BMJ Open

Evaluating Atherosclerosis Prevalence via Coronary Calcium in Executives with Normal LDL Levels- A Cohort Study: The CLEAR Protocol

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2024-094899
Article Type:	Protocol
Date Submitted by the Author:	10-Oct-2024
Complete List of Authors:	Ratrout, Basem M.; Mayo Clinic Katamesh, Basant; Mayo Clinic, General Internal Medicine Lawson, Donna K.; Mayo Clinic VerNess, Christina D.; Mayo Clinic Vincent, Ann; Mayo Clinic, Division of General Internal Medicine Hurt, Ryan T.; Mayo Clinic Bonnes, Sara; Mayo Clinic Adusumalli, Jayanth; Mayo Clinic, General Internal Medicine Schroeder , Darrell ; Mayo Clinic Croghan, Ivana; Mayo Clinic, General Internal Medicine
Keywords:	Coronary heart disease < CARDIOLOGY, Adult cardiology < CARDIOLOGY, CARDIOLOGY

Evaluating Atherosclerosis Prevalence via Coronary Calcium in Executives with Normal LDL Levels- A Cohort Study: The CLEAR Protocol

Basem M. Ratrout, MD, MHA

Basant E. Katamesh, MD

Ann Vincent, MD

Ryan T. Hurt, MD, PhD

Sara L. Bonnes, MD, MS

Jayanth Adusumalli, MBBS, MPH

Donna K. Lawson

Darrell Schroeder, MS

Christina D. VerNess, MA

Ivana T. Croghan, PhD

Author Affiliations: Division of General Internal Medicine (Ratrout, Vincent, Hurt, Bonnes, Adusumalli, VerNess, and Croghan), Research Fellow in the Division of General Internal Medicine (El-Fetouh Katamesh; limited tenure), Division of Hospital Internal Medicine (Lawson), and Department of Quantitative Health Sciences (Schroeder) Mayo Clinic, Rochester, Minnesota.

Corresponding Author: Basem M. Ratrout, MD, MHA, Division of General Internal Medicine, Mayo Clinic, 200 First St SW, Rochester, MN 55905 (Ratrout.Basem@Mayo.edu).

Abstract

Introduction: The coronary artery calcium (CAC) score can stratify risk of coronary artery disease (CAD) in patients with hyperlipidemia, especially when uncertainty exists regarding the use of pharmacotherapy. Although patients with hyperlipidemia often have abnormal CAC scores (i.e. calcified plaque in coronary vessels), it remains unclear what percentage of patients without hyperlipidemia have abnormal CAC scores. Advanced glycation end products (AGEs) have been implicated in atherosclerosis and may be beneficial in assessing cardiovascular risk. However, the relationship among AGE score and other cardiovascular markers has not been fully explored. To evaluate abnormal CAC scores in patients with normal low-density lipoprotein cholesterol (LDL-C) levels ≤100mg/dl. The secondary objective is to explore the associations between the CAC and AGE scores.

Methods and Analysis: We will retrospectively review health records of adult patients seen at the Executive Health Program (Mayo Clinic; Rochester, Minnesota), between September 1, 2023, and March 31, 2024, where all patients were offered the option of a baseline CAC scan. To assess our primary aim, we will determine the percentage of patients with abnormal CAC score among those with normal LDL-C, no established CAD, and not on statins. For our secondary aim, we will examine the potential correlation between the CAC and AGE scores.

Ethics and dissemination: T his study was determined to be exempt from institutional review board approval (ID 24-003921; 45 CFR 46.104d, category/subcategory 4[iii]) at Mayo Clinic, Rochester. The findings of this study will be published in a peer-reviewed journal.

Strengths and limitations of this study:

⇒ This study benefits from comprehensive data collection using multiple data sources, including CAC scores, AGE scores, lipid levels, and cardiac imaging results, providing a multidimensional view of cardiovascular risk.

⇒ This study will ensure the collection of accurate and reliable data by training data collectors, adapting prevalidated research tools, and conducting regular quality checks.

⇒ This single-center study is limited by its reliance of the type of patients that present to unique practice and thus results cannot be generalized to the general population.

⇒ The study is limited by its reliance on patient records, which restricts reviewers to the availability and quality of documented information.

Background

Coronary artery disease (CAD) poses a global health challenge, contributing substantially to morbidity and mortality worldwide (1, 2). CAD manifests through the gradual formation of atherosclerotic plaques within the coronary arteries and require effective risk assessment and management strategies. One risk stratification measure is assessing coronary artery calcium (CAC) through imaging. The CAC scan is a noninvasive, computed tomographic procedure that serves as a highly specific test for coronary atherosclerosis (3). The CAC scan provides a score that quantifies the amount of calcified plaque present in the coronary arteries and helps predict the risk of future cardiovascular events, and thereby help guide clinical decision-making (4, 5).

The American College of Cardiology (ACC) and American Heart Association (AHA) have classified CAC scores into specific ranges (4). A CAC score of 0 signifies a low risk for future cardiovascular events and related mortality (4). Scores between 1-100 indicate a low-to-intermediate risk, while scores between 101-400 suggest an intermediate risk (4). A score above 400 indicates a higher risk for future cardiovascular events (4). In clinical practice, in individuals with higher CAC scores aggressive risk reduction strategies including pharmacotherapy and lifestyle changes are strongly recommended (4, 6).

Current ACC/AHA guidelines recommend CAC scans for patients with an LDL-C above 70 mg/dL with a higher risk estimate of atherosclerotic cardiovascular disease (ASCVD) risk when there is ambiguity regarding pharmacotherapy. However, the utility of CAC scan in individuals with normal LDL-C and low cardiovascular disease risk is not well established. One study by Fernández-Friera et al. reported that normal LDL-C can be linked to subclinical atherosclerosis (7). In this study, 107 of 315 (34%) patients with LDL-C at or below 100 mg/dL exhibited early signs of systemic atherosclerosis (7). Additionally, coronary artery calcification

was reported in 7% of patients with LDL-C ranging 80-90mg/dL and around 4% in patients with LDL-C between 90-100 mg/Dl (7). This association remained significant even in the absence of other cardiovascular risk factors and was evident in low-risk groups based on the 30-year Framingham risk score (7). These findings suggest that relying solely on LDL-C may not be adequate for assessing cardiovascular disease risk and underscore the critical role of LDL-C as a predictor of atherosclerosis, even at levels currently deemed normal (7). This indicates a potential need to reevaluate our understanding of healthy cholesterol levels and their impact on cardiovascular health.

In a second study, Taylor et al (8) examined the predictive capability of CAC detection for CAD in a cohort comprising 2,000 healthy, asymptomatic, active-duty United States Army personnel aged 40 to 50 years. CAC was detected in 22.4% of men and 7.9% of women with low cardiovascular risk and with elevated scores were associated with a 12-fold increased risk of CAD over a 3-year period (8). The study concluded that in young, asymptomatic men, the detection of CAC offered cost-effective independent prognostic value in predicting the development of CAD, beyond the assessment of conventional coronary risk factors (8). In alignment with these two studies, our clinical practice has observed abnormal coronary and vascular calcification on imaging studies in patients with normal LDL-C levels who would not have otherwise qualified for a CAC scan based on current ACC/AHA guidelines. Our clinical observations, combined with the results from these studies, suggest that LDL-C alone may be insufficient to risk stratify patients with asymptomatic ASCVD. We hypothesize that the use of CAC scans could provide additional benefits in the early prediction of heart disease for patients with normal LDL-C levels.

In addition to the CAC score, novel measures for ASCVD risk stratification are being explored. One such measure is the assessment of advanced glycation end products (AGEs). AGEs are harmful compounds formed when proteins or fats combine with sugars in the bloodstream through a process called glycation and are reported to be associated with hyperlipidemia and/or hyperglycemia via mechanisms involving oxidative stress, abnormal inflammatory responses, and endothelial dysfunction (9).They can accumulate in various tissues and organs, contributing to aging and the development of various diseases, including diabetes, cardiovascular diseases, and kidney disease (9). The AGE reader, a noninvasive tool that uses fluorescence techniques that can be easily used in an ambulatory practice to effectively, is one method used in assessing AGEs and forecasts cardiovascular risk (10, 11). A systematic review and meta-analysis by Sharifi-Zahabi et al. to assess the association between AGEs and all-cause mortality reported that higher levels of circulating AGEs were significantly associated with an increased risk of both all-cause and cardiovascular mortality (12). The study reported a 5% and 7% increase in all-cause and cardiovascular mortality respectively with every 100-µg/L increment in AGEs (12). Therefore, incorporating AGEs into cardiovascular risk stratification may improve the prediction of cardiovascular risk (10, 11).

The objective of our study is to determine the percentage of patients with normal LDL-C levels who have abnormal coronary calcium scans. Additionally, we aim to explore potential associations between CAC scores and AGE scores.

Methods

Study Design and Setting

We will retrospectively review the medical records of adult patients who received medical care and completed a CAC study at the Mayo Clinic Executive Health Program (MCEHP) in Rochester, Minnesota, between September 1, 2023, and March 31, 2024. The project is scheduled to commence in the third quarter of 2024, with anticipated completion by December 31, 2025.

Objectives

For all included patients, we will assess and categorize CAC scan results, lipid levels, AGE scores, comorbid conditions, and CVD risk factors. The first component of this record review is to assess the prevalence of abnormal CAC scores among patients with lipid levels equal to or less than 100 mg/dL. The second component of this review will focus on evaluating the correlation between AGEs, CAC score, and lipid levels within the cohort.

Study Sample and sampling Techniques

Study entry criteria will include being an empaneled patient in the MCEHP, having provided authorization for research, completed a CAC scan, and aged 40 to 75 years old. We anticipate that a total of approximately 1,000 patients will meet the inclusion criteria. Bases on a preliminary review of our practice we anticipate that approximately 250 of the 1,000 patients will have normal LDL-C, no established CAD, and not be taking statins. We hypothesize that 5-10% of individuals with normal LDL-C levels will have abnormal CAC scores. With a denominator of 250 and an expected calcium scan abnormality rate of 10% or less, the margin of error for a 95% confidence interval (CI) is expected to be approximately 3.5%, such that any observed calcium abnormality rate (CAR) of 10% or less would yield a confidence interval of

CAR +/- 3.5%. This is based on using nQuery Advisor 7.0 (confidence interval for a proportion using the normal approximation).

Definition:

We define normal LDL-C as ≤100mg/dL. Abnormal coronary calcium will be defined as a CAC score above 0.

Team composition and training:

A comprehensive training reference guide will be created before data extraction. This guide will serve as a vital resource, outlining the project's specifics objectives. Any updates or changes to the project will be promptly incorporated into this guide and communicated to other members of the team. Training sessions will be conducted whenever a new abstractor joins the team, ensuring that each member is well trained in the extraction process. Prior to chart reviews, all members of the reviewing team will undergo training on the study protocol, key definitions, and the use of research tools. They will practice with test cases created by the adjudicators to familiarize themselves with the tools and identify any issues for review.

Record Review and Data Collection Approach

Data for this study will be abstracted from the electronic health record (EHR: Epic software; Epic Systems Corporation), and data will be subsequently collated through REDCap (Research Electronic Data Capture) (13, 14). The data abstraction sheet will be structured into 5 distinct sections: baseline demographic characteristics, laboratory test results, CAC scan and scoring details, cardiac imaging findings, and AGE scores and risk categories, Tables 1-4. The

first section of the data extraction sheet will include baseline demographic characteristics that were already collected at the time of their physical exam as illustrated in table 1. The second section of the data extraction sheet are laboratory variables results collected closest to their CAC scan, Table 2. The third section of the data extraction sheet will detail variables obtained from CAC scans, Table 3. The fourth section of the data extraction sheet will consist of imaging variables, including findings from baseline electrocardiogram, echocardiography, carotid ultrasonography, stress echocardiography, and exercise electrocardiography, Table 4. For the secondary objective of this cohort study, AGE scores and risk categories will be extracted.

Table 1. Data Extraction Sheet for Baseline Demographic Characteristics

Record ID	
Date of executive health visit	
Full name (first, middle initial, last)	
Age (at time of scan)	
Date of birth	
Sex	Male Female Other
Race	American Indian or Alaska Native Asian Native Hawaiian or Other Pacific Islander Black or African American White

	More than 1 race Chose not to disclose Unknown Other
Ethnicity	Hispanic or Latino Non-Hispanic or Latino Unknown
Level of education	8th grade High school graduate or General Educational Development test Some high school but did not graduate Associate's degree (academic) Associate's degree (occupational, technical, or vocational) Some college Bachelor's degree Master's degree Doctorate Professional degree (MD, DDS, DVM, JD) No degree The patient did not answer Unknown
Marital status	Single

	Married Divorced Separated Widowed Life partner Chose not to disclose Unknown
Smoking (any form of tobacco products [e.g., chew])	Never Current, every day Current, some days Former Unknown/unanswered
What type of smoking?	Cigarettes Cigars Pipe Snuff Chew
Does the patient use smokeless tobacco such as chew or snuff?	Yes No
Total packs per year	
Current packs per day	0.25 0.5 0.75

	1 1.5 2 2.5 3 >3 packs Quit
Date patient quit smoking or using smokeless tobacco	
Number of packs per year patient used to smoke	
Comments	
E-cigarette and vaping use	Never Current, every day Current, some days Former Unknown/unanswered
Alcohol use	Yes Not currently Never Chose not to disclose No
Type of drinks and amount per day	Glasses of wine

	Cans of beer Shots of liquor Other (e.g., vodka 750 mL/day) Unknown type
Drinks per week	
Comments	
Illicit drug use	Yes Not currently Never Chose not to disclose No
Type of illicit drug	Amphetamines Anabolic steroids Barbiturates Cocaine Crack cocaine Hashish Heroin LSD (lysergic acid diethylamide) Marijuana MDMA (3,4- methylenedioxymethamphetamine; ecstasy) Methamphetamines

	Opium PCP (phencyclidine) Solvent inhalants Other
Comments	
Family history (select all that apply)	Carotid disease Coronary artery disease Peripheral vascular disease Stroke/transient ischemic attack None of the above Unknown/unanswered
Past medical history	Cancer Chronic kidney disease Chronic liver disease Connective tissue disease Coronary artery disease Diabetes Heart rhythm disorders Hyperlipidemia Hypertension Metabolic syndrome Obesity Obstructive sleep apnea

	Peripheral artery disease Radiotherapy Stroke/transient ischemic attack Thyroid diseases Valvular disease None Unknown/not answered
Medical cannabis use	Yes No
Does the patient exercise?	Yes No Unknown
Minutes per week of exercise	
Days per week of exercise	
Type of exercise	Cardio Strength training Other Unknown
Comments and other types of exercise	
Height, cm	
Weight, kg	
Body mass index	
Neck circumference, cm	

Waist circumference, cm	
Systolic blood pressure (current), mm Hg	
Diastolic blood pressure (current), mm Hg	
Type of executive	Executive Spouse Unknown
Amount of traveling	25% of time 50% of time 75% of time 100% of time None Unknown

Table 2. Data Extraction Sheet for Laboratory Test Results

Date of laboratory tests	
Total cholesterol	
HDL cholesterol	
LDL cholesterol	
Was LDL-C <100 mg/dL?	Yes No
Is the patient on lipid lowering medication?	Yes No
Medication used	Alirocumab

	Atorvastatin Bempedoic acid Evolocumab Ezetimibe Inclisiran Pravastatin Rosuvastatin Simvastatin None
Triglycerides	
Lipoprotein (a)	
Other laboratory tests	
Apolipoprotein A-1	
Apolipoprotein B	
Fasting blood glucose	
Hemoglobin $A1_c$	
MI-Heart Ceramides, Plasma risk score	0-2 low risk 3-6 moderate risk 7-9 increased risk 10-12 high risk Not done
Comments	

Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein.

* Laboratory values closest to the time of CAC scan will be abstracted.

Table 3. Data Extraction Sheet for Coronary Artery Calcium (CAC) Scan and Scoring

Was a CAC scan obtained?	Yes No
Date of scan	
Total CAC score	
Coronary artery measurements	
Left anterior descending	
Circumflex	
Left main vessel	
Right coronary artery	
Percentile ranking	
Incidental findings	Nodules Lymph nodes Granulomas Bony lesions Enlarged aorta Other None
Other findings	

Table 4. Data Extraction Sheet for Cardiac Imaging Findings

Radiographic findings	Calcification Atherosclerosis Negative Not done Other findings
Date of radiograph	
Comments/impressions or other findings on radiography	
Chest CT imaging	Calcification Atherosclerosis Pulmonary nodules Negative Not done Other findings
Date of chest CT	
Comments/impressions or other findings on chest CT	
Carotid US	Atherosclerosis Negative Not done Other findings
Date of carotid US	

Comments/impressions or other findings on carotid US	
Echocardiography	Enlarged aorta Dilated aorta Negative Not done Other findings
Date of echocardiography	
Comments/impressions or other findings on echocardiography	
Stress echocardiography	Enlarged aorta Dilated aorta Negative Not done Other findings
Date of stress echocardiography	
Comments/impressions or other findings on stress echocardiography	
Exercise ECG	Yes No
Type of exercise ECG	Treadmill Bike
Date of exercise ECG	

Comments/impressions or other findings on exercise ECG	
Cardiopulmonary stress test	Yes No
Date of cardiopulmonary stress test	
Comments/impressions or other findings on cardiopulmonary stress test	
Cardiac perfusion stress test	Yes No
Date of cardiac perfusion stress test	
Comments/impressions or other findings on cardiac perfusion stress test	
ECG	Yes No
Date of ECG	
Comments/impressions or other findings on ECG	
Real and ECG age	
Probability of low ejection fraction, %	
Probability of atrial fibrillation, %	

Abbreviations: CT, computed tomography; ECG, electrocardiography; US, ultrasonography.

Quality Assurance

To ensure the accuracy and reliability of the extracted data, wse plan to do random spot checks on the REDCap dashboard. Our objective is to maintain a 10% spot-check rate, equating to approximately 10 checks per page, with each page containing 100 patient records. These spot checks will be assigned randomly to avoid bias and will be conducted by reviewers independent of those who initially extracted the data. This dual-review process is crucial for identifying any discrepancies or errors that may have been overlooked during the initial data extraction phase. If mistakes are identified during the spot checks, they will be promptly corrected. In addition to correcting the errors, individuals responsible for the mistakes will undergo retraining to address any gaps in knowledge.

Data Analysis Plan

Patients will be initially categorized into established CAD or no established CAD, Figure 1. Patients with established CAD will be further stratified by CAC score (0, 1-100, 101-400, and >400). For each CAC score category, the percentage of patients taking pharmacotherapy for hyperlipidemia will be calculated, including the percentage of patients receiving low-intensity, moderate-intensity, high-intensity, and nonstatin therapies.

Patients without established CAD will be first categorized by the use of pharmacotherapy for hyperlipidemia. For those taking pharmacotherapy for hyperlipidemia, the percentage of patients on low-intensity, moderate-intensity, high-intensity statin therapies, and non-statin therapy will be calculated for each CAC score category. Patients not taking pharmacotherapy for hyperlipidemia will be categorized by LDL-C level into 2 groups (≤100, and >100 mg/dL). Each LDL-C group will then be subcategorized by CAC score to determine the percentage of patients in each subcategory. These percentages will be summarized using point estimates and 95%

confidence intervals. This approach will ensure that the analysis accounts for variations in pharmacotherapy for hyperlipidemia use, LDL-C level, and CAC score. A full representation of the data analysis plan is illustrated in Figure 1. Analysis will be conducted using the SAS version 9.4 (SAS Institute Inc, Cary NC) (15).

For the secondary aim of this investigation, correlation analyses and general linear models will be used to assess the association of AGEs, indicative of cumulative metabolic stress, with CAC scores and lipid profiles. For these analyses, distributional assumptions will be assessed with variable transformations (e.g. log transformation) used as appropriate.

Patient and Public Involvement and Ethics

Patient or public involvement was not incorporated in the design, conduct, reporting, or dissemination plans of this research.

Discussion

The rationale behind this study lies in the limitations of traditional lipid-based screening in identifying certain individuals at risk for CAD**.** Asymptomatic individuals with normal LDL-C levels could still have subclinical atherosclerosis, which might be detectable through CAC scanning. By investigating the prevalence of abnormal CAC scores in this specific cohort, we aim to add to the already existing body of literature regarding the added benefit of a CAC scan in patients with normal LDL-C with no prior established cardiovascular disease risk.

In our clinical practice, we have observed that some patients with normal LDL-C levels and no apparent cardiovascular disease risks, who are classified as low-risk, actually have subclinical atherosclerosis. These patients, often seen in primary practices that do not routinely

use CAC scans, might have benefitted from such scans. Incorporating CAC scans could provide a more accurate assessment of coronary atherosclerosis, enabling timely interventions and potentially preventing future cardiovascular events like heart attacks or strokes. This study aims to provide preliminary data on the benefits of routine CAC scans in patients with normal LDL-C levels. Additionally, examining other variables in this study may help identify patients who could benefit from routine CAC scans, regardless of their LDL-C levels or cardiovascular disease risk.

This research initiative is supported by several observations of abnormal CAC score in patient with normal LDL-C≤100mg/dL reported in the literature. In addition, to the observation of abnormal CAC scores reported in patients with low LDL-C≤100mg/dL and low cardiovascular risk reported by Fernández-Friera et al. and Taylor et al. respectively, interim results of two ongoing longitudinal studies support this line of research (7, 8). For example, one currently ongoing longitudinal clinical study, Multi-Ethnic Study of Atherosclerosis [MESA] that is evaluating the risk factors that predict subclinical cardiovascular disease is utilizing CAC scanning as one of its non-invasive tests. Similarly, another ongoing longitudinal study, the Rotterdam study, focused on a wide variety of conditions including CAD and utilizing CAC scanning to risk stratify patients. A subanalysis of these two studies reported that CAC scores had better discriminatory values than polygenic risk scores in the prediction of CAD (16). The integration of CAC scoring into clinical practice could potentially enhance cardiovascular risk prediction beyond that when using traditional cardiovascular risk factors alone (6, 17). CAC scoring could provide early identification of individuals at a higher risk of future cardiovascular events, which would enable clinicians to implement targeted preventive strategies, such as lifestyle modifications, pharmacotherapy, and close monitoring.

Our rationale for assessing the potential association between AGEs and CAC stems from the significant body of evidence linking circulating AGEs to cardiovascular mortality (12). Multiple studies have demonstrated that elevated levels of AGEs are associated with increased risk of cardiovascular events and mortality (18-24). Given this established connection, it is plausible that AGEs may also correlate with the presence and severity of coronary artery calcification. However, no studies to date have examined possible associations between AGEs and CAC which will be unique to this retrospective cohort study. By investigating this potential association, we aim to uncover whether AGEs could serve as a predictive biomarker for coronary artery disease, even in patients with normal LDL-C levels. This could provide valuable insights into the underlying mechanisms of cardiovascular disease and help identify individuals at higher risk, thereby improving prevention and treatment strategies.

The findings of our study could direct future studies to explore using the CAC scan as a screening tool in cardiovascular risk stratification. By investigating the predictive value of CAC scoring in a diverse patient cohort, including patients with normal LDL-C levels, we could potentially uncover areas in which evidence-based clinical guidelines and practices could improve. Moving forward, this study could set the stage for future research aimed at optimizing the clinical utility of CAC scoring and its integration into risk prediction algorithms, along with biomarkers and imaging modalities.

Ethical Consideration

This study underwent expedited review procedures and was determined to be exempt from institutional review board approval (ID 24-003921; 45 CFR 46.104d, category/subcategory 4[iii]). Ethical principles have and will continue to be carefully considered and adhered to, and

only patients who previously consented to the use of their data for research purposes will be included in this cohort. Unique identifiers will be created to document all findings, ensuring the anonymity of the patients whose records are being reviewed. Data will be securely stored on encrypted, institution-approved cloud services, with access limited to the investigating team. Any modifications to the study design will be promptly submitted to the ethics review committee for review.

Dissemination

The results of this study will be disseminated through publication in a peer-reviewed journal and presentations at both national and international academic conferences. Additionally, we aim to promote our findings via social media platforms.

Authors' contributions

All persons listed as contributors met the International Committee of Medical Journal Editors (ICMJE) authorship criteria. Each author has made significant contributions to the writing and revisions to the study and has provided complete assent for publication. B.M.R., B.E.K., A.V., R.T.H., S.L.B., J.A., C.D.V., and I.T.C. were responsible for the conceptualization of the study. A.V., R.T.H., S.L.B., D.K.L., and I.T.C. were responsible for supervising all activities. All authors contributed to designing the methodology. B.M.R., B.E.K., A.V., and D.S. were responsible for calculating the sample size and formulating the data analysis plan. Each author is responsible for the content and has read and approved the final manuscript.

Funding: This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors

Competing interests: The authors declare there are no competing interests.

Patient and public involvement: Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication: Not applicable.

Provenance and peer review: Not commissioned; externally peer reviewed.

References:

1. Vaduganathan M, Mensah GA, Turco JV, Fuster V, Roth GA. The Global Burden of Cardiovascular Diseases and Risk: A Compass for Future Health. J Am Coll Cardiol. 2022;80(25):2361-71.

2. WHO. Cardiovascular diseases (CVDs) 2021 [Available from: https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds).

3. Lo-Kioeng-Shioe MS, Vavere AL, Arbab-Zadeh A, Schuijf JD, Rochitte CE, Chen MY, et al. Coronary Calcium Characteristics as Predictors of Major Adverse Cardiac Events in Symptomatic Patients: Insights From the CORE 320 Multinational Study. J Am Heart Assoc. 2019;8(6):e007201.

4. Arnett DK, Blumenthal RS, Albert MA, Buroker AB, Goldberger ZD, Hahn EJ, et al. 2019 ACC/AHA Guideline on the Primary Prevention of Cardiovascular Disease: A Report of the

American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. Circulation. 2019;140(11):e596-e646.

5. Hussain B, Mahmood A, Flynn MG, Alexander T. Coronary Artery Calcium Scoring in Asymptomatic Patients. HCA Healthc J Med. 2023;4(5):341-52.

6. Schade DS, Hickey M, Eaton RP. Interpreting the Coronary Artery Calcium Score - Critical Information for the Practicing Physician. Am J Med. 2023;136(11):1070-5.

7. Fernández-Friera L, Fuster V, López-Melgar B, Oliva B, García-Ruiz JM, Mendiguren J, et al. Normal LDL-Cholesterol Levels Are Associated With Subclinical Atherosclerosis in the Absence of Risk Factors. J Am Coll Cardiol. 2017;70(24):2979-91.

8. Taylor AJ, Bindeman J, Feuerstein I, Cao F, Brazaitis M, O'Malley PG. Coronary calcium independently predicts incident premature coronary heart disease over measured cardiovascular risk factors: mean three-year outcomes in the Prospective Army Coronary Calcium (PACC) project. J Am Coll Cardiol. 2005;46(5):807-14.

9. Del Turco S, Basta G. An update on advanced glycation endproducts and atherosclerosis. Biofactors. 2012;38(4):266-74.

10. Diagnoptics. Advanced Glycation Endproducts [Available from: https://www.diagnoptics.com/advanced-glycation-endproducts/.

11. Perrone A, Giovino A, Benny J, Martinelli F. Advanced Glycation End Products (AGEs): Biochemistry, Signaling, Analytical Methods, and Epigenetic Effects. Oxid Med Cell Longev. 2020;2020:3818196.

12. Sharifi-Zahabi E, Sharafabad FH, Abdollahzad H, Malekahmadi M, Rad NB. Circulating Advanced Glycation End Products and Their Soluble Receptors in Relation to All-Cause and

Cardiovascular Mortality: A Systematic Review and Meta-analysis of Prospective Observational Studies. Adv Nutr. 2021;12(6):2157-71.

13. Harris PA, Taylor R, Minor BL, Elliott V, Fernandez M, O'Neal L, et al. The REDCap consortium: Building an international community of software platform partners. J Biomed Inform. 2019;95:103208.

14. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. J Biomed Inform. 2009;42(2):377-81.

15. Inc. SI. SAS/STAT® 15.3 User's Guide. Cary, NC: SAS Institute Inc. [Available from: https://documentation.sas.com/api/collections/pgmsascdc/9.4_3.5/docsets/statug/content/sashelp.pdf?locale=en.

16. Khan SS, Post WS, Guo X, Tan J, Zhu F, Bos D, et al. Coronary Artery Calcium Score and Polygenic Risk Score for the Prediction of Coronary Heart Disease Events. Jama. 2023;329(20):1768-77.

17. Tani S, Yagi T, Atsumi W, Kawauchi K, Matsuo R, Hirayama A. Relation between low-density lipoprotein cholesterol/apolipoprotein B ratio and triglyceride-rich lipoproteins in patients with coronary artery disease and type 2 diabetes mellitus: a cross-sectional study. Cardiovasc Diabetol. 2017;16(1):123.

18. Ebert H, Lacruz ME, Kluttig A, Simm A, Greiser KH, Tiller D, et al. Association between advanced glycation end products, their soluble receptor, and mortality in the general population: Results from the CARLA study. Exp Gerontol. 2020;131:110815.

19. Ho JE, Lyass A, Courchesne P, Chen G, Liu C, Yin X, et al. Protein Biomarkers of Cardiovascular Disease and Mortality in the Community. J Am Heart Assoc. 2018;7(14).

20. Jensen LJ, Flyvbjerg A, Bjerre M. Soluble Receptor for Advanced Glycation End Product: A Biomarker for Acute Coronary Syndrome. Biomed Res Int. 2015;2015:815942.

21. Raposeiras-Roubín S, Rodiño-Janeiro BK, Grigorian-Shamagian L, Moure-González M, Seoane-Blanco A, Varela-Román A, et al. Relation of soluble receptor for advanced glycation end products to predict mortality in patients with chronic heart failure independently of Seattle Heart Failure Score. Am J Cardiol. 2011;107(6):938-44.

22. Schwedler SB, Metzger T, Schinzel R, Wanner C. Advanced glycation end products and mortality in hemodialysis patients. Kidney Int. 2002;62(1):301-10.

23. Semba RD, Bandinelli S, Sun K, Guralnik JM, Ferrucci L. Plasma carboxymethyl-lysine, an advanced glycation end product, and all-cause and cardiovascular disease mortality in older community-dwelling adults. J Am Geriatr Soc. 2009;57(10):1874-80.

24. Semba RD, Ferrucci L, Sun K, Beck J, Dalal M, Varadhan R, et al. Advanced glycation end products and their circulating receptors predict cardiovascular disease mortality in older community-dwelling women. Aging Clin Exp Res. 2009;21(2):182-90.

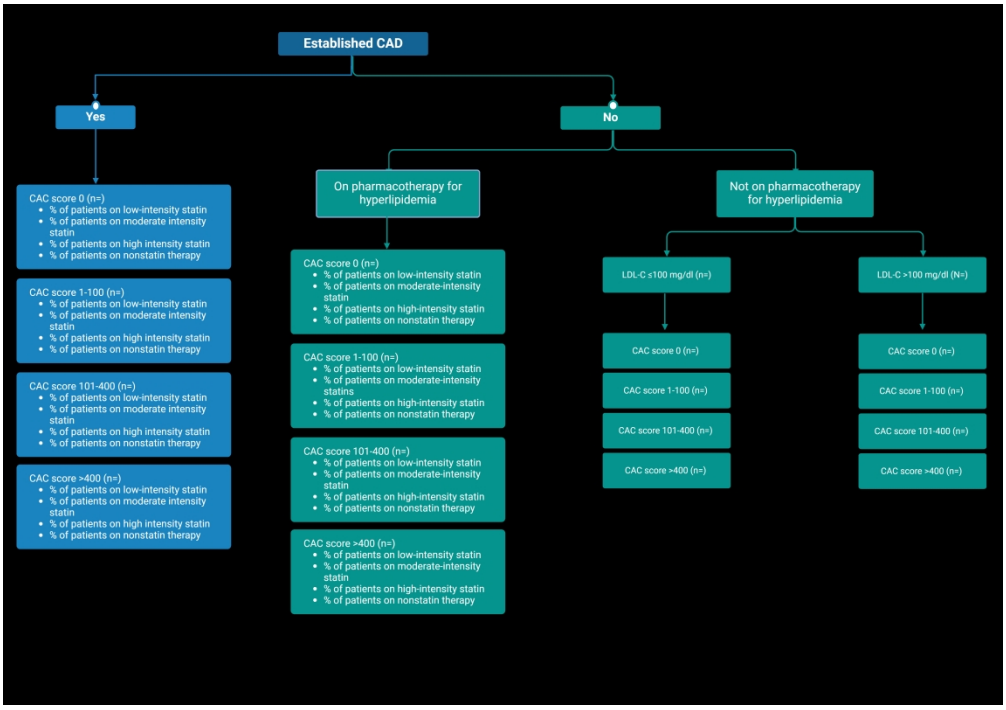

Figure 1: Data Analysis Plan. CAC indicates coronary artery calcium; CAD, coronary artery disease; LDL-C, low-density lipoprotein cholesterol.

645x452mm (236 x 236 DPI)

BMJ Open

Evaluating Atherosclerosis Prevalence via Coronary Calcium in Executives with Normal LDL Levels- A Cohort Study: The CLEAR Protocol

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2024-094899.R1
Article Type:	Protocol
Date Submitted by the Author:	11-Apr-2025
Complete List of Authors:	Ratrout, Basem M.; Mayo Clinic Katamesh, Basant; Mayo Clinic, General Internal Medicine Vincent, Ann; Mayo Clinic, Division of General Internal Medicine Hurt, Ryan T.; Mayo Clinic Bonnes, Sara; Mayo Clinic Adusumalli, Jayanth; Mayo Clinic, General Internal Medicine Lawson, Donna K.; Mayo Clinic Schroeder , Darrell ; Mayo Clinic VerNess, Christina D.; Mayo Clinic Croghan, Ivana; Mayo Clinic, General Internal Medicine
Primary Subject Heading:	Cardiovascular medicine
Secondary Subject Heading:	Epidemiology, Global health
Keywords:	Coronary heart disease < CARDIOLOGY, Adult cardiology < CARDIOLOGY, CARDIOLOGY

Evaluating Atherosclerosis Prevalence via Coronary Calcium in Executives with Normal LDL Levels- A Cohort Study: The CLEAR Protocol

Basem M. Ratrout, MD, MHA

Basant E. Katamesh, MD

Ann Vincent, MD

Ryan T. Hurt, MD, PhD

Sara L. Bonnes, MD, MS

Jayanth Adusumalli, MBBS, MPH

Donna K. Lawson

Darrell Schroeder, MS

Christina D. VerNess, MA

Ivana T. Croghan, PhD

Author Affiliations: Division of General Internal Medicine (Ratrout, Vincent, Hurt, Bonnes, Adusumalli, VerNess, and Croghan), Research Fellow in the Division of General Internal Medicine (El-Fetouh Katamesh), Division of Hospital Internal Medicine (Lawson), and Department of Quantitative Health Sciences (Croghan, Schroeder) Mayo Clinic, Rochester, Minnesota.

Corresponding Author: Basem M. Ratrout, MD, MHA, Division of General Internal Medicine, Mayo Clinic, 200 First St SW, Rochester, MN 55905 (Ratrout.Basem@Mayo.edu).

Abstract

Introduction: The coronary artery calcium (CAC) scan serves as a crucial tool in assessing the risk of coronary atherosclerosis in patients with hyperlipidemia, particularly when there is

ambiguity surrounding pharmacotherapy decisions. In addition to CAC, advanced glycation end products (AGEs), glycated proteins and lipids involved in aging, are emerging as markers for atherosclerosis. However, the relationship between AGEs score and CAC scores has not been evaluated to date. Our primary objective is to evaluate abnormal CAC scores in patients with low and borderline ASCVD risk and normal low-density lipoprotein cholesterol (LDL-C) levels ≤100mg/dl. The secondary objective is to explore potential associations between CAC and AGEs scores.

Methods and Analysis: We will retrospectively review health records of adult patients seen at the General Internal Medicine Executive Health Program (Mayo Clinic; Rochester, Minnesota), between September 1, 2023, and March 31, 2024, where all patients were offered the option of a baseline CAC scan. For our primary aim, we will determine the percentage of patients with low and borderline 10-year ASCVD risk, not receiving pharmacotherapy for hyperlipidemia, have LDL-C levels ≤100 mg/dL, that have an abnormal CAC score. For our secondary aim, we will examine potential associations between CAC and AGEs scores.

Ethics and dissemination: This study was determined to be exempt from institutional review board approval (ID 24-003921; 45 CFR 46.104d, category/subcategory 4[iii]) at Mayo Clinic, Rochester. The findings of this study will be published in a peer-reviewed journal.

Strengths and limitations of this study:

⇒ This study benefits from comprehensive data collection using multiple data sources, including CAC scores, AGEs scores, lipid levels, and cardiac imaging results, providing a multidimensional view of cardiovascular risk.

⇒ This study will ensure the collection of accurate and reliable data by training data collectors, adapting prevalidated research tools, and conducting regular quality checks.

⇒ This single-center study is limited by its reliance on the type of patients that present to unique practice and thus results cannot be generalized to the general population.

⇒ The study is limited by its reliance on patient records, which restricts reviewers to the availability and quality of documented information.

Background

Coronary artery disease (CAD) poses a significant global health challenge, contributing substantially to morbidity and mortality worldwide [1, 2]. CAD manifests through the gradual formation of atherosclerotic plaques within the coronary arteries, necessitating effective risk assessment and management strategies [3]. The American College of Cardiology (ACC) and American Heart Association (AHA) guidelines recommend using 10-year ASCVD risk calculators to estimate cardiovascular risk, categorizing it into three groups: low risk (< 5%), borderline (5% to < 7.5%), intermediate risk (≥ 7.5% to <20%), and high risk (≥ 20% or greater) [4, 5]. Coronary Artery Calcium (CAC) scans are recommended for patients in the intermediate risk category when treatment decisions are unclear [4, 5]. The CAC scan, a noninvasive computed tomographic procedure, quantifies the amount of calcified plaque present in the coronary arteries, aiding in the prediction of future cardiovascular events and guiding clinical decision-making [4-6]. A positive CAC scan indicates the presence of atherosclerosis. While international guidelines differ on the threshold for starting statin therapy, most agree on initiating it when the CAC score exceeds 100 [5]. Notably, CAC scores over 300 are associated with a risk of adverse cardiac events comparable to that of patients with established CAD [8].

Findings from two landmark studies indicate that traditional ASCVD risk models may fail to identify some patients with atherosclerosis, an issue of particular importance especially for those at high risk [8, 9]. The CONFIRM study, which assessed 4511 patients without prior ASCVD, reported that 703 healthy individuals had a score of >300, signifying a high risk for future ASCVD [8]. These patients would have been missed if CAC scans were not part of their clinical evaluation [8]. Similarly, in the MESA study, 480 out of 2174 patients categorized as low ASCVD risk and 356 out of 772 patients with borderline ASCVD risk had a positive CAC

score, with a CAC ≥400 in 20 and 32 patients in these groups respectively [9]. Therefore, adding CAC scoring to traditional risk assessment measures could be a method to identify higher-risk patients who might otherwise be overlooked [10].

In clinical practice, ASCVD risk calculation is often initiated upon observing elevated LDL-C levels, one of the variables inputted into the 10-year ASCVD risk calculation [12]. The 2018 AHA/ACC practice guidelines categorize LDL-C into optimal (below 100 mg/dL), near optimal/above optimal (100-129 mg/dL), borderline high (130-159 mg/dL), high (160-189 mg/dL), and very high (190 mg/dL and above). According to these guidelines, statins are typically recommended for high and very high LDL-C categories regardless of ASCVD risk [12]. The relationship between LDL-C levels and CAC scores is not yet well understood. At least one study has demonstrated evidence that even normal LDL-C levels are associated with subclinical atherosclerosis in the absence of other cardiovascular risk factors [13]. In this study, 4% of patients with LDL-C between 90-100 mg/dL and 7% of patients with LDL-C ranging 80-90 mg/dL, considered optimal LDL-C levels, showed subclinical atherosclerosis [13]. This suggests that some patients with optimal LDL-C may have subclinical atherosclerosis and could benefit from further diagnostic studies such as a CAC [13]. However, there is currently no data to guide clinical scenario such as these.

In addition to 10-year ASCVD risk and LDL-C levels, other biomarkers such as Advanced glycation end products (AGEs), glycated proteins and lipids, are currently being investigated as potential supplementary biomarkers of aging and atherosclerosis [15-17]. AGEs influence atherosclerosis by making LDL-C more atherogenic, promoting oxidative stress and inflammation, and raising blood pressure through vascular stiffness and endothelial dysfunction. A review by Sharifi-Zahabi et al. reported that higher AGEs were significantly associated with

increased all-cause and mortality [21]. Assessing the feasibility of incorporating AGEs into routine cardiovascular evaluations could be worthwhile, but this necessitates further study [20, 22]. The objective of our study is to determine the percentage of patients with low and borderline ASCVD risk, particularly those with normal LDL-C levels who have a positive CAC score. Additionally, we aim to explore potential associations between CAC scores and AGEs scores.

Methods

Study Design and Setting

We will retrospectively review the medical records of adult patients who received medical care and completed a CAC study at the Mayo Clinic Executive Health Program (MCEHP) in Rochester, Minnesota, between September 1, 2023, and March 31, 2024. The project is scheduled to commence in the third quarter of 2024, with anticipated completion by December 31, 2025.

Objectives

For all included patients, we will assess and categorize 10-year ASCVD risk, CAC scores, LDL-C levels, and AGEs.

Study Sample and sampling Techniques

Study entry criteria will include being an empaneled patient aged 40 to 75 years old in the MCEHP, having provided authorization for research, completed a CAC scan (done in all patients

aged 45 and above in our practice), had low or borderline 10-year ASCVD risk, and completed a lipid profile, figure 1. We anticipate that a total of approximately 1,000 patients will meet the inclusion criteria. Based on a preliminary review of our practice we anticipate that approximately 250 of the 1,000 patients will have normal LDL-C, no established CAD, and not be taking pharmacotherapy. We hypothesize that 5-10% of individuals with normal LDL-C levels will have abnormal CAC scores. With a denominator of 250 and an expected calcium scan abnormality rate of 10% or less, the margin of error for a 95% confidence interval (CI) is expected to be approximately 3.5%, such that any observed calcium abnormality rate (CAR) of 10% or less would yield a confidence interval of CAR +/- 3.5%. This is based on using nQuery Advisor 7.0 (confidence interval for a proportion using the normal approximation).

Definition:

Abnormal coronary calcium will be defined as a CAC score above 0. The coronary artery calcification scoring protocol in our institution involves performing an ECG-gated CT scan of the heart. The patient's calcium score is calculated using the Agatston-Janowitz scale, with a threshold of 130 Hounsfield Units (HU) to differentiate calcified plaque from other tissues. We define normal LDL-C as ≤100mg/dL. The calculation of LDL-C in our institution utilizes the Sampson NIH equation. The 10- year ASCVD will be estimated using the ASCVD risk estimator offered by the ACC [23].The AGEs reader, a non-invasive and clinically validated device, will be used to estimate glycated proteins by measuring autofluorescence in human skin tissue [22].

Team composition and training:

A comprehensive training reference guide will be created before data extraction. This guide will serve as a vital resource, outlining the project's specific objectives. Any updates or

changes to the project will be promptly incorporated into this guide and communicated to other members of the team. Training sessions will be conducted whenever a new abstractor joins the team, ensuring that each member is well trained in the extraction process. Prior to chart reviews, all members of the reviewing team will undergo training on the study protocol, key definitions, and the use of research tools. They will practice with test cases created by the adjudicators to familiarize themselves with the tools and identify any issues for review.

Record Review and Data Collection Approach

Data for this study will be abstracted from the electronic health record (EHR: Epic software; Epic Systems Corporation), and data will be subsequently collated through REDCap (Research Electronic Data Capture) [24, 25] [24, 25].. The data abstraction sheet will be structured into 5 distinct sections: baseline demographic characteristics, laboratory test results, CAC scan and scoring details, cardiac imaging findings, and AGEs scores and risk categories, Supplementary1-4. The first section of the data extraction sheet will include baseline demographic characteristics that were already collected at the time of their physical exam as illustrated in Supplementary 1. The second section of the data extraction sheet are laboratory variables results collected closest to their CAC scan, Supplementary 2. The third section of the data extraction sheet will detail variables obtained from CAC scans, Supplementary 3. The fourth section of the data extraction sheet will consist of imaging variables, including findings from baseline electrocardiogram, echocardiography, carotid ultrasonography, stress echocardiography, and exercise electrocardiography, Supplementary 4. For the secondary objective of this cohort study, AGEs scores and risk categories will be extracted. All variables

closest to the CAC date will be collected and their dates recorded in the abstraction sheet. This will help us determine the effect of temporality if any following data abstraction.

Quality Assurance

To ensure the accuracy and reliability of the extracted data, we plan to do random spot checks on the REDCap dashboard. Our objective is to maintain a 10% spot-check rate, equating to approximately 10 checks per page, with each page containing 100 patient records. These spot checks will be assigned randomly to avoid bias and will be conducted by reviewers independent of those who initially extracted the data. This dual-review process is crucial for identifying any discrepancies or errors that may have been overlooked during the initial data extraction phase. If mistakes are identified during the spot checks, they will be promptly corrected. In addition to correcting the errors, individuals responsible for the mistakes will undergo retraining to address any gaps in knowledge.

Data Analysis Plan

Patients will be initially categorized into established CAD or no established CAD, Figure 1. Patients with established CAD will be excluded. Patients without established CAD and those who have completed a coronary calcium study will be categorized according to 10-year ASCVD risk. Patients with low or borderline 10-year ASCVD risk who are not on pharmacotherapy for hyperlipidemia will be categorized by LDL-C levels into two groups: ≤100 mg/dL (group 1) and >100 mg/dL (group 2). Data on CAC scores, other vascular calcifications, and AGEs in this

group will be summarized. Following this we will compare baseline characteristics of group 1 with the other groups: patients with established CAD (group 3), patients with high 10-year ASCVD (group 4), patients with intermediate 10-year ASCVD (group 5), patients with low and borderline 10-year ASCVD on pharmacotherapy (group 6), and patients with low and borderline 10-year ASCVD and LDL>100 (group 2), Figure 1.

Group 1 will be categorized based on the presence or absence of detectable coronary calcium, and two group comparisons will be done. Data will be summarized using median (25^{th}, 75^{th} percentile) for continuous variables and n (%) for categorical variables. Following this, unadjusted, and age- and sex- adjusted, analyses will be performed using logistic regression for group 1. Analysis will be conducted using the SAS version 9.4 (SAS Institute Inc, Cary NC) [26]. For the secondary aim of this investigation, correlation analyses and general linear models will be used to assess the association of AGEs, indicative of cumulative metabolic stress, with CAC scores and lipid profiles. For these analyses, distributional assumptions will be assessed with variable transformations (e.g. log transformation) used as appropriate. Several other variables, such as echocardiogram, carotid ultrasound, cardiac perfusion stress test, and incidental CT findings, Supplementary3 and 4, are being collected as part of the chart review and will be utilized at a later date in the preparation of additional manuscripts.

Patient and Public Involvement and Ethics

Patient or public involvement was not incorporated in the design, conduct, reporting, or dissemination plans of this research.

Discussion

The rationale for this study stems from the limitations of traditional ASCVD risk models in identifying all patients with atherosclerosis, particularly those with normal LDL-C levels [27].

Asymptomatic individuals with normal or borderline 10-year ASCVD risk and normal LDL-C levels may still have subclinical atherosclerosis, detectable through CAC scanning. Clinical observations, along with results from the MESA and CONFIRM studies, demonstrate that atherosclerosis can occur in patients with low cardiovascular risk. Additionally, Fernández-Friera et al. have observed abnormal CAC scores in patients with normal LDL-C levels, suggesting that relying solely on risk models or LDL-C levels may be insufficient for detecting potentially at-risk patients with atherosclerosis. This supports the rationale for our study. These patients are often seen in primary care settings that do not routinely use CAC scans and could benefit from such scans, enabling timely interventions. By investigating the prevalence of abnormal CAC scores in this cohort, we aim to contribute to the existing literature on the potential benefits of CAC scans for these patients.

The rationale for this study arises from the limitations of traditional ASCVD risk models in identifying all patients with atherosclerosis, especially those with normal LDL-C levels. Asymptomatic individuals with normal or borderline 10-year ASCVD risk and normal LDL-C levels may still have subclinical atherosclerosis, detectable through CAC scanning [27]. Clinical observations, results from the MESA and CONFIRM studies that demonstrate that atherosclerosis in patients with low cardiovascular risk and observations of abnormal CAC scores in patients with normal LDL-C levels by Fernández-Friera et al. suggest that relying solely on risk models or LDL-C levels may be inadequate for detecting potentially at-risk patients with atherosclerosis and support the rationale for this study [8, 10, 13]. These patients are often seen in primary care settings that do not routinely use CAC scans and could benefit from such scans enabling timely interventions. By investigating the prevalence of abnormal CAC

scores in this cohort, we aim to contribute to the existing literature on the potential benefits of CAC scans for these patients.

Building on this, evidence suggests a pathophysiological link between AGEs and atherosclerosis, cardiovascular disease, and mortality [15-17]. It is plausible that AGEs may correlate with coronary calcium, a marker for detecting coronary atherosclerosis [21, 30-35]. However, no studies have explored possible associations between AGEs and CAC, which will be unique to this retrospective cohort study. By investigating this potential association, we aim to determine whether AGEs could serve as a predictive biomarker for coronary artery disease, even in patients with normal LDL-C levels. This could provide valuable insights into the underlying mechanisms of cardiovascular disease and help identify individuals at higher risk, thereby improving prevention and treatment strategies.

The findings of our study could pave the way for future research to evaluate the use of CAC scans in cardiovascular risk stratification, potentially enhancing clinical guidelines and practices, and integrating CAC scoring into risk prediction models.

Ethical Consideration

This study underwent expedited review procedures and was determined to be exempt from institutional review board approval (ID 24-003921; 45 CFR 46.104d, category/subcategory 4[iii]). Ethical principles have and will continue to be carefully considered and adhered to, and only patients who previously consented to the use of their data for research purposes will be included in this cohort. Unique identifiers will be created to document all findings, ensuring the anonymity of the patients whose records are being reviewed. Data will be securely stored on encrypted, institution-approved cloud services, with access limited to the investigating team. Any

modifications to the study design will be promptly submitted to the ethics review committee for review.

Dissemination

The results of this study will be disseminated through publication in a peer-reviewed journal and presentations at both national and international academic conferences. Additionally, we aim to promote our findings via social media platforms.

Authors' contributions

All persons listed as contributors met the International Committee of Medical Journal Editors (ICMJE) authorship criteria. Each author has made significant contributions to the writing and revisions to the study and has provided complete assent for publication. B.M.R., B.E.K., A.V., R.T.H., S.L.B., J.A., C.D.V., and I.T.C. were responsible for the conceptualization of the study. A.V., R.T.H., S.L.B., D.K.L., and I.T.C. were responsible for supervising all activities. All authors contributed to designing the methodology. B.M.R., B.E.K., A.V., and D.S. were responsible for calculating the sample size and formulating the data analysis plan. Each author is responsible for the content and has read and approved the final manuscript. B.M.R. is the guarantor of this work and accepts full responsibility for the integrity of the data, the accuracy of the analysis, and the decision to submit for publication.

Funding: This research will be supported by internal division funds, Division of General Internal Medicine, Department of Medicine, Mayo Clinic. Statistical support for this project will be provided by the Mayo Clinic Department of Medicine Research Hub. REDCap will be the

data entry system for this study. The REDCap data entry system was supported in part by the Center for Clinical and Translational Science Grant Number UL1 TR000135 from the National Center for Advancing Translational Sciences (NCATS). Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the National Institutes of Health.

Competing interests: The authors declare there are no competing interests.

Patient and public involvement: Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication: Not applicable.

Provenance and peer review: Not commissioned; externally peer reviewed.

References:

1. Vaduganathan M, Mensah GA, Turco JV, Fuster V, Roth GA. The Global Burden of Cardiovascular Diseases and Risk: A Compass for Future Health. J Am Coll Cardiol. 2022;80(25):2361-71.
2. WHO. Cardiovascular diseases (CVDs) 2021 [Available from: https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds).
3. Mitsis A, Khattab E, Christodoulou E, Myrianthopoulos K, Myrianthefs M, Tzikas S, et al. From Cells to Plaques: The Molecular Pathways of Coronary Artery Calcification and Disease. Journal of Clinical Medicine. 2024;13(21):6352.
4. Arnett DK, Blumenthal RS, Albert MA, Buroker AB, Goldberger ZD, Hahn EJ, et al. 2019 ACC/AHA Guideline on the Primary Prevention of Cardiovascular Disease: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. Circulation. 2019;140(11):e596-e646.

5. Golub Ilana S, Termeie Orly G, Kristo S, Schroeder Lucia P, Lakshmanan S, Shafter Ahmed M, et al. Major Global Coronary Artery Calcium Guidelines. JACC: Cardiovascular Imaging. 2023;16(1):98-117.
6. Lo-Kioeng-Shioe MS, Vavere AL, Arbab-Zadeh A, Schuijf JD, Rochitte CE, Chen MY, et al. Coronary Calcium Characteristics as Predictors of Major Adverse Cardiac Events in Symptomatic Patients: Insights From the CORE 320 Multinational Study. J Am Heart Assoc. 2019;8(6):e007201.
7. Budoff MJ, Kinninger A, Gransar H, Achenbach S, Al-Mallah M, Bax JJ, et al. When Does a Calcium Score Equate to Secondary Prevention?: Insights From the Multinational CONFIRM Registry. JACC Cardiovasc Imaging. 2023;16(9):1181-9.
8. Dzaye O, Razavi AC, Michos ED, Mortensen MB, Dardari ZA, Nasir K, et al. Coronary artery calcium scores indicating secondary prevention level risk: Findings from the CAC consortium and FOURIER trial. Atherosclerosis. 2022;347:70-6.
9. Greenland P, Blaha Michael J, Budoff Matthew J, Erbel R, Watson Karol E. Coronary Calcium Score and Cardiovascular Risk. JACC. 2018;72(4):434-47.
10. Grundy Scott M, Stone Neil J, Bailey Alison L, Beam C, Birtcher Kim K, Blumenthal Roger S, et al. 2018 AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA Guideline on the Management of Blood Cholesterol. JACC. 2019;73(24):e285-e350.
11. Fernández-Friera L, Fuster V, López-Melgar B, Oliva B, García-Ruiz JM, Mendiguren J, et al. Normal LDL-Cholesterol Levels Are Associated With Subclinical Atherosclerosis in the Absence of Risk Factors. J Am Coll Cardiol. 2017;70(24):2979-91.
12. Hegab Z, Gibbons S, Neyses L, Mamas MA. Role of advanced glycation end products in cardiovascular disease. World J Cardiol. 2012;4(4):90-102.
13. Hodgkinson CP, Laxton RC, Patel K, Ye S. Advanced Glycation End-Product of Low Density Lipoprotein Activates the Toll-Like 4 Receptor Pathway Implications for Diabetic Atherosclerosis. Arteriosclerosis, Thrombosis, and Vascular Biology. 2008;28(12):2275-81.
14. Vekic J, Vujcic S, Bufan B, Bojanin D, Al-Hashmi K, Al-Rasadi K, et al. The Role of Advanced Glycation End Products on Dyslipidemia. Metabolites. 2023;13(1).
15. Sharifi-Zahabi E, Sharafabad FH, Abdollahzad H, Malekahmadi M, Rad NB. Circulating Advanced Glycation End Products and Their Soluble Receptors in Relation to All-Cause and Cardiovascular Mortality: A Systematic Review and Meta-analysis of Prospective Observational Studies. Adv Nutr. 2021;12(6):2157-71.
16. Diagnoptics. Advanced Glycation Endproducts Readers [Available from: https://www.diagnoptics.com/advanced-glycation-endproducts/.
17. Perrone A, Giovino A, Benny J, Martinelli F. Advanced Glycation End Products (AGEs): Biochemistry, Signaling, Analytical Methods, and Epigenetic Effects. Oxid Med Cell Longev. 2020;2020:3818196.
18. Cardiology ACo. ASCVD Risk Estimator Plus [Available from: https://tools.acc.org/ascvd-risk-estimator-plus/#!/calculate/estimate/.
19. Harris PA, Taylor R, Minor BL, Elliott V, Fernandez M, O'Neal L, et al. The REDCap consortium: Building an international community of software platform partners. J Biomed Inform. 2019;95:103208.

20. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. J Biomed Inform. 2009;42(2):377-81.
21. Inc. SI. SAS/STAT® 15.3 User's Guide. Cary, NC: SAS Institute Inc. [Available from: https://documentation.sas.com/api/collections/pgmsascdc/9.4_3.5/docsets/statug/content/sashelp.pdf?locale=en.
22. Orringer CE, Blaha MJ, Blankstein R, Budoff MJ, Goldberg RB, Gill EA, et al. The National Lipid Association scientific statement on coronary artery calcium scoring to guide preventive strategies for ASCVD risk reduction. Journal of Clinical Lipidology. 2021;15(1):33-60.
23. Ebert H, Lacruz ME, Kluttig A, Simm A, Greiser KH, Tiller D, et al. Association between advanced glycation end products, their soluble receptor, and mortality in the general population: Results from the CARLA study. Exp Gerontol. 2020;131:110815.
24. Ho JE, Lyass A, Courchesne P, Chen G, Liu C, Yin X, et al. Protein Biomarkers of Cardiovascular Disease and Mortality in the Community. J Am Heart Assoc. 2018;7(14).
25. Jensen LJ, Flyvbjerg A, Bjerre M. Soluble Receptor for Advanced Glycation End Product: A Biomarker for Acute Coronary Syndrome. Biomed Res Int. 2015;2015:815942.
26. Raposeiras-Roubín S, Rodiño-Janeiro BK, Grigorian-Shamagian L, Moure-González M, Seoane-Blanco A, Varela-Román A, et al. Relation of soluble receptor for advanced glycation end products to predict mortality in patients with chronic heart failure independently of Seattle Heart Failure Score. Am J Cardiol. 2011;107(6):938-44.
27. Schwedler SB, Metzger T, Schinzel R, Wanner C. Advanced glycation end products and mortality in hemodialysis patients. Kidney Int. 2002;62(1):301-10.
28. Semba RD, Bandinelli S, Sun K, Guralnik JM, Ferrucci L. Plasma carboxymethyl-lysine, an advanced glycation end product, and all-cause and cardiovascular disease mortality in older community-dwelling adults. J Am Geriatr Soc. 2009;57(10):1874-80.

Figure 1: Enrollment and Screening Process Flowchart

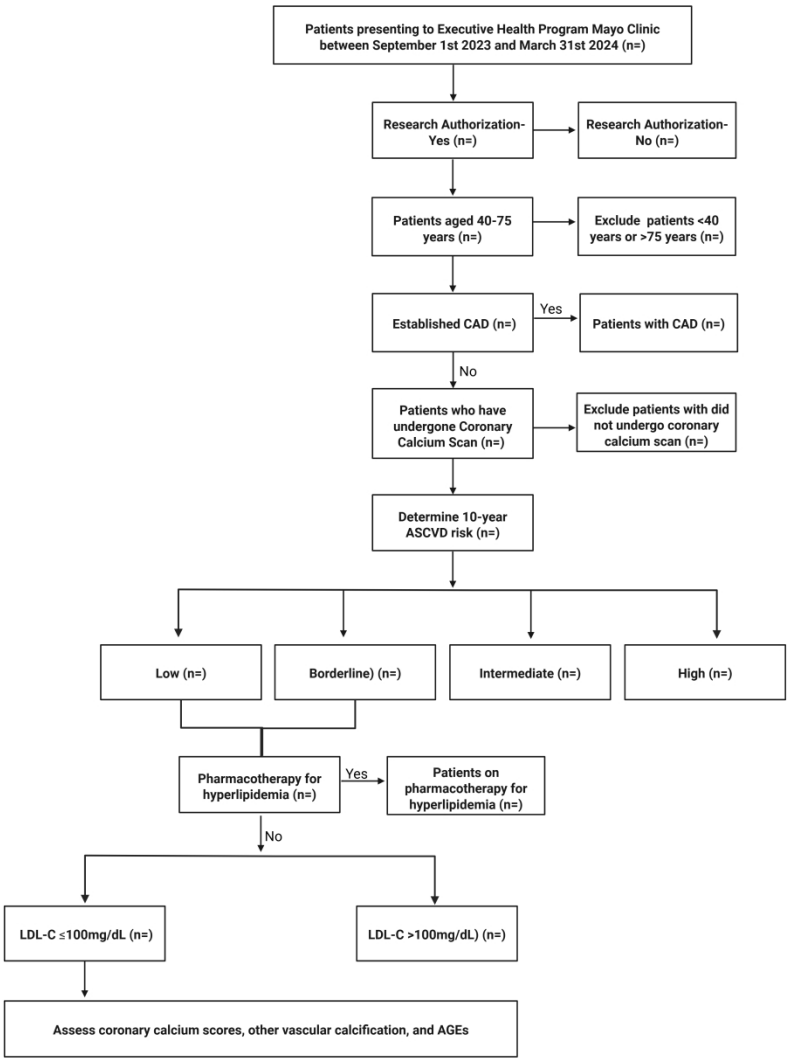

Figure 1: Enrollment and Screening Process Flowchart

336x504mm (236 x 236 DPI)

Supplementary 1. Data Extraction Sheet for Baseline Demographic Characteristics

Record ID	
Date of executive health visit	
Full name (first, middle initial, last)	
Age (at time of scan)	
Date of birth	
Sex	Male Female Other
Race	American Indian or Alaska Native Asian Native Hawaiian or Other Pacific Islander Black or African American White More than 1 race Chose not to disclose Unknown Other
Ethnicity	Hispanic or Latino Non-Hispanic or Latino Unknown
Level of education	8th grade

	High school graduate or General Educational Development test Some high school but did not graduate Associate's degree (academic) Associate's degree (occupational, technical, or vocational) Some college Bachelor's degree Master's degree Doctorate Professional degree (MD, DDS, DVM, JD) No degree The patient did not answer Unknown
Marital status	Single Married Divorced Separated Widowed Life partner Chose not to disclose Unknown

Smoking (any form of tobacco products [e.g., chew])	Never Current, every day Current, some days Former Unknown/unanswered
What type of smoking?	Cigarettes Cigars Pipe Snuff Chew
Does the patient use smokeless tobacco such as chew or snuff?	Yes No
Total packs per year	
Current packs per day	0.25 0.5 0.75 1 1.5 2 2.5 3 >3 packs Quit

Date patient quit smoking or using smokeless tobacco	
Number of packs per year patient used to smoke	
Comments	
E-cigarette and vaping use	Never Current, every day Current, some days Former Unknown/unanswered
Alcohol use	Yes Not currently Never Chose not to disclose No
Type of drinks and amount per day	Glasses of wine Cans of beer Shots of liquor Other (e.g., vodka 750 mL/day) Unknown type
Drinks per week	
Comments	
Illicit drug use	Yes

	Not currently Never Chose not to disclose No
Type of illicit drug	Amphetamines Anabolic steroids Barbiturates Cocaine Crack cocaine Hashish Heroin LSD (lysergic acid diethylamide) Marijuana MDMA (3,4- methylenedioxymethamphetamine; ecstasy) Methamphetamines Opium PCP (phencyclidine) Solvent inhalants Other
Comments	
Family history (select all that apply)	Carotid disease Coronary artery disease

	Peripheral vascular disease Stroke/transient ischemic attack None of the above Unknown/unanswered
Past medical history	Cancer Chronic kidney disease Chronic liver disease Connective tissue disease Coronary artery disease Diabetes Heart rhythm disorders Hyperlipidemia Hypertension Metabolic syndrome Obesity Obstructive sleep apnea Peripheral artery disease Radiotherapy Stroke/transient ischemic attack Thyroid diseases Valvular disease None Unknown/not answered

Medical cannabis use	Yes No
Does the patient exercise?	Yes No Unknown
Minutes per week of exercise	
Days per week of exercise	
Type of exercise	Cardio Strength training Other Unknown
Comments and other types of exercise	
Height, cm	
Weight, kg	
Body mass index	
Neck circumference, cm	
Waist circumference, cm	
Systolic blood pressure (current), mm Hg	
Diastolic blood pressure (current), mm Hg	
Type of executive	Executive Spouse Unknown
Amount of traveling	25% of time

	50% of time 75% of time 100% of time None Unknown

Supplementary 2. Data Extraction Sheet for Laboratory Test Results*

Date of laboratory tests	
Total cholesterol	
HDL cholesterol	
LDL cholesterol	
Was LDL-C <100 mg/dL?	Yes No
Is the patient on lipid lowering medication?	Yes No
Medication used	Alirocumab Atorvastatin Bempedoic acid Evolocumab Ezetimibe Inclisiran Pravastatin Rosuvastatin

	Simvastatin Aspirin Clopidogrel Prasugrel Ticagrelor None
Triglycerides	
Lipoprotein (a)	
Other laboratory tests	
Apolipoprotein A-1	
Apolipoprotein B	
Fasting blood glucose	
Hemoglobin $A1_c$	
MI-Heart Ceramides, Plasma risk score	0-2 low risk 3-6 moderate risk 7-9 increased risk 10-12 high risk Not done
Comments	

Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein.

* Laboratory values closest to the time of CAC scan will be abstracted.

Supplementary 3. Data Extraction Sheet for Coronary Artery Calcium (CAC) Scan and Scoring

Was a CAC scan obtained?	Yes No
Date of scan	
Total CAC score	
Coronary artery measurements	
Left anterior descending	
Circumflex	
Left main vessel	
Right coronary artery	
Percentile ranking*	
Incidental findings	Nodules Lymph nodes Granulomas Bony lesions Enlarged aorta Other None
Other findings	

* For individuals with multiple races recorded in their medical records, the race used in the CAC percentile calculation was determined by the radiologist who issued the CAC report

Supplementary 4. Data Extraction Sheet for Cardiac Imaging Findings

Radiographic findings	Calcification Atherosclerosis Negative Not done Other findings
Date of radiograph	
Comments/impressions or other findings on radiography	
Chest CT imaging	Calcification Atherosclerosis Pulmonary nodules Negative Not done Other findings
Date of chest CT	
Comments/impressions or other findings on chest CT	
Carotid US	Atherosclerosis Negative Not done Other findings
Date of carotid US	

Comments/impressions or other findings on carotid US	
Echocardiography	Enlarged aorta Dilated aorta Negative Not done Other findings
Date of echocardiography	
Comments/impressions or other findings on echocardiography	
Stress echocardiography	Enlarged aorta Dilated aorta Negative Not done Other findings
Date of stress echocardiography	
Comments/impressions or other findings on stress echocardiography	
Exercise ECG	Yes No
Type of exercise ECG	Treadmill Bike
Date of exercise ECG	

Comments/impressions or other findings on exercise ECG	
Cardiopulmonary stress test	Yes No
Date of cardiopulmonary stress test	
Comments/impressions or other findings on cardiopulmonary stress test	
Cardiac perfusion stress test	Yes No
Date of cardiac perfusion stress test	
Comments/impressions or other findings on cardiac perfusion stress test	
ECG	Yes No
Date of ECG	
Comments/impressions or other findings on ECG	
Real and ECG age	
Probability of low ejection fraction, %	
Probability of atrial fibrillation, %	

Abbreviations: CT, computed tomography; ECG, electrocardiography; US, ultrasonography.

BMJ Open

Evaluating Atherosclerosis Prevalence via Coronary Calcium in Executives with Normal LDL Levels in the US- A Cohort Study: The CLEAR Protocol

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2024-094899.R2
Article Type:	Protocol
Date Submitted by the Author:	30-Apr-2025
Complete List of Authors:	Ratrout, Basem M.; Mayo Clinic Katamesh, Basant; Mayo Clinic, General Internal Medicine Vincent, Ann; Mayo Clinic, Division of General Internal Medicine Hurt, Ryan T.; Mayo Clinic Bonnes, Sara; Mayo Clinic Adusumalli, Jayanth; Mayo Clinic, General Internal Medicine Lawson, Donna K.; Mayo Clinic Schroeder , Darrell ; Mayo Clinic VerNess, Christina D.; Mayo Clinic Croghan, Ivana; Mayo Clinic, General Internal Medicine
Primary Subject Heading:	Cardiovascular medicine
Secondary Subject Heading:	Epidemiology, Global health
Keywords:	Coronary heart disease < CARDIOLOGY, Adult cardiology < CARDIOLOGY, CARDIOLOGY

Evaluating Atherosclerosis Prevalence via Coronary Calcium in Executives with Normal LDL Levels in the US- A Cohort Study: The CLEAR Protocol

Basem M. Ratrout, MD, MHA

Basant E. Katamesh, MD

Ann Vincent, MD

Ryan T. Hurt, MD, PhD

Sara L. Bonnes, MD, MS

Jayanth Adusumalli, MBBS, MPH

Donna K. Lawson

Darrell Schroeder, MS

Christina D. VerNess, MA

Ivana T. Croghan, PhD

Author Affiliations: Division of General Internal Medicine (Ratrout, Vincent, Hurt, Bonnes, Adusumalli, VerNess, and Croghan), Research Fellow in the Division of General Internal Medicine (El-Fetouh Katamesh), Division of Hospital Internal Medicine (Lawson), and Department of Quantitative Health Sciences (Croghan, Schroeder) Mayo Clinic, Rochester, Minnesota.

Corresponding Author: Basem M. Ratrout, MD, MHA, Division of General Internal Medicine, Mayo Clinic, 200 First St SW, Rochester, MN 55905 (Ratrout.Basem@Mayo.edu).

Abstract

Introduction: The coronary artery calcium (CAC) scan serves as a crucial tool in assessing the risk of coronary atherosclerosis in patients with hyperlipidemia, particularly when there is

ambiguity surrounding pharmacotherapy decisions. In addition to CAC, advanced glycation end products (AGEs), glycated proteins and lipids involved in aging, are emerging as markers for atherosclerosis. However, the relationship between AGEs score and CAC scores has not been evaluated to date. Our primary objective is to evaluate abnormal CAC scores in patients with low and borderline ASCVD risk and normal low-density lipoprotein cholesterol (LDL-C) levels ≤100mg/dl. The secondary objective is to explore potential associations between CAC and AGEs scores.

Methods and Analysis: We will retrospectively review health records of adult patients seen at the General Internal Medicine Executive Health Program (Mayo Clinic; Rochester, Minnesota), between September 1, 2023, and March 31, 2024, where all patients were offered the option of a baseline CAC scan. For our primary aim, we will determine the percentage of patients with low and borderline 10-year ASCVD risk, not receiving pharmacotherapy for hyperlipidemia, have LDL-C levels ≤100 mg/dL, that have an abnormal CAC score. For our secondary aim, we will examine potential associations between CAC and AGEs scores.

Ethics and dissemination: This study was determined to be exempt from institutional review board approval (ID 24-003921; 45 CFR 46.104d, category/subcategory 4[iii]) at Mayo Clinic, Rochester. The findings of this study will be published in a peer-reviewed journal.

Strengths and limitations of this study:

⇒ This study benefits from comprehensive data collection using multiple data sources, including CAC scores, AGEs scores, lipid levels, and cardiac imaging results, providing a multidimensional view of cardiovascular risk.

⇒ This study will ensure the collection of accurate and reliable data by training data collectors, adapting prevalidated research tools, and conducting regular quality checks.

⇒ This single-center study is limited by its reliance on the type of patients that present to unique practice and thus results cannot be generalized to the general population.

⇒ The study is limited by its reliance on patient records, which restricts reviewers to the availability and quality of documented information.

Background

Coronary artery disease (CAD) poses a significant global health challenge, contributing substantially to morbidity and mortality worldwide [1, 2]. CAD manifests through the gradual formation of atherosclerotic plaques within the coronary arteries, necessitating effective risk assessment and management strategies [3]. The American College of Cardiology (ACC) and American Heart Association (AHA) guidelines recommend using 10-year ASCVD risk calculators to estimate cardiovascular risk, categorizing it into three groups: low risk (< 5%), borderline (5% to < 7.5%), intermediate risk (≥ 7.5% to <20%), and high risk (≥ 20% or greater) [4, 5]. Coronary Artery Calcium (CAC) scans are recommended for patients in the intermediate risk category when treatment decisions are unclear [4, 5]. The CAC scan, a noninvasive computed tomographic procedure, quantifies the amount of calcified plaque present in the coronary arteries, aiding in the prediction of future cardiovascular events and guiding clinical decision-making [4-6]. A positive CAC scan indicates the presence of atherosclerosis. While international guidelines differ on the threshold for starting statin therapy, most agree on initiating it when the CAC score exceeds 100 [5]. Notably, CAC scores over 300 are associated with a risk of adverse cardiac events comparable to that of patients with established CAD [8].

Findings from two landmark studies indicate that traditional ASCVD risk models may fail to identify some patients with atherosclerosis, an issue of particular importance especially for those at high risk [8, 9]. The CONFIRM study, which assessed 4511 patients without prior ASCVD, reported that 703 healthy individuals had a score of >300, signifying a high risk for future ASCVD [8]. These patients would have been missed if CAC scans were not part of their clinical evaluation [8]. Similarly, in the MESA study, 480 out of 2174 patients categorized as low ASCVD risk and 356 out of 772 patients with borderline ASCVD risk had a positive CAC

score, with a CAC ≥400 in 20 and 32 patients in these groups respectively [9]. Therefore, adding CAC scoring to traditional risk assessment measures could be a method to identify higher-risk patients who might otherwise be overlooked [10].

In clinical practice, ASCVD risk calculation is often initiated upon observing elevated LDL-C levels, one of the variables inputted into the 10-year ASCVD risk calculation [12]. The 2018 AHA/ACC practice guidelines categorize LDL-C into optimal (below 100 mg/dL), near optimal/above optimal (100-129 mg/dL), borderline high (130-159 mg/dL), high (160-189 mg/dL), and very high (190 mg/dL and above). According to these guidelines, statins are typically recommended for high and very high LDL-C categories regardless of ASCVD risk [12]. The relationship between LDL-C levels and CAC scores is not yet well understood. At least one study has demonstrated evidence that even normal LDL-C levels are associated with subclinical atherosclerosis in the absence of other cardiovascular risk factors [13]. In this study, 4% of patients with LDL-C between 90-100 mg/dL and 7% of patients with LDL-C ranging 80-90 mg/dL, considered optimal LDL-C levels, showed subclinical atherosclerosis [13]. This suggests that some patients with optimal LDL-C may have subclinical atherosclerosis and could benefit from further diagnostic studies such as a CAC [13]. However, there is currently no data to guide clinical scenario such as these.

In addition to 10-year ASCVD risk and LDL-C levels, other biomarkers such as Advanced glycation end products (AGEs), glycated proteins and lipids, are currently being investigated as potential supplementary biomarkers of aging and atherosclerosis [15-17]. AGEs influence atherosclerosis by making LDL-C more atherogenic, promoting oxidative stress and inflammation, and raising blood pressure through vascular stiffness and endothelial dysfunction. A review by Sharifi-Zahabi et al. reported that higher AGEs were significantly associated with

increased all-cause and mortality [21]. Assessing the feasibility of incorporating AGEs into routine cardiovascular evaluations could be worthwhile, but this necessitates further study [20, 22]. The objective of our study is to determine the percentage of patients with low and borderline ASCVD risk, particularly those with normal LDL-C levels who have a positive CAC score. Additionally, we aim to explore potential associations between CAC scores and AGEs scores.

Methods

Study Design and Setting

We will retrospectively review the medical records of adult patients who received medical care and completed a CAC study at the Mayo Clinic Executive Health Program (MCEHP) in Rochester, Minnesota, between September 1, 2023, and March 31, 2024. The project is scheduled to commence in the third quarter of 2024, with anticipated completion by December 31, 2025.

Objectives

For all included patients, we will assess and categorize 10-year ASCVD risk, CAC scores, LDL-C levels, and AGEs.

Study Sample and sampling Techniques

Study entry criteria will include being an empaneled patient aged 40 to 75 years old in the MCEHP, having provided authorization for research, completed a CAC scan (done in all patients

aged 45 and above in our practice), had low or borderline 10-year ASCVD risk, and completed a lipid profile, figure 1. We anticipate that a total of approximately 1,000 patients will meet the inclusion criteria. Based on a preliminary review of our practice we anticipate that approximately 250 of the 1,000 patients will have normal LDL-C, no established CAD, and not be taking pharmacotherapy. We hypothesize that 5-10% of individuals with normal LDL-C levels will have abnormal CAC scores. With a denominator of 250 and an expected calcium scan abnormality rate of 10% or less, the margin of error for a 95% confidence interval (CI) is expected to be approximately 3.5%, such that any observed calcium abnormality rate (CAR) of 10% or less would yield a confidence interval of CAR +/- 3.5%. This is based on using nQuery Advisor 7.0 (confidence interval for a proportion using the normal approximation).

Definition:

Abnormal coronary calcium will be defined as a CAC score above 0. The coronary artery calcification scoring protocol in our institution involves performing an ECG-gated CT scan of the heart. The patient's calcium score is calculated using the Agatston-Janowitz scale, with a threshold of 130 Hounsfield Units (HU) to differentiate calcified plaque from other tissues. We define normal LDL-C as ≤100mg/dL. The calculation of LDL-C in our institution utilizes the Sampson NIH equation. The 10- year ASCVD will be estimated using the ASCVD risk estimator offered by the ACC [23].The AGEs reader, a non-invasive and clinically validated device, will be used to estimate glycated proteins by measuring autofluorescence in human skin tissue [22].

Team composition and training:

A comprehensive training reference guide will be created before data extraction. This guide will serve as a vital resource, outlining the project's specific objectives. Any updates or

changes to the project will be promptly incorporated into this guide and communicated to other members of the team. Training sessions will be conducted whenever a new abstractor joins the team, ensuring that each member is well trained in the extraction process. Prior to chart reviews, all members of the reviewing team will undergo training on the study protocol, key definitions, and the use of research tools. They will practice with test cases created by the adjudicators to familiarize themselves with the tools and identify any issues for review.

Record Review and Data Collection Approach

Data for this study will be abstracted from the electronic health record (EHR: Epic software; Epic Systems Corporation), and data will be subsequently collated through REDCap (Research Electronic Data Capture) [24, 25] [24, 25].. The data abstraction sheet will be structured into 5 distinct sections: baseline demographic characteristics, laboratory test results, CAC scan and scoring details, cardiac imaging findings, and AGEs scores and risk categories, Supplementary1-4. The first section of the data extraction sheet will include baseline demographic characteristics that were already collected at the time of their physical exam as illustrated in Supplementary 1. The second section of the data extraction sheet are laboratory variables results collected closest to their CAC scan, Supplementary 2. The third section of the data extraction sheet will detail variables obtained from CAC scans, Supplementary 3. The fourth section of the data extraction sheet will consist of imaging variables, including findings from baseline electrocardiogram, echocardiography, carotid ultrasonography, stress echocardiography, and exercise electrocardiography, Supplementary 4. For the secondary objective of this cohort study, AGEs scores and risk categories will be extracted. All variables

closest to the CAC date will be collected and their dates recorded in the abstraction sheet. This will help us determine the effect of temporality if any following data abstraction.

Quality Assurance

To ensure the accuracy and reliability of the extracted data, we plan to do random spot checks on the REDCap dashboard. Our objective is to maintain a 10% spot-check rate, equating to approximately 10 checks per page, with each page containing 100 patient records. These spot checks will be assigned randomly to avoid bias and will be conducted by reviewers independent of those who initially extracted the data. This dual-review process is crucial for identifying any discrepancies or errors that may have been overlooked during the initial data extraction phase. If mistakes are identified during the spot checks, they will be promptly corrected. In addition to correcting the errors, individuals responsible for the mistakes will undergo retraining to address any gaps in knowledge.

Data Analysis Plan

Patients will be initially categorized into established CAD or no established CAD, Figure 1. Patients with established CAD will be excluded. Patients without established CAD and those who have completed a coronary calcium study will be categorized according to 10-year ASCVD risk. Patients with low or borderline 10-year ASCVD risk who are not on pharmacotherapy for hyperlipidemia will be categorized by LDL-C levels into two groups: ≤100 mg/dL (group 1) and >100 mg/dL (group 2). Data on CAC scores, other vascular calcifications, and AGEs in this

group will be summarized. Following this we will compare baseline characteristics of group 1 with the other groups: patients with established CAD (group 3), patients with high 10-year ASCVD (group 4), patients with intermediate 10-year ASCVD (group 5), patients with low and borderline 10-year ASCVD on pharmacotherapy (group 6), and patients with low and borderline 10-year ASCVD and LDL>100 (group 2), Figure 1.

Group 1 will be categorized based on the presence or absence of detectable coronary calcium, and two group comparisons will be done. Data will be summarized using median (25^{th}, 75^{th} percentile) for continuous variables and n (%) for categorical variables. Following this, unadjusted, and age- and sex- adjusted, analyses will be performed using logistic regression for group 1. Analysis will be conducted using the SAS version 9.4 (SAS Institute Inc, Cary NC) [26]. For the secondary aim of this investigation, correlation analyses and general linear models will be used to assess the association of AGEs, indicative of cumulative metabolic stress, with CAC scores and lipid profiles. For these analyses, distributional assumptions will be assessed with variable transformations (e.g. log transformation) used as appropriate. Several other variables, such as echocardiogram, carotid ultrasound, cardiac perfusion stress test, and incidental CT findings, Supplementary3 and 4, are being collected as part of the chart review and will be utilized at a later date in the preparation of additional manuscripts.

Patient and Public Involvement and Ethics

Patient or public involvement was not incorporated in the design, conduct, reporting, or dissemination plans of this research.

Discussion

The rationale for this study stems from the limitations of traditional ASCVD risk models in identifying all patients with atherosclerosis, particularly those with normal LDL-C levels [27].

Asymptomatic individuals with normal or borderline 10-year ASCVD risk and normal LDL-C levels may still have subclinical atherosclerosis, detectable through CAC scanning. Clinical observations, along with results from the MESA and CONFIRM studies, demonstrate that atherosclerosis can occur in patients with low cardiovascular risk. Additionally, Fernández-Friera et al. have observed abnormal CAC scores in patients with normal LDL-C levels, suggesting that relying solely on risk models or LDL-C levels may be insufficient for detecting potentially at-risk patients with atherosclerosis. This supports the rationale for our study. These patients are often seen in primary care settings that do not routinely use CAC scans and could benefit from such scans, enabling timely interventions. By investigating the prevalence of abnormal CAC scores in this cohort, we aim to contribute to the existing literature on the potential benefits of CAC scans for these patients.

The rationale for this study arises from the limitations of traditional ASCVD risk models in identifying all patients with atherosclerosis, especially those with normal LDL-C levels. Asymptomatic individuals with normal or borderline 10-year ASCVD risk and normal LDL-C levels may still have subclinical atherosclerosis, detectable through CAC scanning [27]. Clinical observations, results from the MESA and CONFIRM studies that demonstrate that atherosclerosis in patients with low cardiovascular risk and observations of abnormal CAC scores in patients with normal LDL-C levels by Fernández-Friera et al. suggest that relying solely on risk models or LDL-C levels may be inadequate for detecting potentially at-risk patients with atherosclerosis and support the rationale for this study [8, 10, 13]. These patients are often seen in primary care settings that do not routinely use CAC scans and could benefit from such scans enabling timely interventions. By investigating the prevalence of abnormal CAC

scores in this cohort, we aim to contribute to the existing literature on the potential benefits of CAC scans for these patients.

Building on this, evidence suggests a pathophysiological link between AGEs and atherosclerosis, cardiovascular disease, and mortality [15-17]. It is plausible that AGEs may correlate with coronary calcium, a marker for detecting coronary atherosclerosis [21, 30-35]. However, no studies have explored possible associations between AGEs and CAC, which will be unique to this retrospective cohort study. By investigating this potential association, we aim to determine whether AGEs could serve as a predictive biomarker for coronary artery disease, even in patients with normal LDL-C levels. This could provide valuable insights into the underlying mechanisms of cardiovascular disease and help identify individuals at higher risk, thereby improving prevention and treatment strategies.

The findings of our study could pave the way for future research to evaluate the use of CAC scans in cardiovascular risk stratification, potentially enhancing clinical guidelines and practices, and integrating CAC scoring into risk prediction models.

Ethics and Dissemination

This study underwent expedited review procedures and was determined to be exempt from institutional review board approval (ID 24-003921; 45 CFR 46.104d, category/subcategory 4[iii]). Ethical principles have and will continue to be carefully considered and adhered to, and only patients who previously consented to the use of their data for research purposes will be included in this cohort. Unique identifiers will be created to document all findings, ensuring the anonymity of the patients whose records are being reviewed. Data will be securely stored on encrypted, institution-approved cloud services, with access limited to the investigating team. Any

modifications to the study design will be promptly submitted to the ethics review committee for review.

The results of this study will be disseminated through publication in a peer-reviewed journal and presentations at both national and international academic conferences. Additionally, we aim to promote our findings via social media platforms.

Authors' contributions

All persons listed as contributors met the International Committee of Medical Journal Editors (ICMJE) authorship criteria. Each author has made significant contributions to the writing and revisions to the study and has provided complete assent for publication. B.M.R., B.E.K., A.V., R.T.H., S.L.B., J.A., C.D.V., and I.T.C. were responsible for the conceptualization of the study. A.V., R.T.H., S.L.B., D.K.L., and I.T.C. were responsible for supervising all activities. All authors contributed to designing the methodology. B.M.R., B.E.K., A.V., and D.S. were responsible for calculating the sample size and formulating the data analysis plan. Each author is responsible for the content and has read and approved the final manuscript. B.M.R. is the guarantor of this work and accepts full responsibility for the integrity of the data, the accuracy of the analysis, and the decision to submit for publication.

Funding: This research will be supported by internal division funds, Division of General Internal Medicine, Department of Medicine, Mayo Clinic. Statistical support for this project will be provided by the Mayo Clinic Department of Medicine Research Hub. REDCap will be the data entry system for this study. The REDCap data entry system was supported in part by the Center for Clinical and Translational Science Grant Number UL1 TR000135 from the National

Center for Advancing Translational Sciences (NCATS). Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the National Institutes of Health.

Competing interests: The authors declare there are no competing interests.

Patient and public involvement: Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication: Not applicable.

Provenance and peer review: Not commissioned; externally peer reviewed.

References:

1. Vaduganathan M, Mensah GA, Turco JV, Fuster V, Roth GA. The Global Burden of Cardiovascular Diseases and Risk: A Compass for Future Health. J Am Coll Cardiol. 2022;80(25):2361-71.
2. WHO. Cardiovascular diseases (CVDs) 2021 [Available from: https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds).
3. Mitsis A, Khattab E, Christodoulou E, Myrianthopoulos K, Myrianthefs M, Tzikas S, et al. From Cells to Plaques: The Molecular Pathways of Coronary Artery Calcification and Disease. Journal of Clinical Medicine. 2024;13(21):6352.
4. Arnett DK, Blumenthal RS, Albert MA, Buroker AB, Goldberger ZD, Hahn EJ, et al. 2019 ACC/AHA Guideline on the Primary Prevention of Cardiovascular Disease: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. Circulation. 2019;140(11):e596-e646.
5. Golub Ilana S, Termeie Orly G, Kristo S, Schroeder Lucia P, Lakshmanan S, Shafter Ahmed M, et al. Major Global Coronary Artery Calcium Guidelines. JACC: Cardiovascular Imaging. 2023;16(1):98-117.

6. Lo-Kioeng-Shioe MS, Vavere AL, Arbab-Zadeh A, Schuijf JD, Rochitte CE, Chen MY, et al. Coronary Calcium Characteristics as Predictors of Major Adverse Cardiac Events in Symptomatic Patients: Insights From the CORE 320 Multinational Study. J Am Heart Assoc. 2019;8(6):e007201.
7. Budoff MJ, Kinninger A, Gransar H, Achenbach S, Al-Mallah M, Bax JJ, et al. When Does a Calcium Score Equate to Secondary Prevention?: Insights From the Multinational CONFIRM Registry. JACC Cardiovasc Imaging. 2023;16(9):1181-9.
8. Dzaye O, Razavi AC, Michos ED, Mortensen MB, Dardari ZA, Nasir K, et al. Coronary artery calcium scores indicating secondary prevention level risk: Findings from the CAC consortium and FOURIER trial. Atherosclerosis. 2022;347:70-6.
9. Greenland P, Blaha Michael J, Budoff Matthew J, Erbel R, Watson Karol E. Coronary Calcium Score and Cardiovascular Risk. JACC. 2018;72(4):434-47.
10. Grundy Scott M, Stone Neil J, Bailey Alison L, Beam C, Birtcher Kim K, Blumenthal Roger S, et al. 2018 AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA Guideline on the Management of Blood Cholesterol. JACC. 2019;73(24):e285-e350.
11. Fernández-Friera L, Fuster V, López-Melgar B, Oliva B, García-Ruiz JM, Mendiguren J, et al. Normal LDL-Cholesterol Levels Are Associated With Subclinical Atherosclerosis in the Absence of Risk Factors. J Am Coll Cardiol. 2017;70(24):2979-91.
12. Hegab Z, Gibbons S, Neyses L, Mamas MA. Role of advanced glycation end products in cardiovascular disease. World J Cardiol. 2012;4(4):90-102.
13. Hodgkinson CP, Laxton RC, Patel K, Ye S. Advanced Glycation End-Product of Low Density Lipoprotein Activates the Toll-Like 4 Receptor Pathway Implications for Diabetic Atherosclerosis. Arteriosclerosis, Thrombosis, and Vascular Biology. 2008;28(12):2275-81.
14. Vekic J, Vujcic S, Bufan B, Bojanin D, Al-Hashmi K, Al-Rasadi K, et al. The Role of Advanced Glycation End Products on Dyslipidemia. Metabolites. 2023;13(1).
15. Sharifi-Zahabi E, Sharafabad FH, Abdollahzad H, Malekahmadi M, Rad NB. Circulating Advanced Glycation End Products and Their Soluble Receptors in Relation to All-Cause and Cardiovascular Mortality: A Systematic Review and Meta-analysis of Prospective Observational Studies. Adv Nutr. 2021;12(6):2157-71.
16. Diagnoptics. Advanced Glycation Endproducts Readers [Available from: https://www.diagnoptics.com/advanced-glycation-endproducts/.
17. Perrone A, Giovino A, Benny J, Martinelli F. Advanced Glycation End Products (AGEs): Biochemistry, Signaling, Analytical Methods, and Epigenetic Effects. Oxid Med Cell Longev. 2020;2020:3818196.
18. Cardiology ACo. ASCVD Risk Estimator Plus [Available from: https://tools.acc.org/ascvd-risk-estimator-plus/#!/calculate/estimate/.
19. Harris PA, Taylor R, Minor BL, Elliott V, Fernandez M, O'Neal L, et al. The REDCap consortium: Building an international community of software platform partners. J Biomed Inform. 2019;95:103208.
20. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. J Biomed Inform. 2009;42(2):377-81.

21. Inc. SI. SAS/STAT® 15.3 User's Guide. Cary, NC: SAS Institute Inc. [Available from: https://documentation.sas.com/api/collections/pgmsascdc/9.4_3.5/docsets/statug/content/sashelp.pdf?locale=en.

22. Orringer CE, Blaha MJ, Blankstein R, Budoff MJ, Goldberg RB, Gill EA, et al. The National Lipid Association scientific statement on coronary artery calcium scoring to guide preventive strategies for ASCVD risk reduction. Journal of Clinical Lipidology. 2021;15(1):33-60.

23. Ebert H, Lacruz ME, Kluttig A, Simm A, Greiser KH, Tiller D, et al. Association between advanced glycation end products, their soluble receptor, and mortality in the general population: Results from the CARLA study. Exp Gerontol. 2020;131:110815.

24. Ho JE, Lyass A, Courchesne P, Chen G, Liu C, Yin X, et al. Protein Biomarkers of Cardiovascular Disease and Mortality in the Community. J Am Heart Assoc. 2018;7(14).

25. Jensen LJ, Flyvbjerg A, Bjerre M. Soluble Receptor for Advanced Glycation End Product: A Biomarker for Acute Coronary Syndrome. Biomed Res Int. 2015;2015:815942.

26. Raposeiras-Roubín S, Rodiño-Janeiro BK, Grigorian-Shamagian L, Moure-González M, Seoane-Blanco A, Varela-Román A, et al. Relation of soluble receptor for advanced glycation end products to predict mortality in patients with chronic heart failure independently of Seattle Heart Failure Score. Am J Cardiol. 2011;107(6):938-44.

27. Schwedler SB, Metzger T, Schinzel R, Wanner C. Advanced glycation end products and mortality in hemodialysis patients. Kidney Int. 2002;62(1):301-10.

28. Semba RD, Bandinelli S, Sun K, Guralnik JM, Ferrucci L. Plasma carboxymethyl-lysine, an advanced glycation end product, and all-cause and cardiovascular disease mortality in older community-dwelling adults. J Am Geriatr Soc. 2009;57(10):1874-80.

Figure 1: Enrollment and Screening Process Flowchart

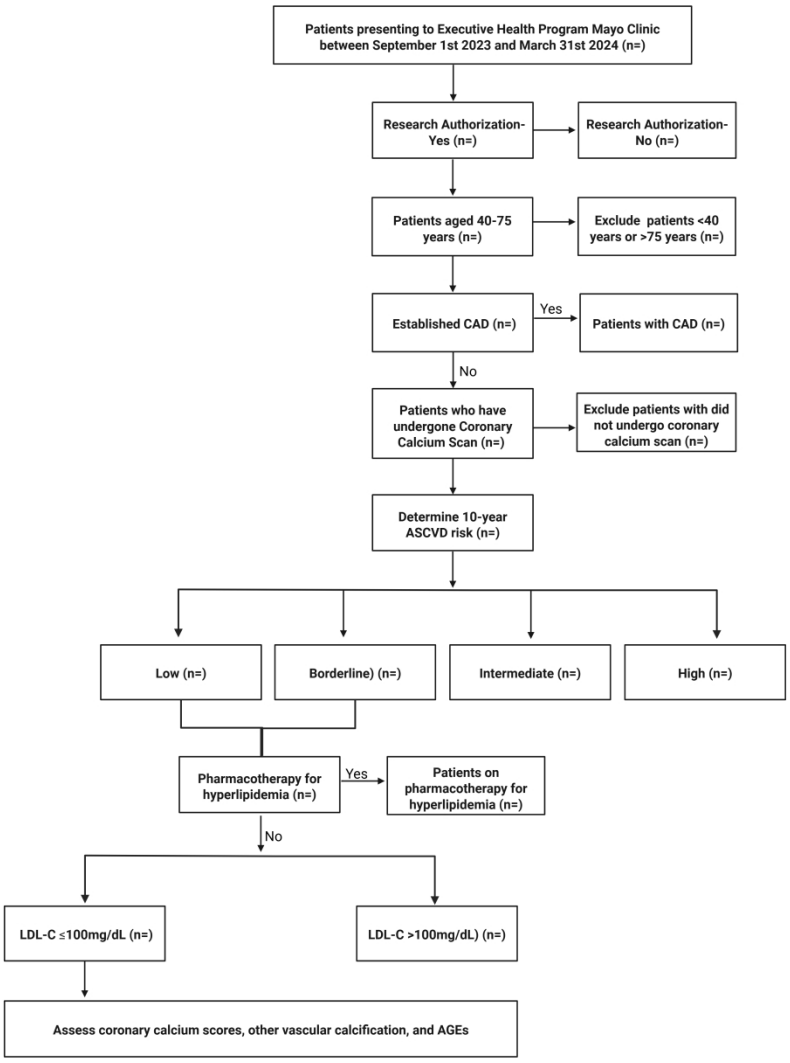

Figure 1: Enrollment and Screening Process Flowchart

336x504mm (236 x 236 DPI)

Supplementary 1. Data Extraction Sheet for Baseline Demographic Characteristics

Record ID	
Date of executive health visit	
Full name (first, middle initial, last)	
Age (at time of scan)	
Date of birth	
Sex	Male Female Other
Race	American Indian or Alaska Native Asian Native Hawaiian or Other Pacific Islander Black or African American White More than 1 race Chose not to disclose Unknown Other
Ethnicity	Hispanic or Latino Non-Hispanic or Latino Unknown
Level of education	8th grade

	High school graduate or General Educational Development test Some high school but did not graduate Associate's degree (academic) Associate's degree (occupational, technical, or vocational) Some college Bachelor's degree Master's degree Doctorate Professional degree (MD, DDS, DVM, JD) No degree The patient did not answer Unknown
Marital status	Single Married Divorced Separated Widowed Life partner Chose not to disclose Unknown

Smoking (any form of tobacco products [e.g., chew])	Never Current, every day Current, some days Former Unknown/unanswered
What type of smoking?	Cigarettes Cigars Pipe Snuff Chew
Does the patient use smokeless tobacco such as chew or snuff?	Yes No
Total packs per year	
Current packs per day	0.25 0.5 0.75 1 1.5 2 2.5 3 >3 packs Quit

Date patient quit smoking or using smokeless tobacco	
Number of packs per year patient used to smoke	
Comments	
E-cigarette and vaping use	Never Current, every day Current, some days Former Unknown/unanswered
Alcohol use	Yes Not currently Never Chose not to disclose No
Type of drinks and amount per day	Glasses of wine Cans of beer Shots of liquor Other (e.g., vodka 750 mL/day) Unknown type
Drinks per week	
Comments	
Illicit drug use	Yes

	Not currently Never Chose not to disclose No
Type of illicit drug	Amphetamines Anabolic steroids Barbiturates Cocaine Crack cocaine Hashish Heroin LSD (lysergic acid diethylamide) Marijuana MDMA (3,4- methylenedioxymethamphetamine; ecstasy) Methamphetamines Opium PCP (phencyclidine) Solvent inhalants Other
Comments	
Family history (select all that apply)	Carotid disease Coronary artery disease

	Peripheral vascular disease Stroke/transient ischemic attack None of the above Unknown/unanswered
Past medical history	Cancer Chronic kidney disease Chronic liver disease Connective tissue disease Coronary artery disease Diabetes Heart rhythm disorders Hyperlipidemia Hypertension Metabolic syndrome Obesity Obstructive sleep apnea Peripheral artery disease Radiotherapy Stroke/transient ischemic attack Thyroid diseases Valvular disease None Unknown/not answered

Medical cannabis use	Yes No
Does the patient exercise?	Yes No Unknown
Minutes per week of exercise	
Days per week of exercise	
Type of exercise	Cardio Strength training Other Unknown
Comments and other types of exercise	
Height, cm	
Weight, kg	
Body mass index	
Neck circumference, cm	
Waist circumference, cm	
Systolic blood pressure (current), mm Hg	
Diastolic blood pressure (current), mm Hg	
Type of executive	Executive Spouse Unknown
Amount of traveling	25% of time

	50% of time 75% of time 100% of time None Unknown

Supplementary 2. Data Extraction Sheet for Laboratory Test Results*

Date of laboratory tests	
Total cholesterol	
HDL cholesterol	
LDL cholesterol	
Was LDL-C <100 mg/dL?	Yes No
Is the patient on lipid lowering medication?	Yes No
Medication used	Alirocumab Atorvastatin Bempedoic acid Evolocumab Ezetimibe Inclisiran Pravastatin Rosuvastatin

	Simvastatin Aspirin Clopidogrel Prasugrel Ticagrelor None
Triglycerides	
Lipoprotein (a)	
Other laboratory tests	
Apolipoprotein A-1	
Apolipoprotein B	
Fasting blood glucose	
Hemoglobin $A1_c$	
MI-Heart Ceramides, Plasma risk score	0-2 low risk 3-6 moderate risk 7-9 increased risk 10-12 high risk Not done
Comments	

Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein.

* Laboratory values closest to the time of CAC scan will be abstracted.

Supplementary 3. Data Extraction Sheet for Coronary Artery Calcium (CAC) Scan and Scoring

Was a CAC scan obtained?	Yes No
Date of scan	
Total CAC score	
Coronary artery measurements	
Left anterior descending	
Circumflex	
Left main vessel	
Right coronary artery	
Percentile ranking*	
Incidental findings	Nodules Lymph nodes Granulomas Bony lesions Enlarged aorta Other None
Other findings	

* For individuals with multiple races recorded in their medical records, the race used in the CAC percentile calculation was determined by the radiologist who issued the CAC report

Supplementary 4. Data Extraction Sheet for Cardiac Imaging Findings

Radiographic findings	Calcification Atherosclerosis Negative Not done Other findings
Date of radiograph	
Comments/impressions or other findings on radiography	
Chest CT imaging	Calcification Atherosclerosis Pulmonary nodules Negative Not done Other findings
Date of chest CT	
Comments/impressions or other findings on chest CT	
Carotid US	Atherosclerosis Negative Not done Other findings
Date of carotid US	

Comments/impressions or other findings on carotid US	
Echocardiography	Enlarged aorta Dilated aorta Negative Not done Other findings
Date of echocardiography	
Comments/impressions or other findings on echocardiography	
Stress echocardiography	Enlarged aorta Dilated aorta Negative Not done Other findings
Date of stress echocardiography	
Comments/impressions or other findings on stress echocardiography	
Exercise ECG	Yes No
Type of exercise ECG	Treadmill Bike
Date of exercise ECG	

Comments/impressions or other findings on exercise ECG	
Cardiopulmonary stress test	Yes No
Date of cardiopulmonary stress test	
Comments/impressions or other findings on cardiopulmonary stress test	
Cardiac perfusion stress test	Yes No
Date of cardiac perfusion stress test	
Comments/impressions or other findings on cardiac perfusion stress test	
ECG	Yes No
Date of ECG	
Comments/impressions or other findings on ECG	
Real and ECG age	
Probability of low ejection fraction, %	
Probability of atrial fibrillation, %	

Abbreviations: CT, computed tomography; ECG, electrocardiography; US, ultrasonography.