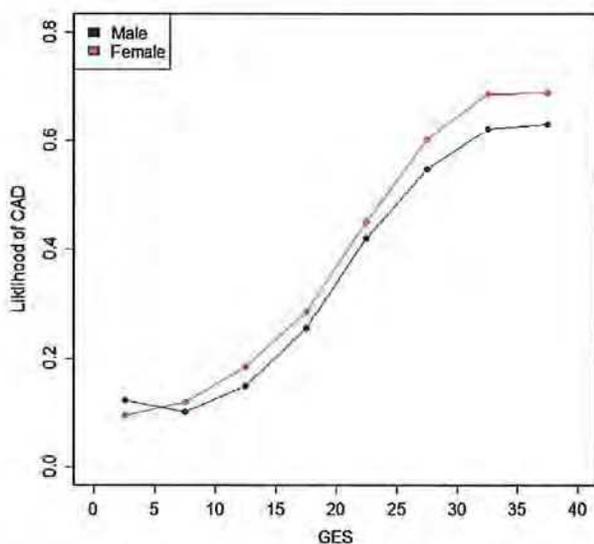
Table II. Chest pain, MPI, and GES association with CAD

	Obstructive disease (OR)		Maximum stenosis (change in % stenosis)		No. of Lesions (change in the number of lesions)		Total plaque burden (change in mm ³)	
	Women	Men	Women	Men	Women	Men	Women	Men
Chest pain association with CAD								
Atypical*	1.13; <i>P</i> = .72	1.52; <i>P</i> = .08	0.0; <i>P</i> = .99	4.2; <i>P</i> = .26	0.18; <i>P</i> = .08	0.14; <i>P</i> = .028	7.0; <i>P</i> = .33	4.9; <i>P</i> = .61
Typical*	1.52; <i>P</i> = .20	2.0; <i>P</i> = .002	2.1; <i>P</i> = .61	13.1; <i>P</i> < .001	0.13; <i>P</i> = .22	0.25; <i>P</i> < .001	11.9; <i>P</i> = .13	34.6; <i>P</i> < .001
MPI as a predictor of CAD								
MPI +†	1.21; <i>P</i> = .53	1.48; <i>P</i> = .09	2.6; <i>P</i> = .49	4.6; <i>P</i> = .21	- 0.08; <i>P</i> = .39	0.16; <i>P</i> = .02	- 4.7; <i>P</i> = .46	14.0; <i>P</i> = .14
GES association with CAD								
GES‡	1.99; <i>P</i> < .001	1.80; <i>P</i> < .001	8.6; <i>P</i> < .001	7.5; <i>P</i> < .001	.19; <i>P</i> < .001	.19; <i>P</i> < .001	14.8; <i>P</i> < .001	14.8; <i>P</i> = .01

*Relative to no angina.

†Relative to MPI-.

‡GES per 10-point increase in score, adjusted for clinical factors (see "Materials and methods").

Figure 2

Probability of obstructive CAD as a function of increasing GES for men (blue) and women (red).

In women, neither typical nor atypical symptoms were associated with significant increases in any of the measures of CAD.

Predictive value of site-interpreted MPI

Site-interpreted MPIs were available in 689 (59%) of 1,160 patients (59% in men and 60% women). An abnormal MPI identified obstructive CAD in 45% of men and 22% of women, and a normal MPI correctly identified the absence of obstructive CAD in 65% of men and 82% of women. In general, MPI positivity was not significantly

associated with any measures of CAD in either men or women (Table II).

Predictive value of the GES

The probability of obstructive CAD increased with increasing GES scores for both men and women (Figure 2). The GES, after accounting for clinical factors, was significantly ($P < .001$) associated with every measure of CAD extent and severity that was assessed for both men and women (Table II). Each 10-point increase in GES was associated with approximately a 2-fold increase in the odds of obstructive disease, as well as an increase in maximum percent stenosis, number of lesions, and total plaque volume. These associations were independently significant in both men and women. Overall, when GES was added to clinical factors, it improved the predictive value for each of the outcome measures (Table III). By multivariable regression analysis, GES was an independent predictor of obstructive CAD in the overall population (odds ratio [OR] 2.53, $P = .001$), as well as in the male (OR 1.99, $P = .001$) and female (OR 3.45, $P = .001$) subgroups separately, whereas MPI was not (Table IV).

Discussion

This study demonstrates that in a contemporary US-based population referred to diagnostic angiography for suspected CAD, only 36.2% of patients and only 22.0% of women had obstructive CAD with a characteristic age-dependent rise in the prevalence of CAD after 60 years of age for women. Although symptoms on presentation were helpful in identifying obstructive CAD in men, symptoms of angina as defined by Diamond and Forrester⁵ were not helpful in predicting CAD in women. In this population referred to angiography, the diagnostic value of MPI as interpreted by clinical sites was modest at best, positively identifying fewer than 50% of men and 25% of women with

Table III. Additive value of MPI and GES to clinical factors

	Obstructive Disease	Max Stenosis	Number of Lesions	Total Plaque Burden
Clinical Model*	$\chi^2 = 87.0, p < .001$	$\chi^2 = 124.7, p < .001$	$\chi^2 = 298.7, p < .001$	$\chi^2 = 85.1, p < .001$
Addition of MPI	$\chi^2 = 6.9, p = .008$	$\chi^2 = 5.7, p = .017$	$\chi^2 = 10.0, p = .002$	$\chi^2 = 3.9, p = .048$
Addition of GES	$\chi^2 = 176.8, p < .001$	$\chi^2 = 200.7, p < .001$	$\chi^2 = 457.0, p < .001$	$\chi^2 = 161.9, p < .001$

* See Methods

Table IV. Independent predictors of obstructive CAD in all patients, female and male

	OR	Lower 95% CI	Upper 95% CI	P
All patients				
GES (per 10 points)	2.535	1.867	3.444	<.001
Typical chest pain	1.94	1.374	2.739	<.001
Sex	1.21	0.161	9.08	.853
Age (per 10 y)	6.298	1.893	20.948	.003
Age ² (per 10 y)	0.867	0.789	0.954	.003
Dyslipidemia	1.601	1.196	2.144	.002
Hypertension	1.45	1.073	1.96	.016
MPI positive	0.876	0.662	1.159	.353
Sex * age interaction	0.969	0.727	1.291	.83
Female				
GES (per 10 points)	3.449	1.972	5.911	<.001
Typical chest pain	1.461	0.783	2.724	.234
Age (per 10 y)	4.665	0.509	42.73	.174
Age ² (per 10 y)	0.869	0.715	1.058	.163
Dyslipidemia	1.692	0.983	2.912	.058
Hypertension	1.675	0.921	3.046	.091
MPI positive	0.737	0.442	1.229	.244
Male				
GES (per 10 points)	1.999	1.35	2.961	.001
Typical chest pain	2.204	1.443	3.367	<.001
Age (per 10 y)	10.018	2.443	41.072	.001
Age ² (per 10 y)	0.84	0.749	0.942	.003
Dyslipidemia	1.603	1.132	2.27	.008
Hypertension	1.379	0.971	1.959	.073
MPI positive	0.946	0.676	1.325	.748

CI, Confidence interval; GES, GES per 10-point increase in score, adjusted for clinical factors (see "Materials and methods").

obstructive CAD; conversely, the false-negative MPI rate was as high as 35% in men and 18.5% in women. An abnormal MPI did not correlate with any measure of severity or extent of CAD in either men or women. In contrast, the GES had a significant association with all measures of CAD extent and severity, with a 2-fold increase in risk of obstructive CAD for every 10-point increment in GES. Furthermore, the GES was the second strongest independent predictor of obstructive CAD (following age) in the overall population and the strongest predictor of obstructive CAD in women, and it offers a reliable alternative to MPI for predicting obstructive CAD based on a simple office-based blood sample.

The accurate diagnosis of obstructive CAD remains challenging in clinical practice, resulting in overreferring to invasive diagnostic coronary angiography.³ Reliance on symptoms in guiding further evaluation of chest pain syndromes is well established in the early description by Diamond and Forrester.⁵ However, this classification was defined in a predominantly male population and is unreliable in women. There has been an emphasis on the atypical nature of anginal symptoms in women, such as vague chest discomfort and/or nausea,⁷⁻⁹ making it difficult to determine when noninvasive or invasive imaging is the most appropriate form of evaluation. The recognition that traditional chest pain classification is less reliable in women is clearly emphasized in the PREDICT trial, where symptoms as defined by D-F could not discriminate the presence of obstructive from nonobstructive CAD in women.

Current scientific guidelines endorse the use of noninvasive cardiac testing for improved detection in symptomatic individuals with intermediate pretest likelihood of CAD.¹⁰⁻¹⁴ However, despite the difficulty of predicting the presence of CAD according to clinical presentation in women, guideline recommendations extend to women with either intermediate or high pretest likelihood of disease,¹¹ which has the potential of further worsening the false-negative rates of noninvasive tests and missing the diagnosis of CAD. At present, CAD assessment for patients with either intermediate or high pretest likelihood of disease is most commonly performed by functional testing with myocardial perfusion single-photon emission computed tomography; however, in women, guidelines still recommend standard treadmill ECG testing for patients without diabetes, with normal baseline ECG, who are able to exercise,¹³ despite the limited diagnostic value of standard treadmill testing in women.^{1,15} Although a number of noninvasive testing modalities are available, each present different limitations and none have perfect accuracy, particularly in women.¹ The overall reported sensitivity and specificity for exercise ECG (52% and 71%),² stress echocardiography (85% and 77%),^{2,12} single-photon emission computed tomography (87% and 64%),² and perfusion magnetic resonance imaging (91% and 81%),^{16,17} compared with angiography as the criterion standard, tend to reflect best case scenarios based on independent central

laboratory evaluations that exclude poor quality cases rather than evaluations in everyday clinical practice. The results of the PREDICT trial highlight the low positive predictive value particularly in women (< 25%) and high false-negative rate (> 35% in men) of MPI when interpreted by clinical sites in the everyday clinical practice setting.

Rationale for blood-based GES in the diagnostic paradigm

The GES evaluated in this study is unique because it is the only gender-specific diagnostic test currently available that risk stratifies patients with obstructive CAD. The results of our analyses show that GES was significantly correlated with obstructive CAD as well as other measures of CAD disease severity and burden for both men and women, whereas MPI was not. This study provides proof of concept that in this patient population, GES can add diagnostic information with the ultimate goal of better identifying the appropriate patient for invasive angiographic referral.⁴

The development of CAD is caused by a complex interaction of genetic and environmental factors. Although traditional clinical risk factors including gender, age, hypertension, and dyslipidemia are well-established predictors of CAD, efforts have now focused on studying biological markers of CAD development. Inflammation plays an important role in the development of atherosclerosis and CAD.^{18,19} This inflammatory process gives rise to systemic indicators of inflammation that have been correlated with the risk of future coronary events at the patient level, such as elevated levels of CRP associated with an increased risk of MI or cardiac death.²⁰⁻²² The incorporation of these markers of inflammation into risk classification models has variably improved the ability to predict cardiovascular risk.²³⁻²⁵ Furthermore, studies have shown that alterations in circulating white blood cell counts, such as increases in neutrophil to lymphocyte ratios, are correlated with increased cardiovascular risk^{26,27} and CAD.²⁸

The recent evidence that gene expression in peripheral blood cells reflects the presence and extent of CAD²⁹ in patients undergoing angiography and can accurately identify patients with obstructive CAD is the first step in a paradigm shift in identifying patient risk based on the biology (including gender-specific factors) of underlying disease rather than ischemic thresholds identified by imaging modalities or performance abnormalities. The GES analyzed herein uses this information and known clinical risk factors (age and gender) with gene expression changes in circulating white blood cells to provide an objective method (not subject to site-to-site interpretation variability) to evaluate for obstructive CAD. Our analysis of the PREDICT study demonstrates that GES is a strong independent predictor of obstructive CAD and

demonstrates the improved ability of GES to identify obstructive CAD, especially in nondiabetic women, compared with clinical presentation algorithms or MPI.

Limitations

Several limitations of this study must be taken into consideration. The results of these analyses can only be applied to the patient cohort meeting the eligibility criteria of the PREDICT study. Thus, this diagnostic test should not be applied to patients with diabetes, with known heart failure, or who have received blood transfusions within the last 2 months. Furthermore, significant CAD determined by angiography (disease severity $\geq 50\%$ by QCA) was the end point used to evaluate this diagnostic test. Thus, this test has not yet been studied to predict the risk of future cardiovascular events. In addition, beyond only a subset of the cohort having MPIs, there is a significant referral bias inherent in the analysis because patients with negative MPI were probably less likely to be referred to diagnostic catheterization, therefore impacting the reported negative predictive value of MPI. Irrespective of these limitations, the comparative validation study applies equally to both MPI and GES, and the positive predictive value should not be greatly impacted by the referral bias because patients with positive MPIs are likely to be referred to diagnostic catheterization given current practice patterns.

Conclusion

The use of a blood-based GES may be particularly helpful in the assessment of obstructive CAD in nondiabetic patients and, in particular, women for whom the use of symptoms and functional testing has proven unreliable. Further studies are needed to validate whether this test or other methods that use individualized genomic data will help promote more efficient and appropriate use of coronary angiography in women.

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The Winners, Category by Category

From Computing Systems to Wireless, the Most Innovative Technologies

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By MICHAEL TOTTY

Updated Sept. 27, 2010 12:01 a.m. ET

(See [Correction & Amplification below](#).)

This year the Innovation Awards judges chose winners in 17 categories. Here's a look at the winning entries.

Computing Systems

Journal Reports

Read the complete [Technology: Innovation Awards report](#).

Plus, get an [update on past winners](#) and read opinions from innovators on [what will win in coming years](#).

Lightfleet Corp., based in Camas, Wash., won in this category for a novel way of connecting computer processors, using beamed light instead of copper or fiber-optic wires.

In big data centers, even the fastest servers get slowed by bottlenecks in the connections between microprocessors, or nodes. Lightfleet's technology aims to eliminate the bottlenecks by replacing the wired switches typically used to manage these connections with a device that sends a data-carrying beam of light to all the nodes at once. The faster transmission of data promises to make it possible, for example, to run Wall Street's high-speed trading operations more efficiently.

The company, founded in 2003, delivered a prototype of its first product earlier this year to [Microsoft \(MSFT +2.49%\)](#) Research, the R&D arm of the computer giant, which will test how it handles different applications. A Lightfleet spokesman says the company expects the first commercial sales by the middle of next year.

RUNNER-UP

Marvell Semiconductor Inc., U.S.: A small, low-power networked home server, called the Plug Computer, that can deliver data and applications to a variety of devices.

Consumer Electronics

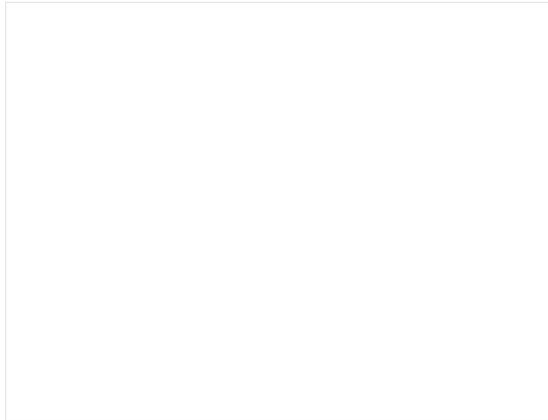
Industrial Technology Research Institute, winner of the overall Gold award, won in this category. (See "[Paper-Thin Screens With a Twist](#)")

RUNNERS-UP

NanoLumens Inc., U.S.: Lightweight digital displays that are flexible, thin and energy efficient. The first product, a 112-inch display, weighs less than 90 pounds, is less than an inch thick and consumes less energy than five light bulbs.

Ford Motor Co. (F +1.46%), U.S.: MyFord Touch, an instrument panel for cars that replaces traditional buttons, knobs and gauges with voice commands, customizable LCD screens and five-way controls on the steering wheel similar to those on cellphones and MP3 players.

Nokia Corp. (NOK +0.51%), Finland: An "augmented reality" browser for mobile devices, called Point & Find, that lets users get information about real-life objects by



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1 **Mac Pro vs. iMac: How Much Computer Do You Need?**



pointing a camera phone at the object.

E-Commerce

New Orleans-based Receivables Exchange LLC won in the e-commerce category—the first winner in this group since 2004—for its online marketplace where small and midsize businesses can auction their receivables.

Smaller companies don't have the same access to financial markets that their larger counterparts do, so it's especially difficult for them to raise short-term working capital. Taking out a loan backed by receivables—known as factoring—is common in some industries. But for most small and midsize businesses, a factoring deal can be costly and often takes a long time to arrange.

Receivables Exchange aims to make it much easier for a company to tap the cash locked in its receivables. A company posts its unpaid invoices on the exchange, which screens the seller to make sure it has a certain minimum revenue and has been in business for at least two years. The screening can be completed within 24 hours and the invoices can be posted the next day. Bidders offer to buy some or all of the posted receivables, and the exchange takes commissions from the buyer and seller.

The company was launched in 2007 by Justin Brownhill, a former investment banker who is now Receivables Exchange's chief executive, and Nicolas Perkin, its president. The exchange hosts between \$1 million and \$5 million in trades each day, a spokeswoman says; it doesn't reveal its revenue.

Energy

InEnTec LLC, based in Bend, Ore., won in the energy category for a process that uses high-temperature plasma gasification to produce synthetic fuel from municipal and industrial waste.

The technology offers a cleaner alternative to using incinerators to burn garbage.

The company's Plasma Enhanced Melter heats the waste in a super-hot plasma. This produces a synthetic gas that can be converted to ethanol, methanol, clean diesel and other transportation fuels. Ash from the process is captured in molten glass, producing an obsidian-like material that can be buried in landfills or used in construction materials. Metals are captured separately and can be recycled.

Plasma gasification isn't a new technology; companies have used it for more than a decade to break down industrial and medical waste. Other companies are planning plasma-gasification plants to convert municipal waste, and a pilot plant from U.K.-based Advanced Plasma Power has been in operation since 2007. But InEnTec says its technology is more energy efficient than other plasma-gasification systems.

InEnTec was formed in 1995 by researchers who had studied and improved the technology in a collaborative effort between the Massachusetts Institute of Technology and the U.S. Department of Energy's Pacific Northwest National Laboratory. Last year, the company created a joint venture with Houston-based [Waste Management Inc.](#) to build and operate plasma-gasification facilities using InEnTec's technology. The first, planned for Arlington, Ore., is scheduled to open by the end of the year, with the capacity to handle 25 tons of waste a day.

RUNNERS-UP

Enphase Energy, U.S.: The Enphase Microinverter System, which converts the direct-current output of solar panels to the alternating current used in homes and businesses. The system includes a meter that collects information about panels' performance and sends it to a website where customers can view the data.

Idaho National Laboratory, U.S.: An efficient, environmentally friendly process for making high-quality biodiesel from waste fats, oils and greases.

Solexant Corp., U.S.: Ultrathin-film inorganic solar photovoltaic cells.

Environment

Desalination promises to deliver virtually unlimited quantities of water to a water-constrained world. But for it to succeed, researchers are going to have to reduce the huge amounts of energy needed to make salt water drinkable.

2 Porsche Takes Wraps Off New 911 Targa Sports Car



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NanoH2O Inc., based in El Segundo, Calif., was voted best in the environment category for a nanotechnology-based reverse-osmosis membrane that promises to reduce the cost of running a typical desalination plant by as much as 25%.

Reverse osmosis, which separates salt and other impurities from salt water by forcing it through a membrane at high pressure, is increasingly favored as a desalination technology. But the pumps that push water through the membranes consume large amounts of energy, and traditional membranes easily are clogged by impurities, reducing their efficiency.

NanoH2O, using technology based on research at the University of California, Los Angeles, weaves nanoparticles into its membranes. The nanoparticles are more permeable to water molecules than the material in traditional membranes, and they resist fouling by bacteria, salt and other contaminants. As a result, the company says, its membranes enable desalination plants to maintain the same levels of production while reducing energy consumption, or to produce 70% more fresh water at current energy levels.

The company says it has begun producing membranes and complete reverse-osmosis modules, which incorporate the membranes and can replace the filters already used in existing desalination plants. It delivered the first products in August.

RUNNERS-UP

Active Water Sciences LLC, U.S.: A portable, self-contained wastewater-treatment system, the Water Phoenix, that can convert municipal wastewater into effluent that meets U.S. Environmental Protection Agency standards in less than 24 hours, producing little to no sludge.

Ceracasa SA and FMC Foret SA, Spain: A porcelain tile, BionicTile, with a photocatalytic glaze that reduces levels of nitrogen oxides and nitric acid in city air.

ClimateWell AB, Sweden: SolarChiller, a solar-powered air-conditioning unit that delivers heating, cooling and hot water to buildings without using electricity.

Health-Care IT

Software called Connect, developed by more than 20 federal agencies led by a program of the U.S. Department of Health and Human Services, won in this category for technology that enables health-care providers to exchange health information electronically.

The health-care industry is moving, albeit slowly, to replace patients' paper records with electronic files that can be easily shared among physicians, hospitals, health-care agencies and others. Two roadblocks stand in the way, though: The cost of electronic records systems and the need to ensure security and patient privacy.

Connect addresses both problems. The software was devised to meet all requirements for maintaining the security and privacy of medical records, including rules for federal agencies that are stricter than those for private health-care companies. And the Federal Health Architecture program, which coordinates health IT activities for several federal agencies, distributes the open-source Connect software free to both government and private health organizations.

In one of the first deployments, the Social Security Administration worked with the state of Virginia's regional health-information network to streamline the process of determining eligibility for disability benefits. Instant access to patients' records cut the time it takes to process disability applications to 46 days from 84.

Though there is other software for exchanging medical records, the Innovation Awards judges praised Connect for its ability to put the technology in the hands of lots of medical providers. The developers "were one of the few people who could move the needle on adoption of these things," says Barry H. Jaruzelski, one of the judges and a partner at Booz & Co.

RUNNERS-UP

Life Image Inc., U.S.: A cloud-based platform for sharing and storing diagnostic images, such as X-rays.

Ingenix, U.S.: Disease Precursor Identification software, which can identify people at risk of developing costly, difficult-to-manage diseases, such as diabetes.

Materials and Other Base Technologies

Cement production pumps a lot of carbon dioxide into the atmosphere. **U.K.-based Novacem Ltd.** was recognized in this category for a new cement-making process that takes in more CO2 than it emits.

The secret is using magnesium oxides instead of calcium carbonates, the main ingredient in Portland cement, the most common type. Magnesium-oxide cements have been around for a long time, but their quality wasn't as good as that of Portland cement, and their manufacture still emitted a lot of CO2.

Novacem, spun out of Imperial College London in 2007, says its cement is as durable as traditional materials and the production process can absorb 100 kilograms of CO2 for each metric ton of cement produced—compared with the roughly 800 kilograms of CO2 emitted in the production of each metric ton of traditional cement.

Novacem plans to begin construction next year of a plant to produce up to 25,000 metric tons of cement a year using the new technology, and to open the first commercial-scale plant by 2015.

RUNNERS-UP

Cambrios Technologies Corp., U.S.: A coating material made of highly conductive silver nanowires that can be used to create a transparent, less costly, bendable thin film for touch screens and other electronic components.

Bolt-A-Blok, U.S.: A building system that uses steel-reinforced concrete blocks that can be easily assembled into houses and other structures by unskilled labor.

MicroGreen Polymers Inc., U.S.: A method for reducing the cost of recycled plastics by adding a gas that expands the length and width of solid polymer sheets.

Medical Devices

Zoom Focus Eyewear LLC, winner of the overall Silver award, won in this category. (See "[A Different Kind of Eyeglasses](#) ")

RUNNERS-UP

MIT Mobility Lab, U.S.: The Leveraged Freedom Chair, a wheelchair designed for use in developing countries that can travel on virtually any terrain.



Enlarge Image

Abbott Labs' MitraClip for heart-valve repairs *Abbott Laboratories*

Abbott Laboratories, ABT -0.06% U.S.: The MitraClip System, a catheter-based device designed to repair damaged heart valves without open-heart surgery.

Aribex Inc., U.S.: The Nomad, a hand-held dental X-ray device. It's rechargeable, can be taken anywhere and allows the operator to stay with the patient during the procedure.

Medicine-Biotech

Counsyl Inc., winner of the overall Bronze award, won in this category. (See "[A Genetic Test for Prospective Parents](#) ")

RUNNERS-UP

CardioDx Inc., U.S.: Corus CAD, a genomic test designed to help clinicians determine, from a simple blood sample, whether a patient with chest pain has a significant blockage in the coronary

arteries.

Pacific Biosciences, U.S.: A DNA sequencer, which reads individual molecules of DNA as they replicate in order to determine an organism's precise genetic code in real time—producing results 20,000 times faster with less overall cost than other systems.

DuPont Qualicon, U.S.: Tests using DuPont's BAX System to detect pathogens in fish and shellfish and E. coli O157:H7 in beef and fresh produce.

Network/Internet Technologies/Broadband

Vidyo Inc., based in Hackensack, N.J., won in this category with its technology for delivering high-quality videoconferencing over the Internet or cellular networks at a fraction of the cost of dedicated "telepresence" systems.

Internet videoconferencing has been around for a few years, but the calls typically are characterized by jerky, low-resolution video. More-realistic, high-resolution videoconferencing systems generally require dedicated communications lines and expensive equipment, limiting their use.

Vidyo uses a new video-compression standard to produce a high-definition videoconferencing product that can work on desktop or laptop computers, tablets and smart phones and travel over the Internet or 3G and 4G cellular networks.

The company introduced its systems, which can include routers and other hardware in addition to software, in 2007. This summer, it licensed software to [Hewlett-Packard Co.](#) ([HPQ +0.42%](#)), which will use the technology to extend its Halo telepresence service to desktop computers and to conference rooms not already set up with dedicated systems.

RUNNER-UP

Microsoft Corp. ([MSFT +2.49%](#)), **U.S.:** An experimental Internet application, called Pivot, designed to help users to explore, organize and visualize collections of data quickly by showing relationships between the information.

Network Security

The Internet is thick with malware—viruses, worms, spyware, Trojan horses. The judges awarded [Symantec Corp.](#) ([SYMC +2.12%](#)), based in Mountain View, Calif., the top prize in the network-security category for a new way to head off these threats: "reputation-based" technology that examines the usage patterns of millions of computers to spot dangers that traditional security products typically miss.

In general, security software identifies malicious software by looking for distinguishing patterns of code or watching for bad behavior—a computer's inexplicably connecting to an unknown server, for example. The problem is that there are so many new malware variants constantly appearing, some of them targeting only a small number of computers, that those techniques can't always spot them before they do mischief.

Symantec's new technology examines the software running on the computers of millions of volunteers, who remain anonymous, to spot possible threats. Based on what these patterns show about a program's source, age, prevalence and other characteristics, the technology assigns a "reputation rating" to every piece of software that it comes across. The technology had been known initially as Quorum but will soon be renamed.

Symantec says that the technology, first incorporated in the company's Norton 2010 security suite that was released in late 2009, is detecting about 10 million new threats a month that are invisible to traditional security methods.

RUNNERS-UP

Panda Security, Spain.: Panda Cloud Antivirus, a free, cloud-based antivirus solution.

Simplified Inc., U.S.: Simplified SinglePoint, a cloud-based service that enables organizations to apply and enforce security policies and controls on cloud applications.

Physical Security

Surveillance cameras generate a prodigious amount of video; unfortunately there's not enough time and manpower to watch it all.

The winner in this category, Israel-based BriefCam Ltd., has developed a fresh solution to the problem: Video Synopsis, which enables a viewer to browse a day's worth of

recording in just a few minutes by creating a summary of all the activities captured by a camera.

Other video-surveillance technologies address the too-much-information problem by fast forwarding through recordings or capturing images only when something happens—using motion detectors, for instance.

BriefCam takes a different approach. Its patented technology pulls out activities recorded over the course of a day—vehicles driving through a security gate, people walking in and out of a building—and compiles the images into a highlight reel in which each vehicle, for instance, follows immediately the one that preceded it through the gate, regardless of how much time actually elapsed between their arrivals. Each vehicle's image carries a time stamp to show when it was recorded, and the user can click on the time stamp to call up that section of the video.

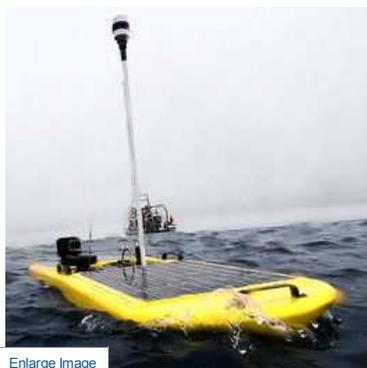
"Five hours of video is not five hours any more," says Shmuel Peleg, developer of the technology and the company's chief scientist. "It's five minutes."

Video Synopsis, licensed from Hebrew University of Jerusalem, where Mr. Peleg is a faculty member, was launched in 2009.

Robotics

Liquid Robotics Inc., based in Sunnyvale, Calif., is the winner in this category for developing an unmanned seagoing craft propelled by the power of ocean waves.

Most unmanned ocean craft can remain at sea for only a short time, relying on batteries to power propellers or pumps. The heavier their payload, the less time they have.



[Enlarge Image](#)

Liquid Robotics' Wave Glider *Liquid Robotics, Inc.*

Thanks to its propulsion system, Liquid Robotics' Wave Glider avoids those limits.

The craft, which consists of a surface buoy and a submerged glider with wing-shaped panels, converts the up-and-down motion of waves into forward thrust, making it possible to propel the buoy indefinitely without relying on batteries or other power sources.

The craft can be controlled remotely via satellite over an Internet connection. Instruments are powered by a solar panel on the surface of the floating buoy. Innovation Awards judge William Webb

says the technology is "simple, novel and very workable."

The vehicle originally was designed by co-inventor Roger Hine, a Silicon Valley engineer and now the company's chief executive, to monitor the activities of humpback whales. It can also be used for tsunami warnings, observing weather and ocean conditions, and national-defense applications. The first craft was sold in 2009.

This summer, [BP](#) (BP -0.28%) PLC deployed two Wave Gliders to the Gulf of Mexico to monitor water quality near the site of the well that exploded in April and spewed millions of gallons of oil into the Gulf.

Semiconductors

InVisage Technologies Inc., based in Menlo Park, Calif., took the prize in the semiconductors category with QuantumFilm, an image sensor for digital cameras that uses semiconducting nanocrystals to capture far more light than traditional sensors.

Inexpensive digital cameras rely on sensors made from silicon that are limited in the amount of light they can capture. This is especially an issue in the smaller sensors used in cellphone cameras.

InVisage replaced silicon in the sensor with quantum dots, semiconducting crystals that are nanometers in size. The product, InVisage says, captures more than 90% of the available light, compared with 25% for a silicon-based sensor.

The technology taps research from the University of Toronto by Ted Sargent, a nanotechnology researcher and InVisage's founder and chief technology officer. The first QuantumFilm prototypes were unveiled in March, and the company says it will deliver sample chips to smart-phone makers by the end of the year; these chips will be used to build prototype devices. The chips could be available in consumer products as early as the end of next year.

RUNNERS-UP

Industrial Technology Research Institute, Taiwan: Slim, flexible sensors. ITRI envisions use of the sensors in such things as electronic musical instruments and weight scales embedded in luggage.

STMicroelectronics, [STM +0.63%](#) Switzerland: The iNemo family of smart multisensor devices, which can be used in new ways to measure movement, pressure, temperature and altitude.

Nanosys Inc., U.S.: QuantumRail, a component that delivers more vibrant color and brightness in notebooks and mobile devices as well as increased energy efficiency.

Software

San Francisco-based Unity Technologies won in this category for a set of game-development tools that make it cheap and easy to create three-dimensional interactive content, including games, training simulations and medical visualizations, for a range of devices from cellphones to game systems.

The software for creating 3D online universes typically requires teams of engineers who spend years creating and refining these tools. As a result, they're often too complex and expensive for small-scale or amateur game developers.

Unity's software simplifies the process of building 3D games and other programs. It includes an easy-to-use editor that can take prefabricated components—rain or falling crates, for example—and combine them with other features to create full game environments.

The software also makes it possible to deploy games on a range of computer systems: Macs or PCs, game consoles from [Sony Corp. \[SNE -0.63%\]\(#\)](#), Nintendo Co. or [Microsoft Corp.](#), and [Apple Inc. \[AAPL +2.36%\]\(#\)](#)'s [iPhone](#) and iPad.

The tools are simple enough for hobbyists or start-up developers; two developers used it to make the popular [Zombievillage USA](#) app for the [iPhone](#). They also are powerful enough for the largest game developers. [Electronic Arts Inc.](#), for example, used Unity to create its Tiger Woods PGA Tour Online game. "What you can create in a short time frame with a low learning curve is pretty revolutionary," says Robert Drost, a computer architect and one of the Innovation Awards judges.

The first version of the software was introduced in 2005, and it currently is being used by more than 200,000 developers. In October 2009, the company began offering at no cost its entry-level version, normally priced at \$200 and intended mainly for hobbyists and small, independent game developers.

Technology Design

The efficient and compact storage of cookware may not be one of the world's great problems, but for anyone who has tried to put away a stack of awkwardly shaped pans with their lids and protruding handles, it's definitely an unmet need.



[Enlarge Image](#)

Gavin Thomson Design's nesting pans *Gavin Thomson Design Ltd.*

Gavin Thomson Design Ltd., based in the U.K., won this category with an elegant solution to this daily annoyance. Mr. Thomson designed a set of three saucepans that nest one inside the other. The largest pan snugly holds the next smaller pan, which holds the smallest one; each permanently attached handle rests inside the hollowed-out grip of the next larger pan, and the lids all fit on top.

The patented design was licensed to

Stellar brands, a unit of Portugal-based Silampos SA, and the first products, called Eazistore, were introduced in March in the U.K. Mr. Thomson's firm is negotiating with housewares brands in North America and Asia to distribute the pans in those regions.

RUNNERS-UP

Smart Lid Systems, Australia: A disposable coffee-cup lid that changes color from brown to red when hot.

Panasonic Avionics Corp., U.S.: An in-flight entertainment system that integrates a touch-screen monitor with a thin, lightweight economy-class seat.

Wireless

Ubiquisys Ltd., based in the U.K., won in the wireless category for a low-priced femtocell—a small cellular base station for use indoors.

Femtocells are designed to address two big, related problems: the poor cellphone coverage typically found inside a house, apartment or office building, and the growing congestion on cellular networks, aggravated by the explosion of data use on the latest smart phones. While femtocells have been around for a few years, their adoption has been limited by their high cost.

The company's G3-mini, introduced in December, is the first femtocell to be sold at a wholesale price under \$100—a price that makes it possible for carriers to provide them to customers free of charge.

Ubiquisys keeps the cost down by providing software that's already proven to work on the leading carrier networks and delivering hardware blueprints to consumer-electronics makers, which can take advantage of their high-volume manufacturing lines to turn out lower-priced gear.

Tokyo-based Softbank Mobile Corp. began offering free G3-mini devices to consumers, retailers and small-office customers in the spring, and the first units were shipped in August.

RUNNERS-UP

Motorola Inc., U.S.: The iSIM, a thin, flexible wafer that attaches to the SIM card in a mobile device. The iSIM enables a host of new mobile applications built by third-party developers.

Shared Spectrum Co., U.S.: Technology that permits two or more networks or applications to share the same radio-frequency band by using channels when they are idle.

Pyxis Mobile Inc., U.S.: Application Studio, which allows companies to create applications for BlackBerry, iPhone, Android and Windows mobile devices from a single configuration with no coding.

Correction & Amplification

In one of the first deployments of the Department of Health and Human Services' Connect software, the Social Security Administration worked with a private regional health-information network in Virginia to streamline the process of determining eligibility for disability benefits. A previous version of this article incorrectly said the health-information network was run by the state of Virginia.

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WSJ In-Depth



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CardioDx Blood-Based Gene Expression Test Honored As One Of *Time Magazine's* Top Ten Medical Breakthroughs of 2010

Innovative Test Also Honored With Technology Innovation Award from The Wall Street Journal

PALO ALTO, Calif. – Dec. 22, 2010 – CardioDx, a pioneer in the field of cardiovascular genomic diagnostics, today announced that Corus™ CAD, the company's blood-based gene expression test, has been honored as one of *Time Magazine's* Top Ten Medical Breakthroughs of 2010.

Corus CAD is the first and only clinically validated blood-based test to help clinicians confidently identify which of their stable symptomatic patients are likely to need further assessment for obstructive coronary artery disease.

"We agree that we are in the midst of one of the most exciting areas of medicine today, and we're pleased that the Corus CAD gene expression test has been recognized by both *The Wall Street Journal*, with a Technology Innovation Award earlier this year, and by *Time Magazine* – two venerable American publications," said David Levison, chief executive officer of CardioDx.

Corus CAD delivers valuable information to cardiologists making decisions about which patients require advanced imaging or potential catheterization, and to primary care clinicians deciding which patients need a specialist referral. These decisions are increasingly important as clinical and regulatory communities recognize the need to reduce radiation exposure from medical imaging.

A study published in the March 11, 2010 issue of the *New England Journal of Medicine* found that in nearly 400,000 patients who underwent elective invasive angiographic procedures, 62% were found to have no obstructive coronary artery blockage. The study authors concluded that current modalities for identifying which patients should undergo elective invasive coronary angiography to diagnose coronary artery disease have limitations, and that better methods are needed for patient risk stratification.

About Corus CAD

Corus CAD is the first and only clinically validated blood-based test for obstructive coronary artery disease. The test involves a routine blood draw conveniently administered in the clinician's office and does not expose patients to risks of radiation or imaging agent intolerance. Corus CAD is a decision-making tool that can help primary care clinicians and cardiologists evaluate whether a non-diabetic patient's symptoms are due to obstructive coronary artery disease. It is the first sex-specific test for obstructive coronary artery disease, accounting for critical biological differences between men and women.

The Corus CAD test procedure uses the RNA levels of 23 genes. Because the RNA levels are increased or decreased when obstructive coronary artery disease is present, Corus CAD is able to measure the likelihood that an individual patient has obstructive coronary artery disease from a simple blood sample.

Corus CAD is commercially available through an innovative patient sample kit that includes everything needed for blood collection and express delivery to the company's CLIA-certified Palo Alto, Calif.

laboratory. Test results are delivered promptly to the clinician's office. Corus CAD is currently available in the United States.

For more information please visit <http://www.cardiodx.com/media-kit/>.

About Gene Expression Testing

Gene expression testing provides valuable tissue and cell-specific information about the molecular mechanisms involved in disease processes, enabling evaluation of an individual patient's disease state, activity, and/or progression at a given point in time. Unlike genetic tests, which measure genetic variations, mutations, traits and predispositions – factors that are constant over a person's lifetime – gene expression testing assesses a dynamic process, integrating both genetic predisposition and additional behavioral and environmental influences on current disease state.

About CardioDx

CardioDx, Inc., a pioneer in the field of cardiovascular genomic diagnostics, is committed to developing clinically validated tests that empower clinicians to better tailor care to each individual patient. Strategically focused on coronary artery disease, cardiac arrhythmia and heart failure, CardioDx is poised to expand patient access and improve healthcare quality and efficiency through the commercialization of genomic technologies. The company was honored as a winner of the *Wall Street Journal's* prestigious Technology Innovation Awards for 2010. Privately held, CardioDx is funded by Kleiner, Perkins, Caufield & Byers, TPG Biotech, Mohr Davidow Ventures, Intel Capital, Pappas Ventures, DAG Ventures, Asset Management Company and GE Capital. For more information, please visit www.cardiodx.com.

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CardioDx Honored as 2012 Gold Edison Award Winner

Corus CAD Wins at the Edison Awards Gala; Awards Celebrate 25 Years of Honoring Innovators and Innovation

PALO ALTO, Calif. – April 30, 2012 – CardioDx, Inc., a pioneer in the field of cardiovascular genomic diagnostics, today announced that Corus[®] CAD has been honored as a gold winner in the Science & Medical category of the internationally-renowned 2012 Edison Awards. The awards, which were presented last week at a gala in New York, symbolize the innovation personified by Thomas Alva Edison, inspiring America's drive to remain at the forefront of creativity and ingenuity in the global economy.

"As the pace of innovation quickens and the 'race to next' becomes ever more competitive, it's increasingly important to take a moment out of our hectic lives to recognize excellence in innovation and greatness in the teams of innovators who make our future. We are honored to present CardioDx with an Edison Award as one of the leading innovators of today and tomorrow," said Thomas Stat, 2012 Edison Awards Steering Committee Chairman.

"We are pleased that the Edison Awards judges have recognized the innovation of Corus CAD, adding to a growing list of accolades for the test," said David Levison, president and CEO of CardioDx. "Clinicians also are increasingly recognizing the clinical utility of Corus CAD as a means to reduce unnecessary procedures and radiation exposure, with more than 25,000 tests ordered to date."

With a simple blood draw, Corus CAD can help primary care clinicians and cardiologists exclude obstructive coronary artery disease as the cause of a stable, non-diabetic patient's symptoms. It is the first sex-specific test for obstructive coronary artery disease, accounting for critical biological differences between men and women. The test is safe and does not expose patients to radiation risks or imaging agent intolerance.

Corus CAD has also been honored as a winner of *The Wall Street Journal's* Technology Innovation Awards and one of *TIME's* Top Ten Medical Breakthroughs.

The ballot of nominees for the Edison Awards is judged by more than 3,000 senior business executives and academics from across the nation whose votes acknowledge the Finalists' success in meeting the award criteria of **Concept, Value, Delivery and Impact**.

Winners of the awards for each category were announced on April 26, 2012 at the Edison Awards Annual Gala, held in the historic ballroom of New York's famed Capitale.

The members of the Edison Awards Steering Committee are senior executives with diverse marketing, scientific, and business backgrounds who monitor the development and successful launch of innovative products each year. The Committee is responsible for bestowing the annual Edison Achievement Awards and serves as a guidepost in developing the list of nominees that is presented to the Edison Award judges.

The 2012 Edison Awards are sponsored by Nielsen, Discovery Communications, Science Channel, USA Today, CSRware, and applepeak. For more information about the Edison Awards and a full list of winners, visit www.edisonawards.com.

About Corus CAD

The Corus CAD test measures the RNA levels of 23 genes from a whole blood sample. Because these RNA levels are altered when obstructive coronary artery disease is present, the Corus CAD score aids clinicians in assessing whether an individual patient's symptoms may be due to obstructive coronary artery disease.

Corus CAD is commercially available through an innovative patient sample kit that includes everything needed for blood collection and express delivery to the company's CLIA-certified Palo Alto, Calif. laboratory. Test results are delivered promptly to the clinician's office. Corus CAD is currently available in the United States.

For more information please visit <http://www.cardiodx.com/media-kit/>.

About CardioDx

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CardioDx Honored as One of *FierceMedicalDevices*' Fierce 15 Most Promising Private Companies

Award Presented at AdvaMed 2012: The MedTech Conference

PALO ALTO, Calif. – Oct. 2, 2012 – CardioDx, Inc., a pioneer in the field of cardiovascular genomic diagnostics, today announced that the company has been named one of *FierceMedicalDevices*' Fierce 15 most promising privately held medical device and diagnostics companies in the world.

FierceMedicalDevices editors chose this year's winners based on their top management teams, notable financial backing, promising technologies and market opportunities. The award was presented today at an event that took place during AdvaMed 2012: The MedTech Conference in Boston, Mass.

"CardioDx was chosen as one of the Fierce 15 based, in part, on its creativity and innovation. The company has paired cutting-edge science with a huge unmet need, and Corus CAD has wowed clinicians, payers and investors alike," said *FierceMedicalDevices* Editor Damian Garde.

"We are honored to be included in *FierceMedicalDevices*' inaugural list of the most promising privately held companies in our industry today," said David Levison, president and CEO of CardioDx. "The value of Corus CAD as a means to reducing unnecessary cardiovascular procedures and radiation exposure for patients is being increasingly recognized by clinicians, with more than 30,000 tests ordered to date. The favorable Medicare coverage decision we recently received further validates the clinical and economic benefits of Corus CAD to patients and the healthcare system."

With a simple blood draw, Corus CAD can safely, accurately and conveniently help primary care clinicians and cardiologists assess whether or not a stable non-diabetic patient's symptoms are due to obstructive coronary artery disease (CAD), enabling many patients to avoid unnecessary invasive procedures and exposure to imaging-related radiation risks or imaging agent intolerance. The test has been clinically validated in multiple independent patient cohorts, including two prospective, multicenter U.S. studies, PREDICT and COMPASS. Additionally, a retrospective, multicenter chart review study and the prospective IMPACT trial at Vanderbilt University demonstrated that Corus CAD use yields statistically significant and clinically relevant changes in patient management decisions in both primary care and cardiology settings.

CardioDx recently announced that Palmetto GBA, a national contractor that administers Medicare benefits, has established coverage for the Corus CAD gene expression test for the evaluation of patients presenting with typical and atypical symptoms suggestive of coronary artery disease. With this decision, the Corus CAD gene expression test is now a covered benefit for more than 40 million Medicare enrollees in the U.S.

Corus CAD has also been recognized by *The Wall Street Journal's* Technology Innovation Awards, honored as a Gold Edison Award recipient, and named one of *TIME's* Top Ten Medical Breakthroughs.

About *FierceMedicalDevices*

An internationally recognized e-newsletter reaching more than 34,000 medical device and diagnostic industry professionals, *FierceMedicalDevices* provides subscribers with a quick authoritative briefing on the day's top stories, with a special focus on clinical studies, FDA/EMA regulations, and post-marketing. A complete list of "Fierce 15" companies is available online at www.fiercemedicaldevices.com.

About CardioDx

CardioDx, Inc., a pioneer in the field of cardiovascular genomic diagnostics, is committed to developing clinically validated tests that empower clinicians to better tailor care to each individual patient. Strategically focused on coronary artery disease, cardiac arrhythmia and heart failure, CardioDx is poised to expand patient access and improve healthcare quality and efficiency through the commercialization of genomic technologies. For more information, please visit www.cardiodx.com.

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CardioDx – 2012 Fierce 15

October 2, 2012

Based: Palo Alto, CA

Founded: 2004

Website: CardioDx.com



*David Levison,
CEO of CardioDx*

The Scoop: With Corus CAD, the company's blood-based gene expression test for obstructive coronary artery disease, CardioDx has a product that has wowed physicians, payers and investors.

What Makes It Fierce: CardioDx has been on the up-and-up of late. Since its launch in 2004, the Palo Alto, CA-based molecular diagnostics company has pulled in more than \$175 million in venture investment, buoyed by another \$58 million round in August. How does a biotech outfit raise that kind of money in a slumping VC climate? CardioDx CEO David Levison says his company took cutting-edge science and matched it with a huge unmet need.

Corus CAD, the company's diagnostic test for obstructive coronary artery disease (CAD), tests the expression of 23 genes in patients, assigning them a 1 to 40 score. The higher the number, the more likely they are to have the ailment. The point: help physicians diagnose patients before prescribing costly imaging procedures that may not be necessary.

And the test, launched in 2009, has succeeded at just that, Levison says. Each year, millions of patients are given noninvasive cardiac imaging after reporting chest pains, and studies have shown that 62% of those who are catheterized don't have the disease to warrant it. "We fit right in there," Levison says. "Our value proposition is that our test should be the gatekeeper to imaging."

Levison isn't alone in that assessment: CardioDx scored a major victory in August, securing Medicare reimbursement for Corus CAD, and the company is optimistic that private insurers will follow suit, opening up an even larger market for the diagnostic.

What To Look For: With all that cash in hand, CardioDx's next move is to expand the adoption of Corus CAD, Levison says. The company has compiled loads of data demonstrating the test's efficacy over the past few years, and now it will make its case to payers. In the long-term, Levison says CardioDx has some ideas on how its technology can be applied to arrhythmia and heart failure.

CardioDx reaches to India with Core partnership

April 25, 2013 | By Damian Garde

[CardioDx](#), a [2012 Fierce 15](#) winner, has partnered with India's [Core Diagnostics](#) to distribute its coronary artery disease test in the country's burgeoning market.

Under the deal, Core will administer the Corus CAD test to patients in India, shipping samples back to CardioDx's lab in California for analysis and turnaround. Neither company disclosed financial terms.

The gene-expression test requires just a blood draw, and CardioDx says it can rapidly determine whether symptoms like chest pains are in fact signs of obstructive coronary artery disease, saving patients from costly imaging procedures and guiding personalized treatment.

For CardioDx, expanding into India opens up a huge market for its innovative diagnostic, and the company is using the \$58 million in venture cash it raised last fall to promote the test around the globe. Since then, CardioDx has been cranking out positive study data, showing Corus CAD to be as effective as the standard of care without the risks inherent in radiation imaging.

Core Diagnostics, a Gurgaon-based startup, opened its doors early this year with the goal of bringing cutting-edge molecular diagnostics to India, absorbing OncoMDx's technology and raising \$5.1 million from Artiman Ventures. Partnering with the likes of CardioDx advances the company's mission of serving a large unmet need in its home country, CEO Mohan Uttarwar said.

"Coronary artery disease is a rising epidemic in India, and about 62 million of the country's population is estimated to suffer from CAD by 2015," Uttarwar said in a statement. "CardioDx's state-of-the-art Corus CAD gene expression diagnostic test will help us spearhead the personalized medicine movement in India and provide diagnostic technology that is more convenient than current diagnostic tests for CAD."

- read the [announcement](#)

Special Report: [CardioDx – 2012 Fierce 15](#)

Related Articles:

[CardioDx CAD Dx passes another post-marketing test](#)
[Startup brings molecular diagnostics to India's swelling market](#)
[CardioDx pulls in \\$58M to expand Dx commercial sales](#)



CardioDx has signed a deal to distribute its Corus CAD test in India. –Courtesy of CardioDx

Source URL: <http://www.fiercemedicaldevices.com/story/cardiody-reaches-india-core-partnership/2013-04-25>

EXHIBIT F

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Health

Bay Area company develops new heart disease test

Friday, October 26, 2012

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PALO ALTO, Calif. (KGO) -- A new technology developed here in the Bay Area could save thousands of patients from having unnecessary tests for heart disease. At the same time it could also help doctors pinpoint those with the highest risk.

Richard Wirtenson was a few strokes from the green, when a strange feeling interrupted his round of golf. He knew he was uncomfortable, but the symptoms were vague, "I got a burning sensation across my chest," Wirtenson said. "It was not a pain."

Wirtenson's doctor, cardiologist Jeffrey Gardino, notes, "It could be gastrointestinal, it could be their lungs, or it could be heart blockage, and my job is listening to them, trying to ferret out more selective symptoms that may pinpoint whether they have heart disease."

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According to Gardino, the goal is not to rush every patient into invasive tests like angiograms, which are highly effective at

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spotting heart blockage, but also carry side effects, "It's a lot of radiation," Gardino said. "Ten years worth of radiation you get in the course of that procedure." He says current options include pre-screening patients like Wirtenson with a cardio stress test, involving a treadmill.

But now, a Bay Area company believes it has an alternative that can help doctors spot which patients are likely to be suffering from heart disease much more quickly. The test was developed by Palo Alto-based CardioDx. It involves a simple blood draw that can be done in a doctor's office. But what happens after that actually involves some extremely sophisticated science,

"Once the blood is drawn from the patient, we bring it into our lab in Palo Alto, and it's a three step process," said CardioDx CEO David Levison. According to Levison, the first step involves automated machines that extract the RNA from the blood cells which is then used to synthesize a complete DNA sample, "And the final step is we put it on the PCR machine which measures the individual levels of 23 genes in our algorithm."

In simpler terms, he says the process is ultimately measuring gene expression. Looking for the activity of specific genes that are typically turned on when a patient's blood vessels are diseased and producing plaques that block the arteries. He says the reading gives doctors a strong indication of whether is patient is suffering from heart disease.

"With our test, we can easily and quickly identify those patients where it's very unlikely to be caused by a blocked coronary artery," Levison said. And he believes the blood test, coupled with other screening methods would significantly reduce the false positives and unnecessary angiograms, while getting patients who do need them a quicker diagnosis.

"Does someone need an invasive test or not? Or is this someone I can watch more closely for the next few months to years before having them committed to doing a test," said Gardino.

In Wirtenson's case, additional tests did eventually lead to an angiogram, which revealed a blockage. He eventually had a stent placed in his artery, allowing him to return to the golf course, "It's a small nine hole course but we really enjoy it," said Wirtenson.

The test runs just under \$1,200. It is now covered under Medicare, but private insurance still varies.

Written and produced by Tim Didion

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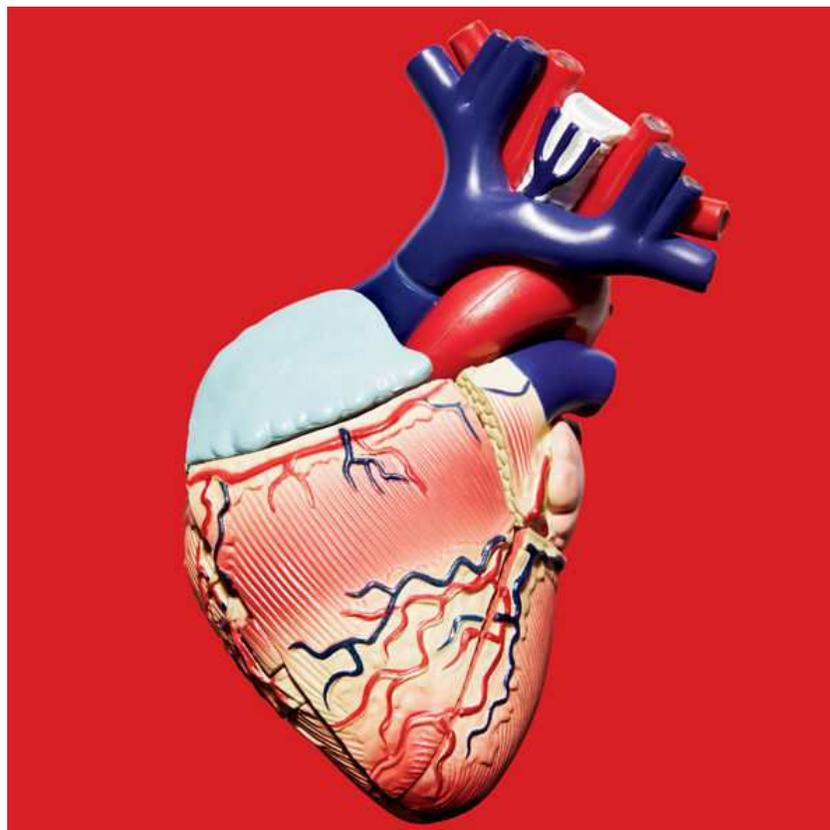
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6 Health Tests that Could Save Your Life

Getting tested now might help you down the road. Ask your doctor about these examinations.

Nancy Ripton

Photography by: Claire Benoist



1) Corus Cad

Heart disease is the leading cause of death for men and women in the United States. This year, more than 1.2 million people will have a heart attack. While there are warning signs and risk factors, heart disease can hit anyone.

Heart attacks and other types of heart disease occur when coronary arteries become clogged with fatty deposits, resulting in coronary artery disease (CAD). Until recently there was no way, outside of traditional imaging tests, to measure artery health, but a new test is changing all that. Imaging scans are invasive and expose people to radiation, limiting their use.

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Corus CAD by Cardio Dx is a gene expression test that uses messenger RNA from a gene set in blood cells to determine whether or not patients have coronary artery blockages. Patients are given a simple blood test that delivers a score on a scale of one to 40. "If they score between one and 15 trial evidence shows that the likelihood of that person having blockage is low," says Deborah L. Kilpatrick, Ph.D., chief commercial officer of Cardio Dx. The higher your score, the more likely you are to have blockages. This knowledge enables your physician to take extra precautions and book follow-up tests that can potentially save your life.

Who should get the test: Any nondiabetic patient with typical or atypical symptoms of coronary artery disease—regardless of age.

Cost: \$1,195; covered by some insurance companies (but if you are uninsured, patient assistance is available).

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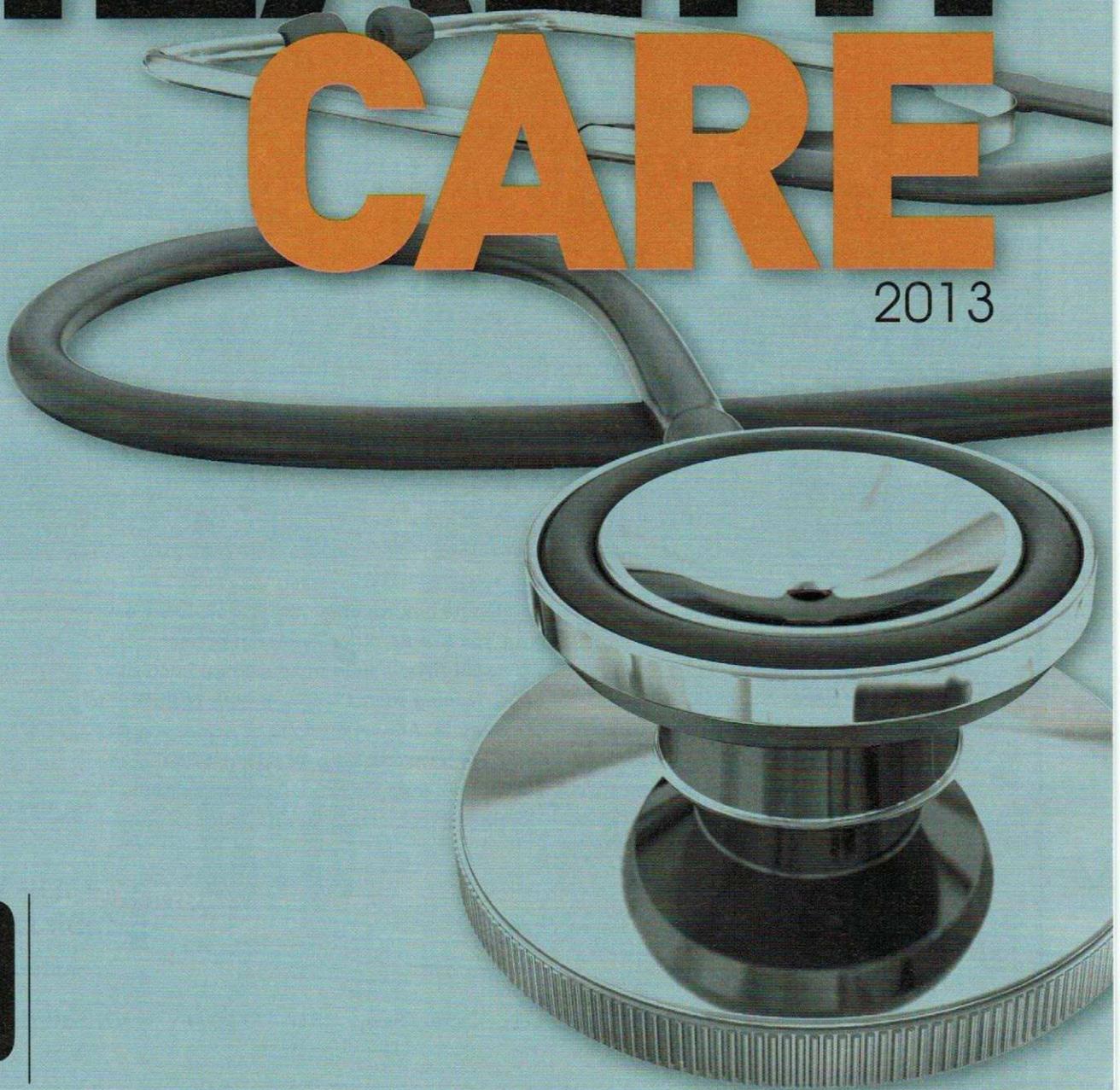
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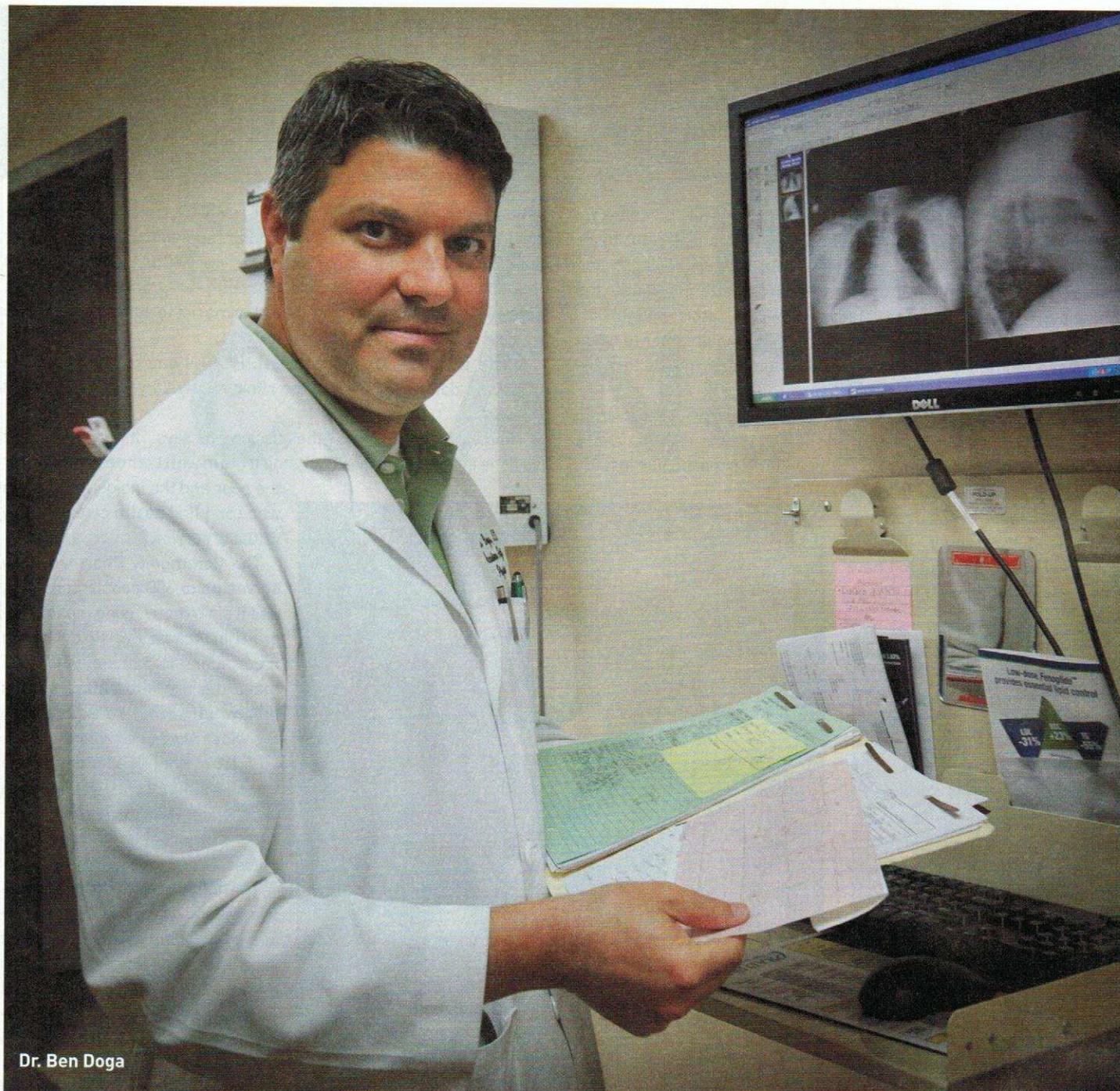
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Dr. Ben Doga

Photo by Robin May

CORUS CAD COMES TO ACADIANA

BY WYNCE NOLLEY

Heat disease remains the leading cause of death for men and women in the United States, but now Acadiana patients who may be at risk for coronary artery disease have access to a new, more effective blood test to measure their artery health called Corus CAD.

Corus CAD is a clinically-validated gene expression test that measures the activity of 23 specific genes in a patient's blood cells that change whenever there is a blockage in the arteries, also known as coronary artery disease, or CAD.

"For a patient, it's very simple," says Missy Kemp, regional manager for CardioDX, the company that developed Corus CAD. "It's a simple, non-fasting blood draw that is done in a clinician's office, and then the clinician gets the results back in three days."

The results come back to the physician in the form of a score,

ranging from 1-40, with low scores between 1-15, intermediate scores from 16-26 and high scores between 26-40. And with this score, a physician can advise patients if they need to go on for further testing, effectively bypassing unnecessary paperwork, time off work, and medical expenses.

"It's actually a very good 'rule out' test," says Dr. Robert St. Armant, a lipidologist and medical director for The Lipid Center at Baton Rouge General Medical Center. "If you have a patient that has a score of 15 or less with the Corus test, you have a 96 percent negative predictive value that that patient has coronary artery disease, so it essentially rules out the likelihood of obstructive coronary artery disease in that patient."

According to Kemp, Corus CAD has been available in Louisiana since 2010, predominantly in Baton Rouge, with the test being introduced into the Lafayette health market in April to clinicians like Dr. Ben Doga of Acadiana Family Physicians and Dr. Kelly Cobb of Nouriche Wellness & Aesthetics.

"It's still fairly new, and commercially it's been available since 2009 but only in selected states," Kemp says. "We have much more traction now in Louisiana; it's currently covered by Medicare, and we're [in talks] with commercial insurance companies."

Corus CAD is also sex specific and takes into account the different symptoms men and women show when CAD may be present. According to St. Armant, men usually display typical symptoms like the "elephant sitting on the chest," pain going into the jaw, numbness of the left arm, sweating and shortness of breath. And because of the difference in the anatomy of coronary arteries, women show more atypical symptoms that may range from uneasiness in the chest, to nausea, pain between the shoulder blades, numbness in the arms and extreme fatigue.

"The clinical utility of the Corus test is that it helps steer you in the right direction of the workup of your patient with these symptoms," says St. Armant. "It means that I'm going to be looking for other causes for their symptoms other than heart disease. So in essence it avoids what could be a very expensive and timely cardiac workup."

Another huge benefit of the Corus CAD is that, unlike other imaging scans, it does not expose patients to ionizing radiation, which can be especially hazardous to women's breast tissue.

One other notable difference is that Corus CAD is not a genetic test, which measures a gene mutation or a gene variation to give a patient a lifelong risk for a certain disease state. St. Armant says the test provides "a biological and molecular picture of the risk of obstructive coronary artery disease in that patient at that point in time."

Corus CAD does have its drawback, namely patients who are excluded from the test — like those who have known CAD, such as a heart attack or a previous myocardial infarction.

Diabetics are also excluded from the test and so are those taking steroids, immunosuppressive or chemotherapeutic agents and those with any chronic inflammatory or connective tissue disease, as they all cause changes in the gene expression.

"So far I'd have to say that of all the patients I've [treated] over the last three years that have had low scores, I do not know of a single one who has subsequently had a major coronary event," says St. Armant. "So that reinforces my confidence in the Corus CAD test."



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