Biomarkers of Key Biological Pathways in CVD

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ABSTRACT

This review provides background on the laboratory design for MESA (Multi-Ethnic Study of Atherosclerosis) as well as the approach used in MESA to select biomarkers for measurement. The research related to the multitude of circulating and urinary biomarkers of inflammation and other novel and emerging biological pathways in MESA is summarized by domain, or pathway, represented by the biomarker. The contributions of MESA biomarkers to our knowledge of these key pathways in the development and progression of atherosclerosis, cardiovascular disease, diabetes, kidney disease, and pulmonary disease are highlighted, as are the contributions of MESA to recommendations for clinical use of several of these biomarkers. In addition, contributions of MESA to multicohort genomics consortia and current collaborations in transomics and metabolomics are noted.

Biomarkers are important in expanding our knowledge about the underlying pathogenesis of disease. MESA (Multi-Ethnic Study of Atherosclerosis) has measured numerous established and novel biomarkers representing a wide variety of biological functions. The evaluation of these biomarkers in MESA has contributed substantially to our understanding of the development and progression of cardiovascular and other diseases.

LABORATORY DESIGN FOR MESA

At the baseline exam (2000 to 2002), 3 groups were designated: group 1 assays were performed on all participants online with results returned to participants; group 2 assays were performed on all participants at the end of the examination; and group 3 assays were performed on a selected subset of participants, also at the end of the examination. Group 1 assays included plasma lipid measurements (total cholesterol, high-density lipoprotein cholesterol [HDL], and calculated low-density lipoprotein [LDL] cholesterol, and triglycerides), serum creatinine, and fasting glucose. Group 2 measurements included urinary albumin and creatinine, total homocysteine, inflammation markers (interleukin-6 [IL-6], C-reactive protein [CRP], and fibrinogen), fasting insulin, several hemostasis and fibrinolysis markers (factor VIII, D-dimer, and others), and individual lipoprotein subclasses by nuclear magnetic resonance (NMR) (LipoProfile-II spectral analysis; Liposcience, Raleigh, NC).

Group 3 was created to allow for extensive phenotyping, which was more experimental in nature and included 1,000 participants randomly selected from the 5,030 MESA participants enrolled prior to February 2002. Due to differences in recruitment rates, this subgroup was 57% women, 46% white, 10% Chinese American, 21% African American, and 23% Hispanic (the full MESA cohort was 53% women, 38% white, 12% Chinese American, 28% African American, and 22% Hispanic). Group 3 assays reflected novel and emerging pathophysiological domains and included biomarkers of inflammation, endothelial function, oxidative damage and stress, atherosclerotic plaque stability, and chronic infection serologies. Subsequently, 1,880 participants were added to group 3 to create a subgroup of 2,880 participants composed of 720 participants from each ethnic group matched for age and sex.

Additional biomarker measurements were conducted by ancillary studies including measurements of circulating immune cells, adipokines, renin and aldosterone, vitamin D metabolites and related analytes, stress hormones, and sex hormones. A comprehensive list of the MESA main and ancillary studies biomarker measurements by specific domain (biological pathway) represented by the biomarker, with the number of measurements available at each examination, is presented in Table 1. Full references for each section are presented in an Online Appendix.

DOMAIN-BASED APPROACH TO BIOMARKER SELECTION

MESA was designed to investigate the prevalence and progression of subclinical cardiovascular disease (CVD) and to identify risk factors for incident clinical CVD in a racially/ethnically diverse population. Therefore, the initial focus of biomarker selection was biological pathways, or domains, intimately involved in the development and progression of CVD. Domains of inflammation, insulin resistance, lipids, hemostasis/fibrinolysis, oxidative damage and stress, endothelial cell function, and several others were selected by committee as key pathways at the beginning of the MESA study. Over time, measurements have expanded to include biomarkers representative of other

The authors report no relationships that could be construed as a conflict of interest.

This research was supported by contracts HHSN268201500003I, N01-HC-95159, N01-HC-95160, N01-HC-95161, N01-HC-95162, N01-HC-95163, N01-HC-95164, N01-HC-95165, N01-HC-95166, N01-HC-95167, N01-HC-95168, and N01-HC-95169 from the National Heart, Lung, and Blood Institute, and by grants UL1-TR-000040 and UL1-TR-001079 from the National Center for Research Resources. Additional support included AHA 0430032N from the American Heart Association; R01 DK080015 from the National Institutes of Diabetes and Digestive and Kidney Diseases; and R01 HL066075, R01 HL074338, R01 HL074406, R01 HL077449, R01 HL077612, R01 HL088451, R01 HL086719, R01 HL093081, R01 HL096875, R01 HL098077, R01 HL10161-01A1, R21 HL109924, R01 HL076831, R21 HL091217, R21 DA024273, and RC1 HL100543 from the National Heart, Lung, and Blood Institute. GlaxoSmithKline and Roche Diagnostics provided investigator-initiated funding.

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GLOBAL HEART

VOL. 11, NO. 3, 2016
ISSN 2211-8160/$36.00.
http://dx.doi.org/10.1016/j.gheart.2016.07.003

TABLE 1. Biomarkers measured in MESA by domain (biological pathway)

Biomarkers by Domain		Number of Measurements at Each Exam				
		Exam				
Domain	Biomarker	1	2	3	4	5
Inflammation	C-reactive protein (high sensitivity)	6,762		1,935	442	501
	Interleukin-6	6,622		1,923		
	Fibrinogen antigen	6,767		1,967	456	517
	White blood cell count				928	2,892
	Interleukin-10	2,810		866		
	Interleukin-2 soluble receptor	2,885				
	Tumor necrosis factor-α soluble receptor 1	2,885	2,372			
	Pentraxin-3	2,838				
	Serum amyloid P	2,863				
	Antihuman heat shock protein-60	998				
	Interleukin-16	824				
	Macrophage migration inhibitory factor	824				
	Macrophage inflammatory protein-1α	824				
	Myeloperoxidase	824				
	Tumor necrosis factor-α		779	1,182		
Insulin resistance	Fasting glucose	6,789	6,184	5,887	5,634	4,587
	Fasting insulin	6,784		1,965		
	Hemoglobin A_1C		6,142			
Lipids/fatty acids	Total cholesterol, HDL cholesterol, LDL cholesterol	6,791	6,185	5,892	5,634	4,582
	Triglycerides	6,971	6,185	5,892	5,634	4,582
	Small HDL 7.3—8.2 nm from NMR*	6,795				
	Medium HDL 8.2—8.8 nm from NMR*	6,795				
	HDL cholesterol (total) from NMR*	6,795				
	HDL particles (total) from NMR*	6,795				
	Mean HDL size from NMR*	6,795				
	Large HDL 8.8—13 nm from NMR*	6,795				
	Large HDL 9.4—14 nm from NMR*	6,786				
	Large LDL 20.5—23 nm from NMR*	6,786				
	Large LDL 21.2—23 nm from NMR*	6,795				
	Large VLDL $>$ 60 nm from NMR*	6,786				
	LDL (total) 18—21.2 from NMR*	6,795				
	Very small LDL 18—19.8 nm from NMR*	6,786				
	Small LDL 18—20.5 nm from NMR*	6,795				
	Medium-small LDL 19.8—21.2 nm from NMR*	6,795				
	LDL particles (total) from NMR*	6,795				
	Mean LDL size from NMR*	6,795				
	Total triglycerides from NMR*	6,786				
	Small 27—35 nm VLDL from NMR*	6,795				
	Medium 35—60 VLDL from NMR*	6,795				
	VLDL triglycerides (total) from NMR*	6,795				
	VLDL particles (total) from NMR*	6,795				
	Mean VLDL size from NMR*	6,795				
	HDL subfractions (HDL-1, -2, -3, -4, -5, -6, -7, -8)	997				
	Remnant-like particle cholesterol	999				
	Cholesterol ester transferase protein activity	982				
	Cholesterol ester transferase protein mass	999				
	Sphingomyelin	6,708				

(continued)

TABLE 1. Continued

Biomarkers by Domain		Number of Measurements at Each Exam				
		Exam				
Domain	Biomarker	1	2	3	4	5
	Apolipoprotein A1	4,679				
	Apolipoprotein B	4,676				
	Lipoprotein(a)	4,676				2,892
	Free fatty acid	6,723				
	14:0 Myristic acid	2,856				
	15:0 Pentadecanoic acid	2,856				
	16:0 Hexadecanoic acid	2,856				
	16:1 9 Cis palmitoleic acid	2,856				
	16:1 9 Transpalmitoleic acid	2,856				
	18:0 Stearic acid	2,856				
	18:1 12 Transoleic acid	2,856				
	18:1 9-11 Transoleic acid	2,856				
	18:1 Cis linoleic acid	2,856				
	18:1 12 Cis linoleic acid	2,856				
	18:1 9 Cis linoleic acid	2,856				
	18:2 C/C linoleic acid	2,856				
	18:2 C/T linoleic acid	2,856				
	18:2 T/C linoleic acid	2,856				
	18:2 T/T linoleic acid	2,856				
	18:3 N3 α-linoleic acid	2,856				
	18:3 M6 γ-linoleic acid	2,856				
	20:0 Arachidonic acid	2,856				
	20:1 N9 gadoleic acid	2,856				
	20:2 N6 eicosanoic acid	2,856				
	20:3 N6 eicosanoic acid	2,856				
	20:4 N6 arachidonic acid	2,856				
	20:5 N3 timnodonic acid	2,856				
	22:0 Behenic acid	2,856				
	22:5 N3 clupanodonic acid	2,856				
	22:6 N3 docosahexaenoic acid	2,856				
	24:1 N9 nervonic acid	2,856				
Hemostasis/ fibrinolysis	D-dimer	6,769			456	515
	Factor VIII activity	6,765				
	Plasminogen activator inhibitor-1	973				
	Plasmin-antiplasmin complex	6,627				
	Tissue factor pathway inhibitor	995				
Oxidative damage	Oxidized LDL cholesterol	999				
	F_2-isoprostanes	390				
Oxidative stress	Salivary cortisol (measured across exams 3 and 4)			1,002		
	Urinary epinephrine† (measured across exams 3 and 4)			1,002		
	Urinary norepinephrine† (measured across exams 3 and 4)			1,002		
	Urinary dopamine† (measured across exams 3 and 4)			1,002		
Renal function	Serum creatinine	6,789	769	5,887	5,634	4,587
	Cystatin C	6,756	770	5,550	5,260	4,581
	Urine creatinine/albumin†	6,789	769	5,887	5,634	4,587

(continued)

TABLE 1. Continued

Biomarkers by Domain		Number of Measurements at Each Exam				
		Exam				
Domain	Biomarker	1	2	3	4	5
	Blood urea nitrogen[†]	6,738				
	Kidney injury molecule-1[†]	686				
	Neutrophil gelatinase-associated lipocalin[†]	686				
Renin-angiotensin-aldosterone system	Plasma renin activity		698	1,103		
	Aldosterone		732	1,158		
Adipokines/ metabolism	Leptin (measured at baseline and across exams 2 and 3)	824	1,960			
	Adiponectin (measured at baseline and across exams 2 and 3)	824	1,967			
	Resistin (measured at baseline and across exams 2 and 3)	824	1,966			
Plaque stability	Lipoprotein phospholipase A_2 mass	5,273				
	Lipoprotein phospholipase A_2 activity	5,353				
	Matrix metalloproteinase-3	999				
	Matrix metalloproteinase-9	999				
	Matrix metalloproteinase-1		2,372			
	Matrix metalloproteinase-2		2,372			
	CD40 ligand	999				
	Soluble tissue factor	993				
Vitamin/mineral	24,25-Dihydroxy vitamin D3	6,473				
	1,25-Dihydoxy vitamin D2	440				
	25-Hydroxy vitamin D		368			
	Parathyroid hormone	6,555				
	Fetuin-A	2,904				
	Fibroblast growth factor-23	6,552				
	Serum calcium	6,514				
	Serum chloride	6,489				
	Serum phosphorus	6,544				
	Serum sodium	6,489				
	Serum bicarbonate	6,489				
	Serum potassium	6,489		650	323	
	Dihydrophylloquinone	1,056				
	Phylloquinone	1,056				
Endothelial cell function	Homocysteine	6,794				
	von Willebrand factor	2,885				
	Soluble intracellular adhesion molecule-1	2,622	2,372		455	512
	Soluble thrombomodulin	997				
	Soluble E-selectin	999			455	516
	Soluble P-selectin	5,974	2,372			
	Soluble L-selectin		2,372			
	Soluble vascular cell adhesion molecule-1		2,372			
	Chemokine ligand 21		2,372			
	E-cadherin		2,372			
	Regulated on Activation, Normal T Expressed and Secreted (RANTES)	824	2,372			
	Stromal derived factor 1α		2,372			
	Secretory leukocyte protease inhibitor		2,372			

(continued)

TABLE 1. Continued

Biomarkers by Domain		Number of Measurements at Each Exam				
		Exam				
Domain	Biomarker	1	2	3	4	5
	Transforming growth factor β1		2,372			
	Tissue inhibitor of metalloproteinases 2		2,372			
	Endothelial progenitor cells (count per 10,000 lymphocytes)				407	
Growth factors/ angiogenesis	Hepatocyte growth factor	5,974				
	Angiopoietin-2	824				
	Epidermal growth factor-1	824				
	Vascular endothelial growth factor (VEGF)	824				
Chemotaxis	Cutaneous T-cell-attracting chemokine (CCL27)	824				
	Eotaxin-3	824				
	Interferon-inducible T-cell alpha chemoattractant (CXCL11)	824				
	Interferon gamma-induced protein-10 (IP-10)	824				
	Monocyte chemoattractant protein-1	824				
	Monocyte chemoattractant protein-2	824				
	Monocyte chemoattractant protein-3	824				
	Monocyte chemoattractant protein-4	824				
Chronic infection	*Chlamydia Pneumoniae* (antibody titer)	6,790				
	Cytomegalovirus (EU/ml)	999				
	Helicobacter Pylori (antibody titer)	999				
	Hepatitis A virus (antibody titer)	999				
	Herpes Simplex virus (EU/ml)	999				
Cardiac function	N-terminal pro-brain natriuretic peptide	5,597		4,694		
	Cardiac troponin T	5,597		4,694		
Apoptosis	Factor activating Exos ligand (FasLigand)	824				
	Soluble factor activating Exos (Fas)	824				
Macrophage activity	Interleukin-18	824				
Vascular remodeling	Tissue inhibitor of metalloproteinase-1 (TIMP-1)	824				
	Tissue inhibitor of metalloproteinase-4 (TIMP-4)	824				
Sex hormones	Dehydroepiandrosterone	6,172				
	Sex hormone—binding globulin	6,172				
	Testosterone	6,167				
	Estradiol	6,170				
Immune cell profiles	% $CD4^+$ lymphocytes that are T cells				917	
	% $CD4^+$ lymphocytes that are Th1 cells				917	
	% $CD4^+$ lymphocytes that are Th2 cells				914	
	% $CD4^+$ lymphocytes that are memory cells				917	
	% $CD4^+$ lymphocytes that are naïve cells				914	
	Natural killer cells				891	
	$\gamma\delta$ cells				919	
	% cells that are monocytes—LPS stimulated expression assay				842	
	% cells that are monocytes—unstimulated expression assay				843	
	Immature granulocytes					1,645
Autoimmunity	Rheumatoid factor IgA	6,738				
	Rheumatoid factor IgM	6,738				

(continued)

TABLE 1. Continued

Biomarkers by Domain		Number of Measurements at Each Exam				
		Exam				
Domain	Biomarker	1	2	3	4	5
Bone morphology	Osteoprotegerin		761			
Liver function	Gamma-glutamyltransferase	6,754				
Blood oxygenation	Red blood cells				928	2,892
	Hemoglobin				927	2,892
	Platelets				922	2,886
Tobacco smoke exposure	Urinary cotinine[†]					3,212

HDL, high-density lipoprotein; LDL, low-density lipoprotein; LPS, lipopolysaccharied; NMR, nuclear magnetic resonance; VLDL, very low-density lipoprotein.
*From NMR LipoProfile-II spectral analysis.
[†]Measured in urine. All other biomarkers measured in serum or plasma unless otherwise noted.

domains such as adaptive immune function and plaque destabilization. Many of these pathways are important not only in CVD, but in other chronic inflammatory diseases such as diabetes, renal disease, and pulmonary disease. These common pathways are of particular interest as they may help define the excess risk of CVD in patients with other inflammatory diseases. As with initial biomarker selection, measurements were vetted by committee with consideration given to potential significance of research findings, impact of sample use on the repository and appropriateness of the MESA cohort for the research.

BIOMARKER DOMAINS MEASURED IN MESA

Inflammation

Inflammation biomarkers measured represent both general inflammation and several specific inflammatory pathways. CRP is perhaps the best known of these systemic inflammation markers. CRP is a nonspecific acute phase protein synthesized by hepatocytes, arterial smooth muscle cells, and adipocytes in response to inflammatory cytokines such as IL-6. Data from MESA have contributed to the status of CRP as a nontraditional marker for CVD risk with clinical utility in screening. CRP was found to identify asymptomatic individuals at higher risk of a CVD event than predicted by traditional risk-screening guidelines. Sex differences in CRP levels were also noted, with women having higher CRP levels than men across all race/ethnic groups, suggesting that clinical cutpoints should be sex-specific [1]. In addition, CRP was found to be an independent predictor of myocardial functional deterioration in asymptomatic individuals with no history of heart disease.

IL-6 is a proinflammatory cytokine with multiple humoral and cellular effects. As a direct regulator of the inflammation response, IL-6 may serve as a link between inflammation and CVD. Studies in MESA support a strong association of IL-6 with left and right ventricular function and endothelial function [2].

Fibrinogen is a major circulating procoagulant protein, a nonspecific acute phase reactant, and also an inflammation biomarker. In MESA, elevated fibrinogen levels were associated with impaired myocardial systolic function supporting the interplay of inflammation, coagulation, and hyperviscosity in the pathogenesis of myocardial dysfunction.

Biomarker measurements also included several novel biomarkers such as pentraxin 3 (PTX3). PTX3 is related to CRP, but is produced at sites of inflammation by vascular endothelial cells, smooth muscle cells, and macrophages and is thought to be a specific marker of vascular inflammation. In MESA, PTX3 was associated with CVD risk factors, subclinical CVD measures, coronary artery calcium (CAC) and incident coronary heart disease (CHD) independent of CRP. Associations of PTX3 with greater right ventricular mass and larger right ventricular end-diastolic volume suggest a functional role for PTX3 in the pulmonary circulation—right ventricular axis [3]. PTX3 has also been associated with kidney dysfunction and highlights the importance of endovascular inflammation in early kidney dysfunction, particularly in African Americans.

Insulin resistance

Levels of glycosylated hemoglobin A_1C, which provide information on glycemic status, were associated with measures of subclinical CVD in nondiabetic MESA participants. These results suggest that a clinical definition of diabetes based on fasting glucose levels alone may not represent the true level of cardiovascular risk due to impairments in glucose regulation.

Lipids and fatty acids

Lipoprotein (protein-lipid complex) particle subclass concentrations were measured by NMR spectroscopy. Small and medium diameter HDL particles were found to be inversely associated with risk of CHD and noncardiovascular, noncancer chronic inflammation—related

death and hospitalization and CHD in MESA, suggesting that smaller HDL particles may have anti-inflammatory properties. Conversely, small LDL particles were associated with increased CHD risk. In addition, LDL particle number was a better estimator of atherosclerotic risk when there was discordance between LDL cholesterol (mass of cholesterol carried by LDL particles) and LDL particle number [4].

Levels of lipoprotein(a) (Lp(a)), a well-characterized subspecies of LDL, are not influenced by lifestyle factors but are instead strongly influenced genetically, leading to noted racial/ethnic differences in circulating Lp(a). Extending these findings, associations of Lp(a) with CHD in MESA suggested that clinical cutoffs for Lp(a) should be race/ethnic-based [5]. In addition, MESA was the lead cohort in a multicohort consortium examining genes for aortic calcification that identified variation in the Lp(a) gene locus (*LPA*) as potentially causative.

Dietary fatty acids have been associated with a number of outcomes in MESA. Pentadecanoic acid, a fatty acid biomarker of dietary dairy intake, was inversely associated with incident CVD and CHD, suggesting a potential cardioprotective role for dietary dairy fat. Similarly, transpalmitoleic acid from dairy fat was associated with lower blood pressure and lower risk of incident diabetes.

Circulating levels of polyunsaturated omega-3 fatty acids derived from dietary seafood were inversely associated with CVD incidence, supporting the hypothesis that increased consumption of omega-3 fatty acid-rich seafood may be beneficial in CVD prevention. Omega-3 fatty acids were likewise inversely associated with lipoprotein-associated phospholipase A_2 (Lp-PLA_2), a biomarker of atherosclerotic plaque stability, indicating a potential mechanism for the cardiovascular benefits of these dietary fatty acids.

Hemostasis and fibrinolysis

Statin use in MESA was associated with lower levels of D-dimer, a fibrin degradation product and biomarker of hemostatic activation, and factor VIII, a procoagulant cofactor, indicating a potential mechanism for statin use to lower incidence of venous thromboembolism [6].

Renal function

Kidney function and injury were assessed using longitudinal measurements of common clinical biomarkers as well as the novel biomarker cystatin C. Cystatin C is a cysteine protease inhibitor secreted by all nucleated cells that is relatively freely filtered at the glomerulus and increasing levels indicate worsening kidney function. A strong nonlinear association of age with cystatin C in MESA suggested that kidney function worsens considerably with age even in those without risk factors for kidney disease. In addition, cystatin C was associated with incident chronic kidney disease independent of microalbuminuria and may have a clinical role in identification of individuals with chronic kidney disease at highest risk for complications. Inverse associations of cystatin C with left ventricular end-diastolic and end-systolic volumes may partially explain the relationship between kidney dysfunction and heart failure. MESA has also made important contributions to understanding racial/ethnic differences in kidney function decline. African Americans and Hispanics had higher rates of kidney function decline than whites did, whereas whites and Chinese Americans had similar rates of decline [7].

Renin-angiotensin-aldosterone system

The renin-angiotensin-aldosterone hormone system regulates plasma sodium concentration and arterial blood pressure and, therefore, plays major roles in hypertension and atherosclerotic CVD, not only through direct effects on blood pressure, but also through effects on cardiac fibrosis and end-organ damage independent of blood pressure. Prior to MESA, no large studies, outside of clinic cohorts with hypertension, had examined racial/ethnic differences in renin or aldosterone levels. In MESA, there were notable racial/ethnic differences in both analytes, with African Americans having lower aldosterone levels, and Hispanics having higher plasma renin activity levels, when compared with other groups. Furthermore, Hispanics appeared to be more sensitive to both hormones with a stronger association between renin or aldosterone and blood pressure. These findings may have important implications in racial/ethnic differences in the diagnosis and treatment of hypertension and resulting morbidity and mortality [8].

Adipokines and metabolism

Adipokines, such as leptin, resistin, and adiponectin, are cytokines secreted by adipose tissue that influence multiple metabolic pathways. In MESA, higher leptin levels were associated with lower risk of all-cause mortality and CVD. MESA has also contributed to the published reports on racial/ethnic differences in circulating levels of adiponectin and leptin and their link to racial/ethnic differences in insulin resistance. Associations of leptin and adiponectin with insulin resistance did not vary significantly among racial/ethnic groups; however, associations of body mass index with adiponectin and leptin differed significantly by race/ethnicity, suggesting roles for leptin and adiponectin in racial/ethnic differences in insulin resistance [9]. Likewise, whereas resistin was associated with CVD across all racial/ethnic groups, significant racial/ethnic interactions were noted; the impact of resistin on CVD may be especially important in Hispanics.

Plaque stability

Lp-PLA_2 is an enzyme responsible for hydrolysis of oxidized phospholipids on LDL particles. Higher Lp-PLA_2 activity within an atherosclerotic plaque is associated with greater vulnerability of the plaque to rupture. Extending previous research to a multi-ethnic cohort, both Lp-PLA_2

mass and activity were associated with increased risk of incident CVD and CHD in MESA, regardless of the presence of subclinical CVD [10].

Matrix metalloproteinase-9 is an enzyme produced by a number of cellular constituents of atherosclerotic plaques, vessel walls, and the myocardium. Matrix metalloproteinase-9 functions in collagen degradation and tissue remodeling. In MESA, matrix metalloproteinase-9 was associated with lower right ventricular mass in individuals free of clinical CVD potentially through prevention of collagen accumulation [11].

Endothelial cell function

Biomarkers of endothelial perturbation include soluble forms of cellular adhesion proteins such as intracellular adhesion molecule-1 and P-selectin. Higher levels of soluble intracellular adhesion molecule-1 were associated with accelerated progression of emphysema in MESA, indicating that neutrophil recruitment to the lung, mediated by intracellular adhesion molecule-1, may play a role in the progression of subclinical emphysema [12]. Similarly, higher levels of P-selectin were associated with peripheral arterial disease, suggesting that leukocytes recruitment to sites of vascular injury, mediated by P-selectin, may contribute to progression of peripheral arterial disease.

Oxidative stress

Measures of oxidative stress include the stress hormone cortisol and catecholamine neurotransmitters (epinephrine, norepinephrine, and dopamine). Sex differences in associations of these measures with diabetes were noted in MESA; women with diabetes had higher total diurnal cortisol exposure than did nondiabetic women, whereas urinary catecholamines were significantly lower in men with diabetes than in nondiabetic men. These findings are intriguing in that they may detect early autonomic neuropathy in diabetic men and subclinical hypothalamic-pituitary-adrenal axis hyperactivity in diabetic women [13].

Oxidative damage

Oxidized LDL cholesterol is a biomarker of oxidative damage. Extending previous findings to younger ages and different ethnic groups, oxidized LDL was associated with CVD risk factors and multiple measures of subclinical CVD, across sex and racial/ethnic groups, in MESA, supporting its role as a biomarker of atherosclerosis initiation and progression [14].

Vitamin and mineral metabolism

MESA also explored the role of mineral metabolism in CVD. Insufficient vitamin D may activate the renin-angiotensin-aldosterone system and stimulate atherogenic cytokine expression, leading to atherosclerosis, whereas excess phosphorus, parathyroid hormone (PTH), and fibroblast growth factor-23 (FGF-23) likely promote medial artery calcification, vascular stiffness, and myocardial hypertrophy.

In MESA, serum 25-hydroxyvitamin D (25[OH]D) concentration varied markedly by race/ethnicity. Lower 25(OH)D was associated with increased risk of CHD among whites and Chinese Americans, but not among African Americans and Hispanics. Lower 25(OH)D was also associated with increased risk of incident CAC, but not other measures of subclinical CVD or heart failure [15]. These findings suggested that insufficient 25(OH)D may be a modifiable risk factor for atherosclerotic CHD, but the ascertainment of vitamin D deficiency, or its biologic impact, may vary by race/ethnicity. Ongoing clinical trials are evaluating the effects of vitamin D supplementation on cardiovascular risk.

Higher serum PTH concentration was associated with arterial stiffness, left ventricular hypertrophy, incident hypertension, and heart failure events, without significant heterogeneity by race/ethnicity [16]. Similarly, higher serum FGF-23 concentration was associated with left ventricular hypertrophy, heart failure events, and incident atrial fibrillation [17]. These findings suggested that PTH, FGF-23, and underlying phosphorus excess may increase risk of heart failure and related clinical outcomes by reducing vascular compliance or promoting myocardial hypertrophy. Medications targeting PTH, FGF-23, and phosphorus are currently being developed and evaluated.

Fetuin-A, a hepatic secretory protein, inhibits arterial calcification in vitro by interacting with calcium and phosphorus to increase their solubility and inhibit precipitation. In humans, fetuin-A circulates at relatively high concentrations and may be a marker of arterial calcification. In a subset of MESA participants, fetuin-A levels were inversely associated with CAC severity, independent of traditional cardiovascular risk factors and kidney function [17].

Chronic infection

A number of chronic infections acquired early in life and not causing obvious illness are implicated in the development and progression of CVD. These pathogens include persistent viruses such as cytomegalovirus and bacterial pathogens such as *Helicobacter pylori*. In MESA, a high antibody response to multiple pathogens was identified as a better marker of inflammation status (levels of circulating inflammation markers) than seropositivity alone. Although associated with inflammation, individual pathogens and pathogen burden (number of positive pathogens) were not associated with measures of subclinical atherosclerosis or CAC, suggesting there was no direct link between infectious burden of these pathogens and subclinical atherosclerosis or subclinical CHD. Although not directly associated with CVD, immune responses to chronic infections may be an important link in the pathway between psychosocial factors and CVD risk as psychosocial

determinants were associated with both pathogen burden and immune response in MESA.

Sex hormones

Levels of endogenous sex hormones were measured to better understand sex differences in CVD and other diseases. In MESA, higher sex hormone—binding globulin levels were associated with less atherogenic lipoprotein profiles, whereas higher endogenous estradiol levels were associated with more atherogenic profiles. Testosterone was associated with favorable lipoprotein profiles in men, but not women, whereas dehydroepiandrosterone had different associations with lipoprotein subclasses in men and women. These findings highlight the potential clinical utility of sex hormones in improving lipoprotein profiles as well as the complexity of their interactions. In addition, associations of testosterone with QT interval in men, but not in post-menopausal women, may explain differences in QT interval duration between men and women. Variations in testosterone level may also contribute to population variability in QT interval duration in men. In men, an androgenic profile was associated with greater carotid distensibility, whereas the opposite was found in women [18]. Associations of sex hormones with waist-to-hip ratio also implicated sex hormones in sex-related differences in central obesity [19].

Cardiac function

Natriuretic peptides have become established markers of long-term cardiovascular prognosis in a variety of clinical settings and even among apparently healthy individuals. In MESA, racial/ethnic differences in N-terminal pro—B-type natriuretic peptide (NT-proBNP) levels were noted; African and Chinese American participants had lower NT-proBNP concentrations compared with whites and Hispanics. Whereas both cardiac troponin T and NT-proBNP were independently associated with increased risk of CVD and CHD, only NT-proBNP provided prognostic information above and beyond traditional risk factors, improving risk prediction and classification when compared with standard risk equations. Change in NT-proBNP levels over several years was also independently associated with CVD regardless of race/ethnicity [20]. NT-proBNP levels in MESA are an integral component of a clinical algorithm developed to predict incident heart failure.

Immune cell profiles

Whereas inflammation biomarkers, such as CRP and IL-6, have elucidated the role of innate immunity and inflammation in atherosclerosis, they do not provide information on the roles of specific cell populations that contribute to CVD development and progression. In particular, $CD4^+$ T helper type 1 (Th1) lymphocytes have been studied extensively and implicated as proatherogenic, whereas Th2 cells are thought to be antiatherogenic. Little information on associations of innate and adaptive immune cells with the progression of atherosclerosis is available from epidemiologic cohorts. The MESA Inflammation study evaluated 11 different cellular phenotypes including Th1 and Th2 cells and $CD4^+$ memory and naïve cells.

Results from variability studies demonstrated that these cellular phenotypes were reproducible and generally stable, indicating, for the first time, their suitability for evaluation in epidemiological research [21].

Th1 cell levels were positively associated with IL-6, CAC, and common carotid intima media thickness, whereas Th2 cell levels were negatively associated with common carotid intima media thickness. These results were consistent with a proatherogenic role for Th1 cells and an antiatherogenic role for Th2 cells, and is the first demonstration of these relationships in a multiethnic population [21]. In addition, the degree of chronic adaptive immune activation, as estimated by higher memory and lower naïve $CD4^+$ cell phenotypes, was associated with subclinical atherosclerosis and type 2 diabetes.

Biomarker-based algorithm for coronary risk assessment

MESA was the validation cohort for a study incorporating a number of biomarkers into a CHD risk assessment model that demonstrated clinical utility in improving risk prediction in intermediate risk patients [22]. Seven biomarkers from the domains of inflammation, chemotaxis, apoptosis, and growth/angiogenesis factors (cutaneous T-cell—attracting chemokine, eotaxin, factor activating Exos ligand, soluble factor activating Exos, hepatocyte growth factor, IL-16, and monocyte chemoattractant protein-3) were included in the model, which demonstrated a net clinical reclassification index of 42.7%, exceeding the clinical net classification indices of established risk factor scores [22].

Participation in multi-cohort consortia

MESA has provided biomarker data to several multicohort consortia involved with genomics research including the CARe (Candidate Gene Association Resource), CHARGE (Cohorts for Heart and Aging Research in Genomic Epidemiology), PAGE (Population Architecture using Genomics and Epidemiology), ESP (Exome Sequencing Project), and, most recently, TOPMed (Trans-omics for Precision Medicine) and the EFRC (Emerging Risk Factor Collaboration), a biomarker meta-analysis. Important meta-analyses completed to date include those for fibrinogen and CRP. In addition, MESA has participated in an international metabolomics consortium, the COMBI-BIO (COMBInational BIOmarkers for subclinical atherosclerosis) for which primary data are currently being analyzed.

SUMMARY

Biomarkers are powerful instruments in the examination of the full spectrum of a disease; from initial development,

through progression to clinical stages. To date, the MESA parent and ancillary studies have measured over 180 biomarkers covering 26 different biological domains. Additional biomarker measurements, utilizing the MESA repository of plasma, serum, and urine samples from each exam, are ongoing. The results of these studies have contributed to, and will continue to contribute to, clinical recommendations utilizing biomarkers for diagnosis and treatment, and a better understanding of racial/ethnic and sex differences in key biological pathways that reflect racial/ethnic and sex differences in disease prevalence and presentation.

Overall, these studies highlight the importance of circulating and urinary biomarkers; they are not merely surrogate endpoints. Biomarkers are able to reflect the entire span of a particular disease from the earliest subclinical manifestations to clinical stages and can broaden our knowledge about the underlying pathogenesis of disease.

ACKNOWLEDGMENTS

The authors thank the other investigators, the staff, and the participants of the MESA study for their valuable contributions. A full list of participating MESA investigators and institutions can be found at http://www.mesa-nhlbi.org.

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