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Comparison of interleukin-6 and high-sensitivity C-reactive protein for cardiovascular risk assessment: findings from the MESA study

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Abstract

Background: Inflammation is a risk factor for major adverse cardiovascular events (MACE). Elevated levels of both high-sensitivity C-reactive protein (hsCRP) and interleukin-6 (IL6) have been associated with MACE. However, few studies have compared IL6 to hsCRP for cardiovascular risk assessment.

Aims: Using the MESA (Multi-Ethnic Study of Atherosclerosis) study cohort, we aim to compare IL6 to hsCRP.

Methods: We divided IL6 and hsCRP by their median values and created 4 groups i.e., low-low, high-low, low-high and high-high. The median follow-up was 14 years.

Results: 6614 (97%) participants had complete baseline IL6 and hsCRP data. The correlation between hsCRP and IL6 was modest ($Rho = 0.53$). An IL6 ≥ 1.2 pg/mL (median) was present in 3309 participants, and a hsCRP ≥ 1.9 mg/L (median) was present in 3339 participants. Compared to participants with low IL6 and low hsCRP, those with high IL6 and high hsCRP were older (64 vs. 60 years), more frequently women (63% vs. 45%), and with more cardiovascular co-morbidities. hsCRP outcome associations lost statistical significance when adjusting for IL6: MACE HR (95%CI) 1.06 (0.93-1.20), $P = 0.39$, whereas IL6 associations remained significant after adjusting for hsCRP: HR (95%CI) 1.44 (1.25-1.64), $P < 0.001$. The C-index of Framingham score for was not improved by hsCRP but improved with IL6. Compared to participants with low IL6 and low hsCRP, those with high IL6, regardless of hsCRP, experienced an increased risk of MACE, heart failure and mortality.

Conclusions: In a diverse and asymptomatic population, IL6 showed a stronger association with atherosclerotic, heart failure and fatal outcomes than hsCRP.

Key-words: Interleukin-6; High-sensitivity C-reactive protein; Cardiovascular risk; General population.

Introduction

Inflammation is an established risk factor for major adverse cardiovascular events (MACE). Elevated levels of both C-reactive protein (CRP) and interleukin-6 (IL6) have been consistently associated with MACE independently of traditional risk factors.¹⁻⁵

Randomized controlled trials (RCTs) have demonstrated that anti-inflammatory therapies that reduce both CRP and IL6 improve cardiovascular outcomes.⁶⁻⁹

Classically, identification of chronic low-grade inflammation has been performed using a high-sensitivity CRP (hsCRP) assay, usually with a ≥ 2 mg/L cutoff.^{6,7}

However, contrary to IL6, Mendelian randomization studies do not support a causal role of CRP in cardiovascular events, and currently, there are no therapies specifically targeting CRP.¹⁰⁻¹² On the other hand, the central role of IL6 in the pathogenesis of multiple inflammatory and cardiovascular diseases is strongly supported by abundant experimental, genetic, and clinical evidence.^{4,5} Moreover, IL6 signalling inhibitors have emerged as relevant treatment options of such disorders.^{6,13}

However, contrary to hsCRP, IL6 is not routinely measured for cardiovascular risk stratification, despite the current technology allowing its widespread determination at a relatively low cost.

Using the MESA (Multi-Ethnic Study of Atherosclerosis) study cohort, we aim to compare hsCRP and IL6 for the assessment of cardiovascular risk.

Methods

The MESA study

The Multi-Ethnic Study of Atherosclerosis (MESA) is a National Heart, Lung, and Blood Institute (NHLBI) funded, community-based, multi-centre cohort study that recruited 6814 adults free of clinical cardiovascular disease (CVD) at baseline, from 4 racial/ethnic groups (Non-Hispanic Whites, African Americans, Hispanics, and Chinese Americans) living in the US.¹⁴

Participants were recruited between 2000 and 2002 from 6 urban areas (New York, Baltimore, Chicago, Los Angeles, Twin Cities and Winston-Salem), and since then have been followed for events at least yearly for up to 15 years. Details of the study methods have been previously published,¹⁴ and further information can be found on the study website (<https://www.mesa-nhlbi.org>) and also on NHLBI/BioLINCC (<https://biolincc.nhlbi.nih.gov/studies/mesa/>).

Participants with baseline (MESA Visit 1) missing values of IL6 or hsCRP were excluded from the present analysis.

Research ethics

The MESA was approved by the institutional ethics review committees of each of the 6 study sites and conforms to the ethical guidelines of the Declaration of Helsinki. In addition, the protocol of the present analysis was approved by the local ethics in research committee at the Faculty of Medicine of the University of Porto, Portugal. All participants provided written informed consent before study entry.

IL6 and hsCRP measurements

IL6 was measured in MESA Visit 1 from blood samples, using an ultrasensitive ELISA (Quantikine HS Human IL6 Immunoassay; R&D Systems, Minneapolis, MN, USA) and quantified in pg/mL. hsCRP was measured by a BNII nephelometer using a particle enhanced immunonephelometric assay (N High Sensitivity CRP, Dade Behring Inc).

Event ascertainment

Full details on the event ascertainment methods used in MESA are available online (<https://www.mesa-nhlbi.org>). All events were adjudicated by a committee of experts. In the present analysis we analysed: 1) the composite of time-to-first of myocardial infarction (MI), ischemic stroke, or cardiovascular death (3P-MACE) as primary outcome; 2) the composite of incident heart failure (HF, defined by the simultaneous presence of symptoms suggestive of HF, a physician diagnosis of HF, and use of medical treatments for HF) or cardiovascular death; 3) cardiovascular death alone; and 4) all-cause mortality.

The median (percentile₂₅₋₇₅) follow-up time for 3P-MACE was 13.8 (9.2-14.6) years and for mortality was 14.1 (13.5-14.7) years.

Statistical analysis

We divided IL6 and hsCRP by their median values. We then created 4 mutually exclusive groups based on the median IL6 and hsCRP values: 1) low IL6 and low hsCRP (i.e., IL6 <1.2 pg/mL and hsCRP <1.9 mg/dL), 2) high IL6 and low hsCRP (i.e., IL6 ≥1.2 pg/mL and hsCRP <1.9 mg/dL), 3) low IL6 and high hsCRP (i.e., IL6 <1.2 pg/mL and hsCRP ≥1.9 mg/L), and 4) high IL6 and high hsCRP (i.e., IL6 ≥1.2 pg/mL and hsCRP ≥1.9 mg/dL). Then we described the characteristics of MESA participants across these categories. We performed outcome associations based on the median values of IL6 and hsCRP, and also using continuous Log2 transformed values while adjusting for the Framingham cardiovascular risk score¹⁵. We studied the association of IL6 and hsCRP with aortic valve calcium (Agatston units). Due to the possible influence of statin use on IL6 levels,¹⁶ sensitivity analyses were performed for statins by testing interactions between the association of IL6 and hsCRP with 3P-MACE and statin use. Additionally, we performed similar analyses to those above described using 4 mutually exclusive groups based on “data-driven” cutoffs from the outcome association analysis across quintiles of IL6 and hsCRP: 1) low IL6 and low hsCRP (i.e., IL6 <0.7 pg/mL and hsCRP <2 mg/dL), 2) high IL6 and low hsCRP (i.e., IL6 ≥0.7 pg/mL and hsCRP <2 mg/dL), 3) low IL6 and high hsCRP (i.e., IL6 <0.7 pg/mL and hsCRP ≥2 mg/L), and 4) high IL6 and high hsCRP (i.e., IL6 ≥0.7 pg/mL and hsCRP ≥2 mg/dL). These “data-driven” cutoffs were chosen based on the association of hsCRP and IL6 quintiles with the study primary outcome of 3P-MACE. For hsCRP the association with the primary outcome became statistically significant in quintile 3; thus, we used the cutoff of 2 mg/dL which is both close to the median value of quintile 3 and is also a commonly used cutoff for entry selection in clinical trials targeting inflammation. For IL6 we used the cutoff of 0.7 pg/mL because it is the maximum value of quintile 1 and the associations became statistically significant above quintile 1 (i.e., in quintile 2). **Supplementary Table 1.** C-indexes were determined and compared using the method described by Harrell and colleagues.¹⁷ Comparison of net reclassification index (NRI) was performed for the primary outcome by comparing the “base” model including the Framingham cardiovascular risk score and an “enhanced” model adding IL6 or hsCRP. Analyses were performed using Stata® (StataCorp. 2023. Stata Statistical Software: Release 18. College Station, TX: StataCorp LLC). P-values <0.05 were considered statistically significant.

Results

Participant's characteristics

Of the 6814 MESA participants, a total of 6614 (97%) participants who had complete baseline IL6 and hsCRP data were included in the analysis. The median (percentile₂₅₋₇₅) IL6 levels were 1.21 (0.78-1.89) pg/mL and the median hsCRP levels were 1.91 (0.84-4.20) mg/L. An IL6 ≥ 1.2 pg/mL was present in 3309 participants, whereas 3339 participants had an hsCRP ≥ 1.9 mg/L.

The correlation between hsCRP and IL6 was modest (Spearman Rho =0.53).

The 4 mutually exclusive groups based on the median IL6 and hsCRP values included: 1) 2331 participants with low IL6 and low hsCRP, 2) 1008 participants with high IL6 and low hsCRP, 3) 978 participants with low IL6 and high hsCRP, and 4) 2297 participants with high IL6 and high hsCRP. Compared to participants with low IL6 and low hsCRP, those with high IL6 and high hsCRP were older (64 vs. 60 years), more frequently women (63% vs. 45%), African American and Hispanic (34% vs. 21% and 27% vs 17%, respectively), with higher Framingham cardiovascular risk score (13.7 vs. 10.0 points), more cardiovascular co-morbidities such as hypertension, diabetes *mellitus*, obesity, and dyslipidemia, higher heart rate (64 vs. 60 bpm), lower eGFR (76 vs. 80 ml/min/1.73m²), and higher aortic calcium score (6% vs. 3% with ≥ 100 Agatston units). Participants with high IL6 and low hsCRP were remarkably similar to those with high IL6 and high hsCRP, whereas participants with low IL6 and high hsCRP resembled more like participants with low IL6 and low hsCRP. **Table 1.**

The 4 groups based on “data-driven” cutoffs from the quintile outcome associations included: 1) 1109 participants with low IL6 and low hsCRP, 2) 2331 participants with high IL6 and low hsCRP, 3) 213 participants with low IL6 and high hsCRP, and 4) 2961 participants with high IL6 and high hsCRP.

Outcome associations of hsCRP and IL6

During follow-up 1118 3P-MACE events occurred, 616 (18.8%) of 3275 participants with hsCRP ≥ 1.9 mg/L experienced 3P-MACE events (event rate =1.7 per 100py) vs. 502 (15.0%) of 3339 with hsCRP < 1.9 mg/dL (event rate =1.3 per 100py); the corresponding Framingham risk score adjusted HR (95%CI) for participants with

hsCRP ≥ 1.9 mg/L vs. hsCRP < 1.9 mg/L was 1.26 (1.12-1.42), $P < 0.001$, and the Framingham risk score adjusted HR (95%CI) per doubling in hsCRP concentration was 1.08 (1.04-1.12), $P < 0.001$. The associations lost statistical significance when further adjusting for IL6: hsCRP ≥ 1.9 mg/L vs. hsCRP < 1.9 mg/L HR (95%CI) 1.06 (0.93-1.20), $P = 0.39$, and per doubling in hsCRP concentration HR (95%CI) 1.01 (0.97-1.05), $P = 0.61$. **Table 2.**

For IL6, 697 (21.1%) of 3305 participants with IL6 ≥ 1.2 pg/mL experienced 3P-MACE events (event rate = 1.7 per 100py) vs. 421 (12.7%) of 3309 with IL6 < 1.2 pg/mL (event rate = 0.8 per 100py); the corresponding Framingham risk score adjusted HR (95%CI) for participants with IL6 ≥ 1.2 pg/mL vs. IL6 < 1.2 pg/mL was 1.50 (1.33-1.70), $P < 0.001$, and the Framingham risk score adjusted HR (95%CI) per doubling in IL6 concentration was 1.27 (1.20-1.35), $P < 0.001$. The associations remained statistically significant after further adjustment for hsCRP: IL6 ≥ 1.2 pg/mL vs. IL6 < 1.2 pg/mL HR (95%CI) 1.44 (1.25-1.64), $P < 0.001$, and per doubling in IL6 concentration HR (95%CI) 1.26 (1.17-1.36), $P < 0.001$. **Table 2.**

Similar findings were observed for the composite of cardiovascular death or incident HF and cardiovascular death alone where hsCRP associations lost statistical significance after IL6 adjustment and IL6 remained significant despite hsCRP adjustment. Interestingly, higher hsCRP was associated with a lower risk of all-cause mortality when adjusting for IL6, whereas IL6 remained associated with a higher risk of all-cause mortality when adjusting for hsCRP. **Table 2.** No significant statistical interactions were found between hsCRP and IL6 for any of the studied outcomes: interaction P values for 3P-MACE = 0.55, cardiovascular death or HF hospitalization = 0.36, cardiovascular death = 0.27, and all-cause mortality = 0.29. **Table 2 (legend).**

The C-index of Framingham cardiovascular risk score for 3P-MACE was not improved by adding hsCRP but improved with the addition of IL6: Framingham risk score C-index (95%CI) = 0.714 (0.699-0.727); Framingham risk score + hsCRP C-index (95%CI) = 0.716 (0.701-0.730), Δ C-index Framingham risk score vs. Framingham risk score + hsCRP P-value = 0.23; and Framingham risk score + IL6 C-index (95%CI) = 0.721 (0.707-0.734), Δ C-index Framingham risk score vs. Framingham risk score + IL6 P-value = 0.006. Adding hsCRP to Framingham risk score + IL6 did not change C-index = 0.721 (0.707-0.734) i.e., same result as

Framingham risk score + IL6; however, adding IL6 to Framingham risk score + hsCRP improved C-index from 0.716 (0.701-0.730) to 0.721 (0.707-0.734), Δ C-index P-value =0.007.

Dividing hsCRP and IL6 in similarly distributed quintiles of participants, we observed that a low IL6 provided a more correct classification of participants with a true low risk of 3P-MACE compared to low hsCRP, and a high IL6 provided a more correct classification of participants with a true high risk of 3P-MACE compared to high hsCRP. **Figure 1.** IL6 provided greater NRI of the model including the Framingham risk score than hsCRP (IL6 continuous NRI =0.16, 95%CI 0.10 to 0.24 and hsCRP continuous NRI =0.11, 95%CI 0.04 to 0.18; Δ NRI +5% in favour of IL6, P <0.001).

Overall results remained similar when using the “data-driven” IL6 cutoff of 0.7 pg/mL and the hsCRP cutoff of 2 mg/L. **Supplementary Tables 2, 3, 4** and **Supplementary Figure 1.** The associations between IL6 and hsCRP levels and 3P-MACE did not vary among statin users (n =979) and nonusers (n =5632): IL6-by-statin interaction P =0.82 and hsCRP-by-statin interaction P =0.48. **Supplementary Table 1 (legend).**

Compared to participants with low IL6 and low hsCRP (i.e., both below median), those with high IL6 (\geq 1.2 pg/mL) regardless of low or high hsCRP experienced an increased risk of 3P-MACE, cardiovascular death or incident HF, and all-cause death, whereas the risk of outcome events for participants with low IL6 and high hsCRP was generally not statistically different from the risk observed among participants with low IL6 and low hsCRP. **Table 3** and **Graphical Abstract.**

Regarding the associations of hsCRP and IL6 with aortic valve calcium, high hsCRP was not associated with increased aortic valve calcium (Agatston units) whereas high IL6 was. **Table 4** and **Supplementary Table 5.**

Discussion

This study, enrolling participants from diverse ethnic backgrounds free of clinical CVD at baseline, showed that IL6 was associated with atherosclerotic, HF and fatal outcomes independently of the Framingham risk score and hsCRP, whereas hsCRP was not, particularly after adjustment for IL6. Moreover, IL6 showed a strong

association with outcomes independently of hsCRP, while hsCRP associations lost statistical significance after IL6 adjustment. In fact, elevated IL6 drove outcome associations irrespective of hsCRP concentrations. It is also important to highlight that the correlation between IL6 and hsCRP was modest (around 0.5), suggesting that the information provided by these two biomarkers is substantially different, and many participants who experienced adverse events would be excluded from risk assessment if solely high hsCRP was used for risk stratification. Additionally, compared to hsCRP, a low IL6 better classified patients as having true low risk and a high IL6 better classified patients as having true high risk. These findings expand previous results from our group demonstrating that IL6 was associated with adverse cardiovascular outcomes independently of hsCRP, and had a stronger association with adverse outcomes than hsCRP in patients hospitalized with acute HF.¹⁸

It is well-established that elevated hsCRP predicts cardiovascular events, particularly in populations with a relatively high cardiovascular risk,^{19,20} and risk scores containing hsCRP are now commonly used in routine practice.^{21,22} Beyond its role as a prognostic marker, a hsCRP ≥ 2 mg/L has also been proposed to select patients who might benefit from statin therapy even when their LDL cholesterol levels are not increased.²³⁻²⁷

Like hsCRP, the association of IL6 with adverse cardiovascular outcomes is now well-established, including in populations with a low cardiovascular risk as in the MESA study.^{1,28} Circulating IL6 is mainly produced by monocytes/macrophages but can also be expressed by several cells types in the cardiovascular system, some of which (e.g., endothelial cells) also express the IL6 receptor. This contrasts with CRP, which is mainly produced in the liver in response to IL6 and IL-1 β and does not integrate the causal pathway leading to cardiovascular events.^{4,5} Moreover, experimental studies have demonstrated that IL6 plays a critical role in microvascular dysfunction, vascular remodelling, atherosclerotic plaque development and myocardial ischemia.²⁹⁻³¹ This evidence may explain IL6 superior sensitivity in detecting low-grade cardiovascular inflammation, thus supporting its preferential association with cardiovascular outcomes, compared to hsCRP. Additionally, it highlights the potential of direct IL6 inhibition as a mean to improve cardiovascular outcomes.³²

A strategy targeting a specific inflammatory pathway to reduce cardiovascular risk was tested in the 10,061-participant CANTOS (Canakinumab Anti-Inflammatory Thrombosis Outcomes Study) trial, demonstrating that IL1 β inhibition with canakinumab reduced MACE despite no effect on cholesterol, blood pressure, or coagulation.⁷ Importantly, the magnitude of risk reduction with canakinumab vs. placebo was dependent on the magnitude of IL6 reduction achieved after the first dose. Specifically, canakinumab-treated participants who achieved on-treatment IL6 levels below the study median value of 1.65 pg/mL experienced a 32% relative reduction in MACE, a 52% relative reduction in cardiovascular mortality, and a 48% relative reduction in all-cause mortality. On the other hand, those with on-treatment IL6 levels \geq 1.65 pg/mL after taking the first dose of canakinumab did not experience significant benefit for any of these outcomes.³³ These findings suggest that lowering IL6 may be an important target for reducing cardiovascular events.³⁴ This hypothesis is currently being tested in the ZEUS (Ziltivekimab Cardiovascular Outcomes Study; NCT05021835) trial comparing the IL6 inhibitor ziltivekimab vs. placebo among patients with chronic kidney disease and elevated CRP (a population with a high inflammatory and atherothrombotic risk).³⁵ It is worth highlighting that the median on-treatment IL6 value of 1.65 pg/mL in CANTOS was higher than the median IL6 of 1.21 pg/mL observed in MESA, which can be explained by the higher cardiovascular risk of patients enrolled in CANTOS who must have had an MI plus elevated hsCRP to enter the trial. Despite these differences in IL6 levels, in the placebo-group of CANTOS the association of IL6 with cardiovascular outcomes was also linear, supporting an association with increased risk of events even with small elevations in IL6 levels.³³

A direct comparison of IL6 with hsCRP was performed in the Cardiovascular Inflammation Reduction Trial (CIRT), a placebo-controlled RCT for the evaluation of low-dose methotrexate in the prevention of recurrent cardiovascular events, enrolling patients with a prior MI or multivessel coronary disease who additionally had diabetes or metabolic syndrome.³⁶ Over a median follow-up of 5 years, IL6 was a stronger predictor of all-cause mortality than hsCRP.⁹ A finding that replicates our observations in a population with a higher cardiovascular risk.

In our study, IL6 was associated with aortic valve calcification and hsCRP was not. Previous studies have reported positive associations between IL6 and coronary

artery calcium, and increased visit-to-visit blood pressure variability.³⁷⁻³⁹

Notwithstanding, to the best of our knowledge, our study is the first to report a positive association between IL6 and aortic valve calcification, suggesting that IL6 pathway may be involved in the progression of aortic valve calcification.⁴⁰

Limitations

Our study has some limitations. This is an observational study and no causality between IL6 elevations and cardiovascular outcomes can be assumed from the present work; still, prior Mendelian randomization studies support a causal association between IL6 and MACE.^{4,5} Many other studies reported strong associations between IL6 and adverse cardiovascular outcomes. However, the finding that elevated IL6 levels have prognostic significance in patients with low hsCRP is original. In fact, IL6 has been strongly associated with cardiovascular outcomes irrespective of hsCRP levels. Given the exploratory nature of our study we did not perform adjustment for test multiplicity; still, the consistency of the associations across different outcomes in this and other studies supports the robustness of the data. This is a population free of clinical CVD at baseline, and these findings may not be applicable to populations with symptomatic CVD. hsCRP assays are widely available and are generally less expensive than IL6 ones; therefore, hsCRP assessment is still easier to perform; however, technological advances are allowing reliable IL6 determination at a relatively low cost. These data require further replication and confirmation across different populations.

Conclusion

In a population free of clinical CVD at baseline, IL6 showed a stronger association with atherosclerotic, heart failure and fatal outcomes, as well as aortic valve calcification, compared to hsCRP.

Disclosures

JPF is a consultant for Boehringer Ingelheim and AstraZeneca. He has received research support from Novartis, Boehringer Ingelheim, AstraZeneca and Bayer through his institution. FVN has received consulting or speaker fees from Bayer, Daiichi Sankyo and Ultragenyx. JSN has received consulting or speaker fees from AstraZeneca, BIAL, Boehringer Ingelheim, Lilly, Merck, and Novo Nordisk. All other authors report not having relevant conflicts of interest to disclose.

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Data access

The access to the MESA (Multi-Ethnic Study of Atherosclerosis) database was provided by the NHLBI/BioLINCC with ethical approval from the Faculty of Medicine of the University of Porto.

Consent for publication

All authors approved the manuscript and consent its publication.

Availability of data and materials

The MESA (Multi-Ethnic Study of Atherosclerosis) database can be fully available from NHLBI/BioLINCC upon reasonable request.

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Table 1. Participants characteristics by IL6 and hsCRP categories based on median values

Variable/Categories	Low IL6 Low hsCRP	High IL6 Low hsCRP	Low IL6 High hsCRP	High IL6 High hsCRP	P- value
N.	2331	1008	978	2297	
Age, years	60 (52, 68)	66 (56, 74)	59 (53, 67)	64 (55, 71)	<0.001
Male sex	1290 (55.3%)	582 (57.7%)	385 (39.4%)	856 (37.3%)	<0.001
Race					<0.001
Caucasian	972 (41.7%)	386 (38.3%)	414 (42.3%)	793 (34.5%)	
Chinese	491 (21.1%)	122 (12.1%)	72 (7.4%)	108 (4.7%)	
African American	483 (20.7%)	259 (25.7%)	288 (29.4%)	775 (33.7%)	
Hispanic	385 (16.5%)	241 (23.9%)	204 (20.9%)	621 (27.0%)	
Framingham score, points	10.0 (5.3, 18.4)	15.6 (7.3, 29.4)	10.0 (5.3, 18.4)	13.7 (7.3, 25.3)	<0.001
Hypertension	776 (33.3%)	494 (49.0%)	435 (44.5%)	1247 (54.3%)	<0.001
Diabetes <i>mellitus</i>	199 (8.5%)	143 (14.2%)	97 (9.9%)	393 (17.1%)	<0.001
Smoking					
No	1285 (55.3%)	487 (48.5%)	492 (50.4%)	1065 (46.6%)	<0.001
Past	814 (35.0%)	398 (39.6%)	350 (35.8%)	854 (37.4%)	
Current	226 (9.7%)	119 (11.9%)	135 (13.8%)	367 (16.1%)	
Heart rate, bpm	60 (55, 66)	62 (55, 69)	63 (57, 69)	64 (58, 71)	<0.001
SBP, mmHg	128 (117, 141)	133 (121, 149)	131 (120, 144)	136 (125, 149)	<0.001
DBP, mmHg	74 (68, 81)	76 (69, 82)	75 (68, 82)	75 (69, 82)	<0.001
Waist circumference, cm	92 (84, 99)	97 (89, 105)	97 (88, 105)	104 (95, 114)	<0.001
BMI, kg/m ²	25.5 (23.1, 28.2)	27.1 (24.4, 30.2)	27.8 (25.1, 30.8)	30.4 (27.0, 34.7)	<0.001
Triglycerides, mg/dL	101 (72, 147)	108 (75, 155)	122 (85, 173)	119 (85, 173)	<0.001
Total cholesterol, mg/dL	192 (171, 214)	185 (166, 208)	201 (178, 223)	192 (169, 216)	<0.001
LDL cholesterol, mg/dL	116 (97, 136)	112 (93, 130)	120 (99, 140)	116 (95, 137)	<0.001
HDL cholesterol, mg/dL	50 (42, 60)	47 (40, 59)	50 (41, 62)	46 (39, 57)	<0.001
eGFR, ml/min/1.73m ²	79.9 (69.3, 89.2)	76.6 (64.2, 87.3)	78.6 (68.6, 90.5)	76.2 (65.1, 88.4)	<0.001
Lp(a), mg/dL	15.7 (7.4, 36.8)	15.6 (6.4, 39.6)	19.6 (8.0, 43.8)	18.4 (7.7, 41.5)	<0.001
hsCRP, mg/L	0.8 (0.4, 1.2)	1.0 (0.7, 1.4)	3.2 (2.4, 4.6)	4.9 (3.2, 8.9)	<0.001
IL6, pg/mL	0.7 (0.5, 0.9)	1.7 (1.4, 2.2)	0.9 (0.7, 1.1)	2.0 (1.6, 2.9)	<0.001
Ao valve calcium (Agatston)					<0.001
0	2092 (89.7%)	818 (81.2%)	881 (90.1%)	1942 (84.6%)	
1-99	170 (7.3%)	107 (10.6%)	72 (7.4%)	218 (9.5%)	
≥100	69 (3.0%)	83 (8.2%)	25 (2.6%)	135 (5.9%)	
ACEi/ARB	298 (12.8%)	203 (20.1%)	143 (14.6%)	543 (23.6%)	<0.001
Beta-blocker	190 (8.2%)	107 (10.6%)	85 (8.7%)	249 (10.8%)	0.008
Statins	348 (14.9%)	179 (17.8%)	140 (14.3%)	312 (13.6%)	0.020

Legend: SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; eGFR, estimated glomerular filtration rate; Lp, lipoprotein; hsCRP, high-sensitivity C-reactive protein; IL6, interleukin-6; Ao, aortic; ACEi/ARB, angiotensin converting enzyme inhibitor/angiotensin receptor blocker.

Low biomarker: CRP <1.9 mg/L (below median) or IL6 <1.2 pg/mL (below median).

High biomarker: CRP ≥1.9 mg/L (above median) or IL6 ≥1.2 pg/mL (above median).

Table 2. Outcome associations of hsCRP and IL6 based on median values

Outcome/ Biomarker	Low biomarker Events n/N (%) IR per 100py	High biomarker Events n/N (%) IR per 100py	High vs. Low HR (95%CI) ¹ P-value	High vs. Low HR (95%CI) ² P-value	Log2 HR (95%CI) ¹ P-value	Log2 HR (95%CI) ² P-value
High-sensitivity C-reactive protein (hsCRP)						
3P-MACE	502/3339 (15.0) 1.3 (1.2-1.4)	616/3275 (18.8) 1.7 (1.5-1.8)	1.26 (1.12-1.42) P <0.001	1.06 (0.93-1.20) P =0.39	1.08 (1.04-1.12) P <0.001	1.01 (0.97-1.05) P =0.61
CVD/HF	237/3339 (7.1) 0.6 (0.5-0.6)	301/3275 (9.2) 0.8 (0.7-0.9)	1.26 (1.06-1.49) P =0.008	0.99 (0.82-1.19) P =0.93	1.10 (1.04-1.16) P =0.001	1.0 (0.94-1.06) P =0.92
CVD	130/3339 (3.9) 0.3 (0.2-0.4)	165/3275 (5.0) 0.4 (0.3-0.5)	1.25 (0.99-1.58) P =0.055	0.95 (0.74-1.22) P =0.68	1.07 (1.00-1.15) P =0.056	0.95 (0.88-1.03) P =0.25
ACM	581/3330 (17.5) 1.3 (1.2-1.4)	662/3263 (20.3) 1.6 (1.5-1.7)	1.14 (1.02-1.27) P =0.021	0.85 (0.75-0.96) P =0.008	1.04 (1.01-1.08) P =0.014	0.92 (0.89-0.96) P <0.001
Interleukin-6 (IL6)						
3P-MACE	421/3309 (12.7) 1.0 (0.9-1.1)	697/3305 (21.1) 1.9 (1.8-2.1)	1.50 (1.33-1.70) P <0.001	1.44 (1.25-1.64) P <0.001	1.27 (1.20-1.35) P <0.001	1.26 (1.17-1.36) P <0.001
CVD/HF	176/3309 (5.3) 0.4 (0.4-0.5)	362/3305 (11.0) 0.9 (0.9-1.0)	1.74 (1.45-2.08) P <0.001	1.68 (1.37-2.05) P <0.001	1.37 (1.25-1.50) P <0.001	1.38 (1.24-1.53) P <0.001
CVD	91/3309 (2.8) 0.2 (0.1-0.2)	204/3305 (6.2) 0.5 (0.4-0.6)	1.85 (1.44-2.37) P <0.001	1.88 (1.43-2.47) P <0.001	1.43 (1.27-1.62) P <0.001	1.49 (1.30-1.72) P <0.001
ACM	405/3303 (12.3) 0.9 (0.8-1.0)	838/3290 (25.5) 2.0 (1.9-2.2)	1.84 (1.63-2.08) P <0.001	1.96 (1.72-2.24) P <0.001	1.42 (1.34-1.51) P <0.001	1.52 (1.43-1.63) P <0.001

Legend: hsCRP, high sensitivity C-reactive protein; IL6, interleukin-6; HR, hazard ratio; CI, confidence interval; 3P-MACE, composite of cardiovascular death, stroke, or myocardial infarction; CVD, cardiovascular death; HF, heart failure; ACM, all-cause mortality.

Low biomarker: CRP <1.9 mg/L (below median) or IL6 <1.2 pg/mL (below median). High biomarker: CRP ≥1.9 mg/L (above median) or IL6 ≥1.2 pg/mL (above median).

¹ Model adjusted Framingham risk score.

² Model adjusted Framingham risk score plus IL6 if hsCRP was the independent variable or mode adjusted Framingham risk score plus hsCRP if IL6 was the independent variable.

Interaction P-values hsCRP-by-IL6 (continuous): 3P-MACE =0.55; CVD/HF =0.36; CVD =0.27; ACM =0.29.

Table 3. Outcome associations of hsCRP and IL6 category combinations based on median values

Outcome/ Biomarker combinations	Low IL6-Low hsCRP Events n/N (%) IR per 100py	High IL6-Low hsCRP Events n/N (%) IR per 100py	Low IL6-High hsCRP Events n/N (%) IR per 100py	High IL6-High hsCRP Events n/N (%) IR per 100py	High IL6-Low hsCRP vs. Low- Low HR (95%CI) ¹ P-value	Low IL6-High hsCRP vs. Low- Low HR (95%CI) ¹ P-value	High IL6-High hsCRP vs. Low- Low HR (95%CI) ¹ P-value
3P-MACE	278/2331 (11.9) 0.8 (0.6-0.9)	224/1008 (22.2) 1.6 (1.4-1.7)	143/978 (14.6) 0.9 (0.6-1.3)	473/2297 (20.6) 1.7 (1.6-1.9)	1.62 (1.35-1.93) P <0.001	1.27 (1.03-1.55) P =0.022	1.62 (1.40-1.88) P <0.001
CVD/HF	115/2331 (4.9) 0.3 (0.2-0.4)	122/1008 (12.1) 0.7 (0.6-0.8)	61/978 (6.2) 0.2 (0.1-0.5)	240/2297 (10.5) 0.8 (0.7-0.9)	2.00 (1.54-2.58) P <0.001	1.31 (0.96-1.78) P =0.090	1.84 (1.47-2.30) P <0.001
CVD	56/2331 (2.4) 0.2 (0.1-0.2)	74/1008 (7.3) 0.4 (0.3-0.4)	35/978 (3.6) 0.2 (0.1-0.4)	130/2297 (5.7) 0.4 (0.4-0.5)	2.41 (1.70-3.43) P <0.001	1.54 (1.01-2.34) P =0.056	2.01 (1.46-2.75) P <0.001
ACM	286/2328 (12.3) 0.7 (0.6-0.9)	295/1002 (29.4) 1.6 (1.5-1.8)	119/975 (12.2) 0.6 (0.4-0.9)	543/2288 (23.7) 1.7 (1.5-1.9)	2.06 (1.75-2.42) P <0.001	1.01 (0.82-1.26) P =0.89	1.75 (1.52-2.02) P <0.001

Legend: hsCRP, high sensitivity C-reactive protein; IL6, interleukin-6; HR, hazard ratio; CI, confidence interval; 3P-MACE, composite of cardiovascular death, stroke, or myocardial infarction; CVD, cardiovascular death; HF, heart failure; ACM, all-cause mortality.

Low biomarker: CRP <1.9 mg/L (below median) or IL6 <1.2 pg/mL (below median).

High biomarker: CRP ≥1.9 mg/L (above median) or IL6 ≥1.2 pg/mL (above median).

The “Low-Low” is the referent group.

¹ Model adjusted Framingham risk score.

Table 4. Associations of hsCRP and IL6 (medium values) with aortic valve calcium

Ao valve calcium*/ Biomarker	Low biomarker Events n/N (%)	High biomarker Events n/N (%)	High vs. Low OR (95%CI)¹ P-value
High-sensitivity C-reactive protein (hsCRP)			
0	2910/3339 (87.2)	2823/3273 (86.3)	Referent
1-99	277/3339 (8.3)	290/3273 (8.9)	1.01 (0.84-1.20) P =0.93
≥100	152/3339 (4.6)	160/3273 (4.9)	0.99 (0.79-1.25) P =0.95
Interleukin-6 (IL6)			
0	2973/3309 (89.9)	2760/3303 (83.6)	Referent
1-99	242/3309 (7.3)	325/3303 (9.8)	1.13 (0.94-1.35) P =0.19
≥100	94/3309 (2.8)	218/3303 (6.6)	1.81 (1.40-2.35) P <0.001

Legend: hsCRP, high sensitivity C-reactive protein; IL6, interleukin-6; OR, odds ratio (multinomial logistic regression); CI, confidence interval; Ao, aortic.

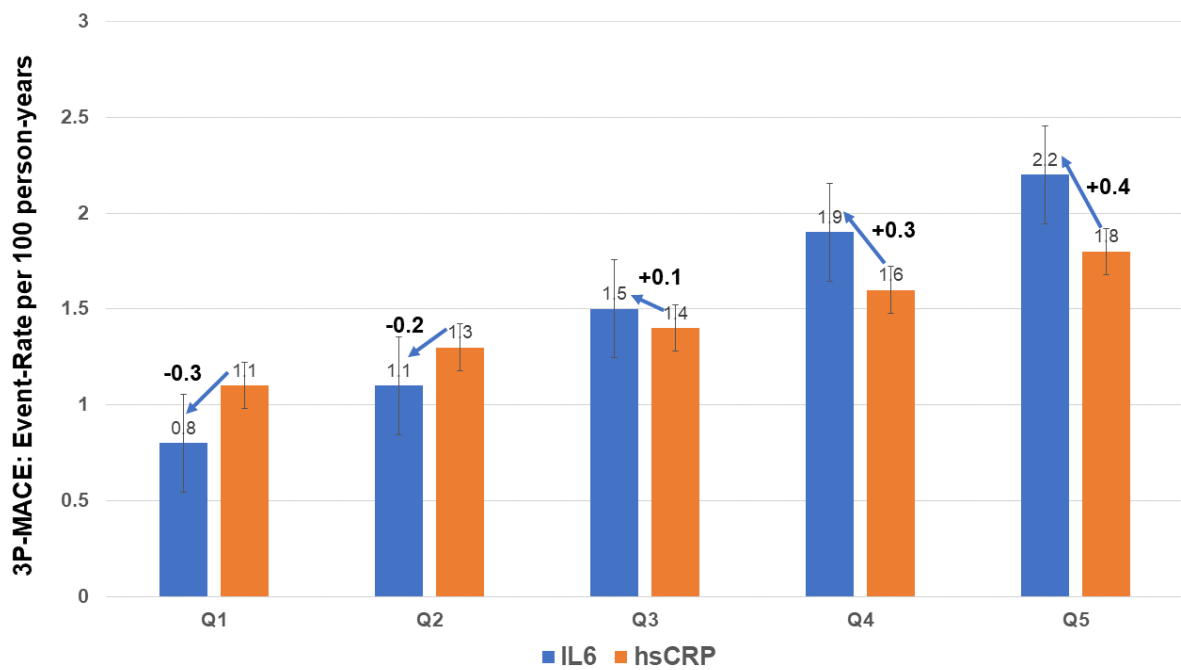
*Agatston units

Low biomarker: CRP <1.9 mg/L (below median) or IL6 <1.2 pg/mL (below median).

High biomarker: CRP ≥1.9 mg/L (above median) or IL6 ≥1.2 pg/mL (above median).

¹ Model adjusted Framingham risk score.

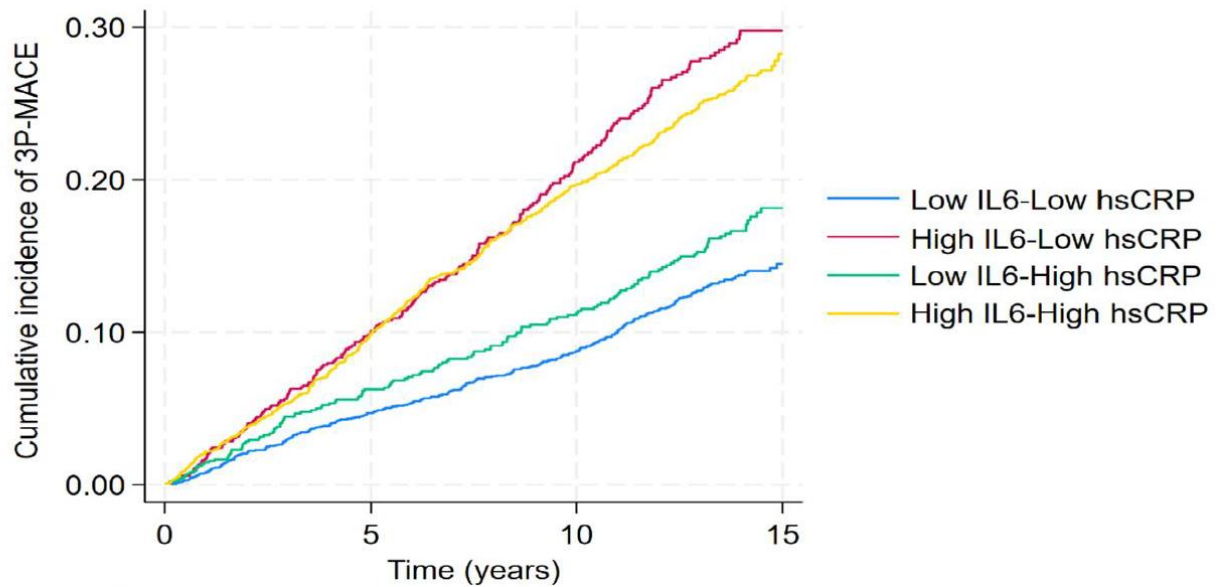
Figure 1. Event-rates across quintiles of IL6 and hsCRP



Legend: hsCRP, high sensitivity C-reactive protein; IL6, interleukin-6; 3P-MACE, composite of cardiovascular death, stroke, or myocardial infarction; Q, quintile.

Graphical Abstract. 3P-MACE association of hsCRP and IL6 category combinations

Graphical Abstract: Patients with high IL6 experience higher risk of cardiovascular events, irrespective of hsCRP levels



	Number at risk			
Low IL6-Low hsCRP	2328	2104	1885	223
High IL6-Low hsCRP	1002	834	651	55
Low IL6-High hsCRP	975	867	760	101
High IL6-High hsCRP	2288	1880	1511	180

based on median values

Caption: Patients with high IL6 (above median) experience higher risk of cardiovascular events, irrespective of hsCRP levels.

Legend: hsCRP, high sensitivity C-reactive protein; IL6, interleukin-6; 3P-MACE, composite of cardiovascular death, stroke, or myocardial infarction.
 Low biomarker: CRP <1.9 mg/L (below median) or IL6 <1.2 pg/mL (below median).
 High biomarker: CRP ≥1.9 mg/L (above median) or IL6 ≥1.2 pg/mL (above median).