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Short Report

Inflammatory biomarkers relationship with major adverse cardiovascular events and hypertensive-mediated organ damage, in subjects without established cardiovascular disease

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1. Introduction

Increasing evidence indicates that chronic low-grade inflammation plays a central role in the pathophysiology of atherosclerosis and contributes to residual cardiovascular (CV) risk beyond conventional factors [1]. Inflammatory biomarkers have been associated with major adverse CV events (MACE) [2] while, their relationship with hypertension-mediated organ damage (HMOD) were less frequently evaluated [3].

The present study aims to evaluate, in subjects without established

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CV diseases, the association between high-sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL-6), IL-18, osteoprotegerin (OPG), calprotectin and Tumor-Necrosis Factor α (TNF-α) and long-term MACE and subclinical HMOD evaluated cross-sectionally at multiple organ levels (aortic, carotid, cardiac, and renal).

2. Methods

Between September 2009 and February 2014, 1935 subjects aged 18 to 88 years were consecutively included in the study conducted at the Primary Prevention Unit of S. Gerardo Hospital in Monza, Italy. Participants were identified either through the hospital's outpatient clinic for arterial hypertension or among healthy volunteers from the institutional blood donor registry.

Individuals were deemed ineligible if they were younger than 18 years, pregnant, or breastfeeding. Further exclusion criteria comprised any previous major cardiovascular or cerebrovascular event—such as myocardial infarction, angina, heart failure, stroke, transient ischemic attack, or peripheral artery disease with intermittent claudication—as well as any medical condition likely to compromise the accurate evaluation of cardiac or vascular target organ damage (e.g., atrial fibrillation, a high burden of supraventricular or ventricular ectopy, or prior endovascular stenting of the carotid, aortic, or femoral arteries). Subjects with chronic obstructive pulmonary disease, active substance use disorder, or having a diagnosis of malignancy or auto-immune diseases (as well as anti-inflammatory therapies) were also excluded.

Inflammatory biomarkers were available in a subgroup of patient due to serum quantity available for dosages. In particular, hs-CRP and IL-6 measurements were available in 804 patients while 460 of them also had IL-18, OPG, calprotectin and TNF-α. On these patients we conducted the present analysis. Although there were no specific selection criteria for biomarkers evaluation, some significant differences were seen in analysed subgroup (detailed in supplementary material and Supplementary Table 1 and 2).

The study protocol was approved by institutional ethics review committees of the institution involved and all participants provided informed written consent after being informed of its nature and purpose.

Evaluated HMOD were arterial stiffness (pulse wave velocity - PWV), carotid atherosclerosis (plaque and intima media-thickness - IMT), cardiac remodeling (left ventricular mass index – LVMI – and left ventricular hypertrophy - LVH), and renal damage (glomerural filtration rate – GFR and chronic kidney disease - CKD). HMOD were analyzed both as a continuous (that give information across the entire spectrum of values) and as a categorical (defining the presence or the absence of HMOD) variable. In this latter case contemporary HMOD consensus statement was used for threshold definition.

Long-term outcome assessment for all-cause mortality and CV events was conducted through linkage with the administrative healthcare databases of the Milan and Brianza Agencies for Health Protection (ATS). Each participant was prospectively monitored for a median duration of 10.7 years (I-III quartiles 9.7–11.3 years).

CV outcomes were evaluated as a 3-point (CV death, myocardial infarction and stroke) and a 4-point MACE (also with coronary, carotid and peripheral arterial revascularization).

See supplementary material for details regarding laboratory methods, outcomes and HMOD evaluations.

2.1. Statistical analysis

Distribution of continuous variables was summarized using mean and standard deviation (SD, when normally distributed) or median and quartiles (when skewed). Categorical variables were expressed as counts and percentages. T-test (or Mann-Whitney test for skewed variables) was used for continuous variables while Chi-square for categorical ones. Standardized mean differences (SMD) were also reported.

The crude incidence of CV events was estimated using the Aalen-Johansen method considering all-cause death without CV event as a competing risk and time to first events was considered. Univariate and multivariable Cox models for the relationship between MACE and inflammatory biomarkers (one model each) were fitted and adjusted for pre-specified confounders (age, sex, LDL cholesterol, systolic BP, smoking, ACE-inhibitors, angiotensin receptor blockers, statins, diabetes mellitus, BMI, family history of CV events and GFR). Estimates (hazard ratio -HR- and 95% confidence interval -CI) were reported per one SD of the log-transformed biomarker.

The association between inflammatory biomarkers and HMOD binary outcomes was assessed using Mann-Whitney test while Spearman correlation analyses was used for continuous outcomes. Q-values controlling false discovery rate (FDR) using Benjamini-Hocberg method for all analysis of association were also calculated. Multivariable logistic (for categorical variables) or linear (for continuous one) regression was adjusting for previously stated covariates.

All statistical analyses were performed using R, with significance set at a two-tailed p-value < 0.05.

3. Results

The demographic and clinical features of the population were detailed in Supplementary Table 3 and described in supplementary material. Inflammatory biomarkers distributions were right-skewed (Supplementary Figure. 1)

During follow-up 75 CV events, 10 CV deaths and 31 non-CV deaths were observed. Multivariable fully-adjusted models (Fig. 1, panel A) showed a significant association between MACE and IL-6 (3-point MACE HR and 95% CI: 1.486, 1.074;2.057, $p = 0.017$; 4-point MACE: 1.519, 1.086;2.124, $p = 0.015$), hs-CRP (3-point MACE 1.291, 1.016;1.641, $p = 0.037$; 4-point MACE: 1.314, 1.035;1.668, $p = 0.025$) and calprotectin (3-point and 4-point MACE 4.048, 1.058;15.493, $p = 0.041$). Full multivariable models for each inflammatory biomarkers were reports in Supplementary Table 4–9.

These results were also confirmed at Aalen-Johansen crude incidence curves of MACE with inflammatory biomarkers dichotomized according to the median value (Fig. 1, panel B).

Regarding HMOD, at fully adjusted multivariable analysis (Fig. 1, panel C) positive significant associations were found for hs-CRP and calprotectin and carotid plaque, TNF-α and PWV (both continuous and categorical) while a negative association was found between IL-18 and ejection fraction. Further detailed results regarding MACE and HMOD were reported in supplementary materials.

4. Discussion

The main finding of the present study was that, among evaluated biomarkers, IL-6, hs-CRP and calprotectin were significantly and independently associated with subsequent MACE in subjects without established CV diseases.

Our findings are consistent with previous evidence. In fact, IL-6 and hs-CRP are the two inflammatory biomarkers more strongly associated with an increased risk of myocardial infarction, stroke, and CV death [4]. Furthermore, while mostly evaluated for its association with inflammatory bowel disease, serum calprotectin role as a biomarker of residual inflammatory risk is emerging [5].

Regarding the other evaluated biomarkers, previous literature results are more heterogeneous with relationship that vanish or appears attenuated after covariates adjustement [6,7].

Furthermore, we found some significant and consistent associations between inflammatory biomarkers and HMOD. In particular, a positive significant association were confirmed for hs-CRP and calprotectin and carotid plaque and TNF-α and PWV (both continuous and categorical) while a negative association was found between IL-18 and ejection fraction. Our results confirm previously published one and, taken together, these findings suggest a possible role for inflammation in these

Panel A: univariable and multivariable Cox models on the association between inflammatory bio markers and the major adverse cardiovascular events endpoints. Table show one model for each biomarker and each outcome adjusted by age, sex, LDL cholesterol, SBP, smoking, ACE-I, ARBs, statins, diabetes mellitus, BMI, family history of CV events and GFR. Values reported as estimated Hazard Ratio for one standard deviation unit increase of the log-transformed biomarker with 95% confidence intervals.

Inflammatory biomarkers	Composite endpoint: 3-point MACE		Composite endpoint: 4-point MACE	
	Univariable Cox models HR (95%CI) p-value	Multivariable Cox model HR (95%CI) p-value	Univariable Cox models HR (95%CI) p-value	Multivariable Cox model HR (95%CI) p-value
Log(IL-6), per 1 SD increase	1.764 (1.084;2.870) p=0.022	1.486 (1.074;2.057) p=0.017	1.865 (1.035-3.066) p=0.014	1.519 (1.086;2.124) p=0.015
Log(hs-CRP), per 1 SD increase	1.287 (1.023;1.618) p=0.031	1.291 (1.016;1.641) p=0.037	1.269 (1.017;1.584) p=0.035	1.314 (1.035;1.668) p=0.025
Log(IL-18), per 1 SD increase	0.975 (0.718;1.324) p=0.872	0.964 (0.619;1.502) p=0.871	0.975 (0.718;1.324) p=0.872	0.964 (0.619;1.502) p=0.871
Log(Osteoprotegerin), per 1 SD increase	1.794 (1.081;2.976) p=0.024	1.995 (0.913;4.359) p=0.083	1.794 (1.081;2.976) p=0.024	1.995 (0.913;4.359) p=0.083
Log(Calprotectine), per 1 SD increase	3.098 (1.341;7.156) p=0.008	4.048 (1.058;15.493) p=0.041	3.098 (1.341;7.156) p=0.008	4.048 (1.058;15.493) p=0.041
Log(TNF-alpha), per 1 SD increase	0.973 (0.708;1.338) p=0.867	0.996 (0.587;1.691) p=0.99	0.973 (0.708;1.338) p=0.867	0.996 (0.587;1.691) p=0.99

Panel C: Multivariable linear regression models to evaluate the association between each biomarker and HMOD (one model for each biomarker and each outcome) adjusted by age, sex, LDL cholesterol, SBP, smoking, ACE-I, ARBs, statins, diabetes mellitus, BMI, family history of CV events and GFR (not for GFR as outcome). Values reported as estimated mean difference in the outcome for one standard deviation / odds ratio unit increase of the log-transformed biomarker with 95% confidence intervals.

Biomarker	Continuous HMOD				
	IMT	LVMI	LVEF	PWV	GFR
Log(IL-6), per 1 SD increase	0.01 (-0.001;0.021)	-0.414 (-1.604;0.777)	-0.004 (-0.319;0.311)	-0.037 (-0.149;0.076)	0.102 (-0.931;1.135)
Log(hs-CRP), per 1 SD increase	0.013 (0.001;0.025)	-0.461 (-1.637;0.716)	0.053 (-0.274;0.381)	-0.024 (-0.14;0.093)	0.085 (-0.977;1.148)
Log(IL-18), per 1 SD increase	0.004 (-0.013;0.021)	-0.848 (-2.405;0.709)	-0.538 (-0.977;-0.098)	-0.016 (-0.167;0.135)	1.639 (0.275;3.002)
Log(Osteoprotegerin), per 1 SD increase	0.012 (-0.004;0.027)	-0.618 (-2.046;0.81)	-0.172 (-0.584;0.241)	-0.006 (-0.148;0.136)	1.234 (-0.053;2.522)
Log(Calprotectine), per 1 SD increase	-0.003 (-0.02;0.013)	0.366 (-1.085;1.816)	-0.026 (-0.441;0.389)	0.012 (-0.13;0.155)	-0.941 (-2.246;0.364)
Log(TNF-alpha), per 1 SD increase	0.005 (-0.013;0.022)	-0.922 (-2.556;0.713)	0.039 (-0.432;0.509)	-0.159 (-0.312;-0.006)	0.4 (-1.015;1.815)

Biomarker	Categorical HMOD			
	IMT HMOD	Carotid Plaque	PWV HMOD	CKD
Log(IL-6), per 1 SD increase	1.165 (0.874;1.552)	1.097 (0.89;1.352)	0.998 (0.796;1.252)	0.982 (0.671;1.439)
Log(hs-CRP), per 1 SD increase	1.253 (0.906;1.734)	1.279 (1.002;1.632)	1.097 (0.838;1.435)	1.252 (0.755;2.077)
Log(IL-18), per 1 SD increase	1.2 (0.71;2.028)	0.859 (0.594;1.242)	1.017 (0.652;1.585)	1.819 (0.647;5.114)
Log(Osteoprotegerin), per 1 SD increase	1.095 (0.588;2.038)	1.479 (0.9;2.429)	1.147 (0.655;2.009)	0.456 (0.12;1.729)
Log(Calprotectine), per 1 SD increase	0.915 (0.515;1.625)	1.976 (1.016;3.84)	0.886 (0.525;1.495)	0.473 (0.17;1.321)
Log(TNF-alpha), per 1 SD increase	3.792 (0.593;24.248)	0.89 (0.61;1.3)	0.617 (0.405;0.939)	0.235 (0.074;0.75)

List of abbreviations (for all panel): LDL = Low-Density Lipoprotein; SBP = Systolic Blood Pressure; ACE-I = Angiotensin-Converting Enzyme Inhibitors; ARBs = Angiotensin Receptor Blockers; BMI = Body Mass Index; CV = CardioVascular; GFR = Glomerular Filtration Rate; IL = Interleukin; hsCRP = high sensitivity C-Reactive Protein; TNF = Tumor Necrosis Factor; IMT = Intima-Media Thickness; LVMI = Left Ventricular Mass Index; PWV = Pulse Wave Velocity; HMOD = Hypertension Mediated Organ Damage; MACE = Major Adverse Cardiovascular Events; LVEF = Left Ventricular Ejection Fraction; CKD = Chronic Kidney Disease.

Panel B: Aalen-Johansen crude incidence curves of MACE stratified by biomarkers dichotomized according to the median value. On both endpoints, death due to other causes was considered a competing event.

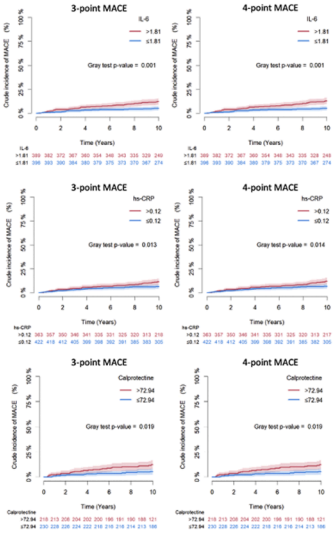

Fig. 1. Panel A: univariate and multivariable models on the association between inflammatory biomarkers and the major adverse cardiovascular events endpoints. Panel B: Aalen-Johansen crude incidence curves of major adverse cardiovascular events. Panel C: multivariable linear regression models to evaluate the association between each inflammatory biomarkes and hypertension mediated organ damage.

HMOD pathogenesis with the needs for further specific future investigations.

Our study presents some elements of strengths being the first one the large sample size of the population evaluated, which guarantees adequate power in identifying the association between inflammatory biomarkers, HMOD and CV events/death. Furthermore, a simultaneous assessment of multiple inflammatory biomarkers together with a comprehensive, multiorgan evaluation of HMOD (vascular, cardiac, and renal) has not been previously investigated in subjects without established CV diseases. Finally, we performed a very extended prospective follow-up.

However, our paper has also several limitations that need to be acknowledged. Firstly, the inflammatory biomarkers assessment was done only at baseline precluding causal inference and temporal variability analysis. The same also applies to HMOD evaluation determining the impossibility to evaluate longitudinal assessment of organ damage progression or regression. Secondly, despite the high total number of subjects in the whole population some subgroup presents such a low number of subjects (i.e. LVH): this sample size can limit our power to detect important associations or findings. Furthermore, IMT measurements were performed manually, lacking the precision of automated echo-tracking systems. Moreover, in the multifactorial context linking inflammation with HMOD and subsequent CV events and mortality, is indeed difficult to assess all parameters that can influence the relationship and some of them have not been evaluated. A very important limitation is that significant baseline differences were observed between participants with and without available inflammatory biomarker measurements (missing not at random is plausible), as detailed in the Methods section. This may have influenced the cross-sectional analyses of HMOD although it is very unlikely that the substantially negative results could be different in the whole population. Furthermore, the lack of differences in CV events and mortality according to biomarker availability mitigates the concern of a major selection bias influencing the prognostic findings.

5. Conclusions

In subjects without established CV diseases, IL-6, hs-CRP and calprotectin were significantly and independently associated with subsequent MACE. Furthermore, some association were found with HMOD suggesting a potential role of inflammation in their pathogenesis. However, unmeasured confounders, measurement limitations, low prevalence of advanced HMOD, and insufficient power may have limited our analyses.

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Compliance with ethical standard and ethical approval

The study protocol complies with the Helsinki Declaration and was approved by the San Gerardo Hospital Ethics Committee and all participants provided informed written consent.

CRediT authorship contribution statement

Chiara Tognola: Conceptualization. **Davide Paolo Bernasconi:** Data curation. **Paola Rebora:** Conceptualization. **Rita Cristina Myriam Intravaia:** Writing – original draft, Conceptualization. **Costantino Mancusi:** Formal analysis. **Valeria Visco:** Funding acquisition. **Arturo Cesaro:** Methodology. **Enrica Golia:** Funding acquisition. **Fucile Ilaria:** Conceptualization. **Piera Merlini:** Conceptualization. **Maddalena Ardissino:** Conceptualization. **Elvira Inglese:** Visualization, Validation. **Romano Danesi:** Methodology. **Fabrizio Oliva:** Formal analysis, Data curation. **Magda Rognoni:** Conceptualization. **Luca Cavalieri d'oro:** Conceptualization. **Antonio Russo:** Software, Project administration, Methodology. **Paolo Calabrò:** Formal analysis, Data curation, Conceptualization. **Nicola De Luca:** Conceptualization. **Cristina Giannattasio:** Writing – review & editing, Writing – original draft,

Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Alessandro Maloberti:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ajpc.2026.101640.

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