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Inflammatory biomarkers and risk of cardiovascular events in patients undergoing coronary angiography

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**Background** Inflammation, measured by traditional biomarkers such as C-reactive protein, has been linked to cardiovascular (CV) events. Recent technological advancement has allowed for measuring larger numbers of inflammatory biomarkers. A contemporary evaluation with established and novel biomarkers of inflammation is needed.

**Methods** 1,090 individuals who underwent coronary angiography were enrolled. Twenty-four inflammatory biomarkers were collected prior to angiography. Unsupervised machine learning cluster analyses determined unique patterns of inflammatory biomarkers. Cox proportional hazard regression assessed both association of inflammatory biomarker clusters and individual biomarker associations with major adverse cardiovascular events (MACE; non-fatal myocardial infarction or stroke, and CV death) during a median follow-up of 3.67 years.

**Results** Four distinct clusters were recognized. Incremental increases in inflammatory biomarkers were observed from cluster 1 to cluster 4. During follow-up, 263 MACE were ascertained. Considering cluster 1 as a reference, study participants with inflammatory cluster 2 (Hazard ratio [HR] 1.55, 95% confidence interval [CI]: 1.01-2.37), cluster 3 (HR 1.89, CI: 1.25-2.85), and cluster 4 (HR 2.93, CI: 1.95-4.42) were at increased risk of MACE. Interleukin (IL)-1α, IL-6, IL-8, IL-10, IL-12, Adhesion molecule-1 high-sensitivity C-reactive protein, ferritin, myeloperoxidase, macrophage inflammatory protein (MIP)-1α, MIP 3, and macrophage colony-stimulating factor-1 were independently associated with MACE.

**Conclusions** Among persons undergoing coronary angiography procedures, distinct clusters of inflammatory biomarker distributions with significant prognostic meaning may be identified. These results may identify unique targets for anti-inflammatory treatments aimed at CV disease. (Am Heart J 2022;252:51-59.)
ously measuring a pool of related inflammatory biomarkers might be more informative.

To address this, using data available from samples collected in the Catheter-Sampled Blood Archive in Cardiovascular Diseases (CASABLANCA) study, we aimed to determine whether inflammatory biomarker patterns of distribution exist in persons undergoing coronary angiography, and whether such patterns are associated with the risk of future major adverse cardiovascular events (MACE; non-fatal myocardial infarction, non-fatal stroke, and CV mortality). Furthermore, we aim to investigate the association of each inflammatory biomarker with MACE independent of lipid levels and other conventional risk factors.

**Methods**

All study procedures were approved by the Mass General/Brigham Institutional Review Board. No extramural funding was used to support this work.

**Study design and participants**

The design of the CASABLANCA study has been described previously (ClinicalTrials.gov Identifier: NCT00842868). Briefly, 1,251 patients undergoing coronary and/or peripheral angiography with or without intervention between 2008 and 2011 were prospectively enrolled at the Massachusetts General Hospital in Boston, MA. Patients were referred for angiography for various acute and non-acute indications, including acute coronary syndromes, heart failure, abnormal stress tests, stable chest pain, claudication, and routine pre-operative evaluation. The final group of study participants (n = 1,090) was achieved after the exclusion of those who underwent only peripheral angiography (n = 153) and those with missing values for biomarker levels (n = 8).

**Follow-up**

Medical record review from the time of enrolment to the end of follow-up was performed. Median follow-up was 3.67 years with a maximum follow-up of 8 years. For identification of clinical endpoints, a review of medical records as well as phone follow-up with patients and/or managing physicians was performed. The Social Security Death Index and/or postings of death announcements were used to confirm vital status. A panel of investigators adjudicated each clinical endpoint; a detailed definition of endpoints for CASABLANCA was previously published. Specific to this analysis, we evaluated associations between inflammation and time to incident MACE (non-fatal myocardial infarction, non-fatal ischemic stroke, CV mortality).

**Biomarker testing**

A total of 15 mL of blood was obtained immediately before the angiographic procedure through a centrally placed vascular access sheath. The blood was immediately centrifuged for 15 minutes, serum and plasma aliquoted on ice, and frozen at −80°C until biomarker measurement. The samples for this study were analyzed after the first freeze-thaw cycle and were derived from the baseline blood draw. In this study, we evaluated high sensitivity C-reactive protein (hs-CRP; Siemens, Newark, DE) and myeloperoxidase (MPO; Siemens, Newark, DE). Additionally, Luminex xMAP technology platform (Luminex Corporation, Austin, Texas) was used to measure concentrations of the inflammatory markers listed in Supplemental Table 1. The Luminex approach uses multiplexed, microsphere-based assays in a single reaction vessel and is accomplished by assigning each protein-specific assay a recognizable microsphere-based fluorescence signature. To do this, assay-specific capture antibodies are conjugated covalently to each unique set of microspheres and bound to the protein of interest followed by addition of assay-specific detection antibodies together with a streptavidin-labeled fluorescent “reporter” molecule; the bound amount of fluorescence generated is proportionate to the protein level. A minimum of 100 individual microspheres from each unique set were analyzed.

**Statistical analysis**

Unsupervised machine learning using K-means clustering was used to assess the clusters of inflammatory biomarker concentrations among study participants. The elbow curve method was used to select the optimal number of clusters (Supplementary Figure 1). Analysis of variance and chi-square test were used to compare the baseline characteristics of study participants across clusters for continuous and categorical variables, respectively. Overall, 3.3% of the data were missing. We used Multivariate Imputation via Chained Equations (MICE) package in R for data imputation. We implemented Cox proportional hazard regression to investigate the association of each inflammatory cluster with MACE. Multivariate models were adjusted for age, sex, hypertension, diabetes mellitus, chronic kidney disease, history of coronary artery disease, low-density lipoprotein, high-density lipoprotein, acute coronary syndrome, and statin use. Variables selection was based on their significance in prior CASABLANCA sub-studies. The proportional hazard assumption in the Cox model was assessed with the Schoenfeld residual test, and all proportionality assumptions were appropriate. Finally, to assess the most influential inflammatory biomarker associated with MACE, we conducted Cox regression with the least absolute shrinkage and selection operator (LASSO) penalization, which can help to reduce the dimensions of the model. To determine the penalty factor (lambda), a tenfold cross-validated error plot of the LASSO model was constructed. The optimal lambda was determined by choosing the most regularized and parsimonious model within 1 stan-
Table I. Baseline characteristics of the study population and subdivided by inflammation cluster.

| Inflammation clusters | 1 (N = 285) | 2 (N = 331) | 3 (N = 261) | 4 (N = 231) | P-value |
|-----------------------|-------------|-------------|-------------|-------------|---------|
| Age                   | 66.3 (11.5) | 64.8 (11.0) | 64.2 (11.2) | 68.3 (11.4) | <.001   |
| Sex, male             | 778 (71.4%) | 226 (79.3%) | 237 (75.7%) | 152 (58.2%) | <.001   |
| Race, white           | 1017 (93.3%)| 271 (95.1%) | 297 (94.9%) | 241 (92.3%) | .48     |
| Clinical history      |             |             |             |             |         |
| Hypertension          | 799 (73.3%) | 184 (64.6%) | 222 (70.9%) | 206 (78.9%) | <.001   |
| Diabetes Mellitus     | 265 (24.3%) | 50 (17.5%)  | 67 (21.4%)  | 77 (29.5%)  | .001    |
| Heart failure         | 227 (20.5%) | 44 (15.4%)  | 56 (17.9%)  | 65 (24.9%)  | .001    |
| CAD                   | 558 (51.2%) | 151 (53.0%) | 127 (40.6%) | 153 (58.6%) | <.001   |
| CKD                   | 134 (12.3%) | 12 (4.2%)   | 24 (7.7%)   | 42 (16.1%)  | <.001   |
| Smoker                | 153 (14.0%) | 27 (9.5%)   | 48 (15.3%)  | 36 (13.8%)  | <.001   |
| Atrial fibrillation   | 210 (19.3%) | 40 (14.0%)  | 53 (16.9%)  | 63 (24.1%)  | .07     |
| CVA/TIA               | 110 (10.1%) | 25 (8.8%)   | 23 (7.3%)   | 32 (12.3%)  | .01     |
| Hx of PCI             | 298 (27.3%) | 88 (30.9%)  | 62 (19.8%)  | 81 (31.0%)  | .01     |
| Hx of DES             | 711 (65.2%) | 163 (57.2%) | 217 (69.3%) | 168 (64.4%) | .009    |
| Hx of CABG            | 189 (17.3%) | 44 (13.4%)  | 38 (12.1%)  | 61 (23.4%)  | .006    |
| Presentation          |             |             |             |             | <.001   |
| Asymptomatic/stable   | 864 (79.3%) | 250 (87.7%) | 247 (78.9%) | 201 (77.0%) | .16     |
| Unstable Angina       | 133 (12.2%) | 30 (10.5%)  | 30 (9.6%)   | 50 (19.2%)  | .20     |
| Acute MI              | 93 (8.5%)   | 5 (1.8%)    | 36 (11.5%)  | 10 (3.8%)   | .003    |
| Medications           |             |             |             |             |         |
| ACEi                  | 433 (39.7%) | 116 (40.7%) | 130 (41.5%) | 102 (39.1%) | .84     |
| ARB                   | 164 (15.0%) | 26 (9.1%)   | 37 (11.8%)  | 59 (22.6%)  | <.001   |
| Bbblocker             | 769 (70.6%) | 194 (68.1%) | 209 (66.8%) | 188 (72.0%) | .09     |
| MRA                   | 46 (4.2%)   | 7 (2.5%)    | 13 (4.8%)   | 15 (5.7%)   | .40     |
| Loop diuretic         | 232 (21.3%) | 23 (8.1%)   | 51 (16.3%)  | 77 (29.5%)  | <.001   |
| Nitrates              | 210 (19.3%) | 51 (17.9%)  | 45 (14.4%)  | 76 (29.1%)  | <.001   |
| CCB                   | 259 (23.8%) | 46 (16.1%)  | 78 (24.9%)  | 81 (31.0%)  | .002    |
| Aspirin               | 824 (75.6%) | 230 (80.7%) | 231 (73.8%) | 199 (76.2%) | .12     |
| Statin                | 779 (71.5%) | 215 (75.4%) | 217 (69.3%) | 193 (73.9%) | .18     |
| Clopidogrel           | 247 (22.7%) | 73 (25.6%)  | 56 (17.9%)  | 62 (23.8%)  | .20     |
| Warfarin              | 167 (15.3%) | 33 (11.6%)  | 35 (11.2%)  | 50 (19.2%)  | .003    |
| Biomarkers            |             |             |             |             |         |
| hs-cTnl               | 4.8 (2.2-14)| 3.1 (1.7-6.9)| 4.7 (2.2-17)| 4.3 (2.2-10)| <.001   |
| NT-proBNP            | 1500 (540-4200)| 960 (400-2000)| 1100 (360-2900)| 1800 (690-4700)| <.001   |
| Golectin 3            | 19 (15-25)| 17 (14-21)| 18 (15-22)| 21 (17-28)| <.001   |
| Soluble ST2           | 36 (27-48)| 33 (26-42)| 34 (26-44)| 36 (27-47)| <.001   |
| suPAR                 | 3.6 (2.5-5.2)| 2.7 (2.1-3.6)| 3.0 (2.3-4.3)| 4.1 (3.2-6.4)| <.001   |
| Osteopontin           | 28 (20-42)| 23 (18-31)| 24 (17-32)| 30 (22-42)| <.001   |
| Adiponectin           | 3.7 (2.4-6.0)| 3.5 (2.4-5.7)| 3.3 (2.1-4.8)| 4.0 (2.6-6.3)| <.001   |
| KIM-I                 | 150 (98-240)| 110 (74-160)| 140 (96-210)| 170 (110-290)| <.001   |
| Cystatin C            | 0.76 (0.63-0.95)| 0.69 (0.61-0.78)| 0.73 (0.62-0.87)| 0.85 (0.66-1.0)| <.001   |

CAD, coronary artery disease; CKD, chronic kidney disease; CVA/TIA, cerebrovascular event/transient ischemic attack; Hx, history; PCI, percutaneous coronary intervention; DES, drug eluting stent; CABG, coronary artery bypass graft; MI, myocardial infarction; ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; MRA, mineralocorticoid receptor antagonist; CCB, calcium channel blocker; hs-cTnl, high sensitive cardiac troponin; NT-proBNP, N terminal-pro B natriuretic peptides; suPAR, soluble plasminogen activator receptor; KIM, kidney injury molecule.

Table I details the baseline characteristics of study participants across clusters. Hyper-tension, diabetes mellitus, heart failure, coronary artery disease, chronic kidney disease, atrial fibrillation, and previous revascularization were most prevalent among cluster 4, which also tended to have the highest concentrations of inflammatory markers, the high-sensitive car-

standard error from the minimum. All P-values reported were 2-sided. A P-value < .05 was considered statistically significant. All statistical analyses were performed using the R version 3.6.2 (R Foundation for Statistical Computing, Vienna, Austria. URL: https://www.R-project.org/).

Results

Table I details the baseline characteristics and biomarker concentrations of the study participants. Following clustering, 4 patterns of inflammatory biomarkers were identified; Supplementary Figure 2 demonstrates the distribution of inflammatory biomarkers across these 4 clusters. From Cluster 1 to Cluster 4, we observed a significant incremental increase in the level of IL-1a receptor, IL-6, IL-6 receptor, IL-8, IL-12, IL18, fibrinogen, MIPs, MCP, and MCSF-1. Table I details the baseline characteristics of study participants across clusters. Hypertension, diabetes mellitus, heart failure, coronary artery disease, chronic kidney disease, atrial fibrillation, and previous revascularization were most prevalent among cluster 4, which also tended to have the highest concentrations of inflammatory markers, the high-sensitive car-
Table II. Angiographic characteristics of the study population as a function of inflammation

| Angiography results | All individuals (N = 1090) | 1 (N = 285) | 2 (N = 313) | 3 (N = 261) | 4 (N = 231) | P-value |
|---------------------|-----------------------------|-------------|-------------|-------------|-------------|---------|
| ≥ 30% coronary stenosis in ≥ 2 vessels | 704 (64.6%) | 180 (63.2%) | 192 (61.3%) | 174 (66.7%) | 158 (68.4%) | .45 |
| ≥ 30% coronary stenosis in ≥ 3 vessels | 550 (50.5%) | 145 (50.9%) | 145 (46.3%) | 132 (50.6%) | 128 (55.4%) | .35 |
| ≥ 50% coronary stenosis in ≥ 2 vessels | 572 (52.5%) | 150 (52.6%) | 148 (47.3%) | 143 (54.8%) | 131 (56.7%) | .23 |
| ≥ 50% coronary stenosis in ≥ 3 vessels | 388 (35.6%) | 104 (36.5%) | 97 (31.0%) | 92 (35.2%) | 95 (41.1%) | .19 |
| ≥70% coronary stenosis in ≥ 2 vessels | 438 (40.2%) | 108 (37.9%) | 117 (37.4%) | 114 (43.7%) | 99 (42.9%) | .45 |
| ≥70% coronary stenosis in ≥ 3 vessels | 246 (22.6%) | 62 (21.8%) | 58 (18.5%) | 70 (26.8%) | 56 (24.2%) | .19 |
| Acute coronary syndrome | All individuals (N = 226) | 1 (N = 35) | 2 (N = 66) | (N = 60) | (N = 65) | |
| ≥ 30% coronary stenosis in ≥ 2 vessels | 174 (77.0%) | 27 (77.1%) | 50 (75.0%) | 52 (80.0%) | .97 |
| ≥ 30% coronary stenosis in ≥ 3 vessels | 141 (62.4%) | 22 (62.9%) | 40 (60.0%) | 36 (60.0%) | 43 (66.2%) | .96 |
| ≥ 50% coronary stenosis in ≥ 2 vessels | 138 (61.1%) | 23 (65.7%) | 35 (53.0%) | 37 (61.7%) | 43 (66.2%) | .59 |
| ≥ 50% coronary stenosis in ≥ 3 vessels | 95 (42.0%) | 15 (42.9%) | 22 (33.3%) | 24 (40.0%) | 34 (52.3%) | .29 |
| ≥70% coronary stenosis in ≥ 2 vessels | 107 (47.3%) | 15 (42.9%) | 27 (40.9%) | 30 (50.0%) | 35 (53.8%) | .62 |
| ≥70% coronary stenosis in ≥ 3 vessels | 65 (28.8%) | 6 (17.1%) | 13 (19.7%) | 20 (33.3%) | 26 (40.0%) | .04 |

Figure 1

Association of inflammatory cluster groupings with cardiovascular outcomes. Models were adjusted for age, sex, history of diabetes mellitus, hypertension, chronic kidney disease, coronary artery disease, acute coronary syndrome as well as statin use and concentrations of low density and high-density lipoprotein cholesterol. MACE: major adverse cardiovascular outcome (MI, stroke, and CV mortality), MI: myocardial infarction, CV: cardiovascular, HR: hazard ratio, CI: confidence interval.
Event rate based on Kaplan-Meier curve across the clusters. Patients in cluster 4 had higher rate of adverse events; A) Major adverse cardiovascular events, B) myocardial infarction C) cardiovascular death D) all-cause death.

diac troponin and NT-proBNP. Prevalence of acute coronary syndromes (unstable angina pectoris or acute myocardial infarction) was also highest in cluster 4, however there were no differences in angiographic results between clusters (Table II).

During a median of 3.67 years of follow-up, 263 MACE events, 171 MI events, 124 CV mortality, and 166 all-cause mortality events were ascertained. Figure 1 illustrates the association of clusters with adverse clinical outcomes. In a multivariate model, considering cluster 1 as a reference, patients with cluster 2 (Hazard ratio [HR] 1.55, 95% confidence interval [CI] 1.01-2.37), cluster 3 (HR 1.89, 95% CI 1.25-2.85) and cluster 4 (HR 3.00 (95% CI 1.95-4.42) were at increased risk of MACE. Notably, the HR (95% CI) of CV mortality was 3.05 (1.47-6.33) and 2.52 (1.21-5.22) for clusters 2 and 3, and 5.12 (2.52-10.44) for cluster 4. Similar differences in all-cause mortality were seen with cluster 4 demonstrating a nearly 2-fold higher cause-independent risk for death. Figure 2 depicts the time to incident MACE across the clusters.

Cluster 4 was associated with incident type 1 MI (HR 3.01, 95% CI 1.10-8.23) and type 2 MI (HR 3.84, 95% CI 1.98-7.42). Data are shown in Supplementary Table 2.

Lastly, the adjusted association of each individual inflammatory biomarker with MACE was evaluated and presented in Supplementary Table 3. Numerous inflammatory markers remained associated with MACE, even after adjusting for non-inflammatory risk factors, including the low density and the high density lipoprotein cholesterol (LDL-C and HDL-C). Figure 3 illustrates the most influential clinical and inflammatory markers associated with MACE according to LASSO regression. MCSF-1, IL-8, IL-1α, prevalent diabetes mellitus, and age were most strongly linked to adverse outcome.

Discussion

In this prospective cohort of patients who underwent diagnostic and/or therapeutic coronary angiography through measurement of a pool of inflammation
biomarkers, we identified 4 patterns of biomarker concentrations (Graphical abstract). Irrespective of lipid profile, conventional CV risk factors and presenting syndrome, we were able to show several of these inflammatory biomarker clusters had a higher risk of MACE, with correspondingly higher risk for mortality, particularly in those with the greatest degrees of inflammation. Out of the 24 inflammatory biomarkers evaluated, elevated concentrations of MCSF-1, IL-8, and IL-1α were most highly associated with poor prognosis.

Coronary artery disease is highly prevalent and often morbid. In this regard, many secondary preventive measures have been developed to alleviate the high risk associated with the diagnosis. Aggressive reduction of LDL-C, employment of newer diabetes agents that lower atherothrombotic complications, the use of more intensive anti-thrombotic therapy with potent antiplatelet or the low-dose direct oral anticoagulants along with improvements in techniques for revascularization have all lowered the rate of the adverse outcomes following diagnosis of coronary atherosclerosis, however significant residual risk still exists even when correcting for such conventional risk factors. Some of the residual risk may be attributed to the observed high inflammatory status among patients with atherosclerosis despite receiving optimal medical treatment. Despite the accepted role of inflammation in the development, progression and complication of coronary atherosclerosis, the efforts to improve prognosis of those with coronary artery disease through targeting candidate inflammatory processes have returned mixed results. Nonetheless, many ongoing randomized clinical trials are investigating the efficacy of anti-inflammatory medications in patients with atherosclerosis.

Interleukins—cytokines expressed by the white blood cells that mediate communication between cells—are striking targets to modulate the inflammatory process. The Canakinumab Antiinflammatory Thrombosis Outcomes Study (CANTOS) trial showed that in patients with prior MI and hs-CRP >2 mg/dl, canakinumab (an interleukin-1β monoclonal antibody) lowered the risk of recurrent cardiovascular events. Targeting somewhat further downstream, in a phase II clinical trial of high-risk CV patients, Ziltivekimab (a novel IL-6 ligand in-
hibitor) markedly reduced multiple biomarkers of systemic inflammation and thrombosis that promote the atherothrombotic process, including hs-CRP, fibrinogen, serum amyloid A protein, secretory phospholipase A2, and lipoprotein (a). Lastly, colchicine has emerged as a potentially useful treatment option for cardiovascular disease, with 2 recent randomized clinical trials suggesting its use significantly reduces cardiovascular events in patients with chronic coronary disease.\textsuperscript{13} Data indicate that colchicine treatment reduced IL-1β, IL-6, and IL-18 concentrations in ACS patients.\textsuperscript{14}

Despite promising results regarding manipulation of inflammation in CV disease, not all anti-inflammatory trials had positive findings. For example, in the Cardiovascular Inflammation Reduction Trial,\textsuperscript{15} the low-dose methotrexate did not result in a reduction of CV events in patients with stable atherosclerosis and was associated with a reduction in white cell counts, liver injury, and a higher incidence of non-basal cell skin cancers compared to the placebo group during 2.3 years of follow-up. The discrepancy between these findings implies the need to understand in more depth the complex cytokine and chemokine signaling networks involved in atherosclerosis; rather than targeting in an indiscriminate fashion, a more gainful approach might be to identify and treat those most likely to respond in a favorable fashion. Much as has been shown with canakinumab in CANTOS where optimal outcomes were achieved in those demonstrating a shift in the inflammasome after treatment,\textsuperscript{16} individualizing use of anti-inflammatory therapies might be expected to be even more effective. To approach this question most appropriately, a better understanding of the collective distribution of inflammatory pathways and their relative clinical meaning is needed.

To better explore this question, we sought to identify patterns of characteristic biomarker signatures among study participants undergoing coronary angiography in the CASABLANCA study. Using 24 inflammatory biomarkers, we found 4 clusters among patients who underwent coronary angiography. In a stepwise manner from cluster 1 to cluster 4, we observed incremental increases in inflammatory biomarkers. Thus, these clusters represent varying degrees of inflammation severity. The extent of coronary artery disease was generally similar across clusters; however, among those who presented with acute coronary syndrome, we observed a higher prevalence of cluster 4 in those with at least 70% or more coronary stenosis in 3 or more vessels. This finding implies systemic inflammation activation during the extensive myocardial infarction and supports the theory of inflammation in acute myocardial infarction. Furthermore, inflammation clusters stratified the future CV risk of patients who underwent coronary angiography even when adjusted for conventional CV risk factors, LDL-c concentration, and presenting diagnosis. Thus, clinicians may use this machine learning clustering approach for assessing CV risk among patients who undergo coronary angiography.

On an individual level, we noted several biomarkers associated with MACE, even in fully adjusted models; these include MCSF-1, IL-8, and IL-1α. MCSF-1 was possibly the most highly associated with the risk of adverse outcomes among inflammatory biomarkers. Macrophage proliferation within the atherosclerotic plaques is vital in the progression of atherosclerosis and bench research studies have illuminated the role of MCSF-1 in plaque progression.\textsuperscript{17} Moreover, the clinical studies showed MCSF-1 is a harbinger of elevated CV risk in patients with stable angina and acute coronary syndrome possibly due to plaque instability.\textsuperscript{18,19} IL-8 has many biological effects, including recruitment and activation of monocytes and neutrophils.\textsuperscript{20} In a study investigating 10 inflammatory biomarkers, IL-8 was the only marker associated with cardiovascular events independently.\textsuperscript{21} IL-1α is a member of the interleukin-1 superfamily that regulate and initiate inflammatory responses in sterile inflammation.\textsuperscript{22} A growing number of bench studies have proposed IL-1α as a mediator of the development of atherosclerotic plaque.\textsuperscript{23} It is of note that monoclonal antibody therapies have been developed against MCSF-1\textsuperscript{24}, IL-8\textsuperscript{25} and IL-1α\textsuperscript{26} but CV applications for these treatments remain relatively unexplored.

Our study has several limitations. First, we did not have follow-up measures of inflammatory biomarkers. Previous research has shown that those with persistent inflammation have higher CV risk\textsuperscript{3}; we could not distinguish between the transient and the persistent inflammation in this study. Second, 93% of our study population were Caucasian and male-predominant. The effect of ethnicity and sex on the inflammatory profile cannot be assessed in this study. Third, while providing a convenient means to group individuals and guarantees convergence, hard clustering approaches such as k-means may be less robust than soft clustering approaches wherein individuals may reside in more than one grouping. Fourth, we did not have any data regarding underlying inflammatory conditions and the use of anti-inflammatory medications. Both factors may influence inflammatory biomarker levels and confound the risk of MACE. Fifth, our primary event was MACE (CV death, MI, stroke). A few non-cardiovascular deaths occurred during the follow-up that might be considered competing events against MACE. Our results may be interpreted in the lack of presence of any competing events. Finally, although our study is statistically well-powered, external validation and studies examining long-term consequences of inflammatory phenotypes are required.

In conclusion, among study participants undergoing coronary angiography, we were able to identify discrete inflammatory biomarker distributions; these inflammatory “phenotypes” have specific CV risks. Increase in IL-1α, IL-6, IL-8 IL-10, IL-12, IL-18, hs-CRP, Ferritin, MPO,
MIP1α, MIP 3, and MCSF1, independent of lipid measures and other conventional CV risk factors were associated with an increased risk of MACE, CV mortality, and all-cause mortality. In a most parsimonious model, MCSF-1, IL-8, and IL-1α each had significant prognostic meaning. Future clinical trials may investigate the efficacy of medications developed to target these specific inflammatory markers in patients with coronary artery disease.

**Contributorship statement**

JJ and HG contributed to the conception or design of the work. RM and JJ contributed to the acquisition, analysis, or interpretation of data for the work. RM and JJ drafted the manuscript. CMP, RK and HG critically revised the manuscript. All gave final approval and agree to be accountable for all aspects of work ensuring integrity and accuracy.

**Author disclosures**

Dr. van Kimmenade has received research grants from Novartis. Dr. Gaggin has received research grants support from Roche Diagnostics, Jana Care, Ortho Clinical, Novartis, Pfizer, Ahlylam, Akcea; consulting income from Amgen, Eko, Merck, Roche Diagnostics, Pfizer; Stock ownership for Eko; Research payments for clinical end-point committees from Radiometer. She has also received research payment for clinical end-point committees from Baim Institute for Clinical Research for Abbott, Siemens, and Beckman Coulter. Dr. Januzzi is supported by the Hutter Family Professorship; is a Trustee of the American College of Cardiology; is a board member of Imbia Pharmaceuticals; has received grant support from Abbott Diagnostics, Applied Therapeutics, Innolife, and Novartis; has received consulting income from Abbott Diagnostics, Boehringer-Ingelheim, Janssen, Novartis, Roche Diagnostics; and participates in clinical end-point committees/data safety monitoring boards for AbbVie, Siemens, Takeda, and Vifor. The rest of the authors have no disclosures.

**Conflict of interest**

None reported.

**Supplementary materials**

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ahj.2022.06.004.

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