Check for updates

Received: 6 February 2024 | Revised: 10 August 2024 | Accepted: 3 September 2024

DOI: 10.1111/cts.70036

ARTICLE

Association between aortic calcification and cytokine levels in patients with peripheral artery disease

Ville Rantasalo[1,2] | **Dan Laukka**[3,4] | **Veikko Nikulainen**[2] | **Juho Jalkanen**[2] | **Jarmo Gunn**[1] | **Tuomas Kiviniemi**[5] | **Harri Hakovirta**[1,6,7]

[1]Department of Surgery, University of Turku, Turku, Finland

[2]Division of Gastroenterology and Urology, Department of Vascular Surgery, Turku University Hospital, Turku, Finland

[3]Department of Clinical Neurosciences, University of Turku, Turku, Finland

[4]Department of Neurosurgery, Neurocenter, Turku University Hospital, Turku, Finland

[5]Turku University Hospital, Heart Center, Turku, Finland

[6]Satasairaala, Pori, Finland

[7]King Faisal Specialist Hospital and Research Center, Medina, Saudi Arabia

Correspondence
Ville Rantasalo, Turku University Hospital, Kiinamyllynkatu 4-8, PO Box 52, Turku 20500, Finland.
Email: vheran@utu.fi

Abstract

Aortic calcification—a marker of advanced atherosclerosis in large arteries—associates with cardiovascular mortality and morbidity. Little is known about the soluble inflamJarmatory profiles involved in large artery atherosclerosis. We investigated the correlation between aortic calcification in the abdominal aorta and cytokine levels in a cohort of peripheral artery disease patients. Aortic calcification index was measured from computed tomography exams and circulating cytokine levels were analyzed from blood serum samples of 156 consecutive patients prior to invasive treatment of peripheral artery disease. The study included 156 patients (mean age 70.7 years, 64 (41.0%) women). The mean ankle-brachial index (ABI) was 0.64 and the mean aortic calcification index (ACI) was 52.3. ACI was associated with cytokines cutaneous T-cell-attracting chemokine CTACK (β 23.08, SE 5.22, $p < 0.001$) and monokine induced by gamma-interferon MIG (β 9.40, SE 2.82, p 0.001) in univariate linear regression. After adjustment with cardiovascular risk factors, CTACK and MIG were independently associated with ACI, β 17.9 (SE 5.22, $p < 0.001$) for CTACK and β 6.80 (SE 3.33, p 0.043) for MIG. CTACK was significantly higher in the patients representing the highest ACI tertile (highest vs. middle, 7.53 vs. 7.34 Tukeys HSD p-value 0.023 and highest vs. lowest tertile 7.53 vs. 7.29, Tukeys HSD p-value 0.002). MIG was significantly higher in the highest tertile versus lowest (7.65 vs. 7.30, Tukeys HSD p-value 0.027). Cytokines CTACK and MIG are associated with higher ACI, suggesting that CTACK and MIG reflect atherosclerotic disease burden of the aorta. This might further suggest the possible association with other cardiovascular morbidities.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Soluble inflammatory profile of atherosclerosis has been investigated, but to our best knowledge, the circulating cytokine profile has not been correlated to the degree of aortic calcification before.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.
© 2024 The Author(s). *Clinical and Translational Science* published by Wiley Periodicals LLC on behalf of American Society for Clinical Pharmacology and Therapeutics.

WHAT QUESTION DID THIS STUDY ADDRESS?

This study investigates serum cytokine levels in patients with peripheral artery disease, in relation to the degree of aortic calcification.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

Cytokines CTACK and MIG are associated with increased aortic calcification.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

Soluble inflammatory profile of aortic calcification might have distinctive features. Cytokines seen in atherosclerosis and aortic calcification might overlap with cytokines detected in other diseases like psoriasis.

INTRODUCTION

Low-grade inflammation and accumulation of lipid-rich calcified plaques in artery walls are the cornerstones of atherosclerotic disease. Clinical problems arise from narrowed arteries or ruptured plaques. Chronic inflammation is characterized by inflammatory cells of both innate and adaptive immunity and several cytokines that direct their expression and function.

The pathophysiology of atherosclerosis includes dysfunction of the endothelial layer, which is characterized by an imbalance in the nitric oxide synthesis, faulty signaling of the endothelial cells, and interrupted endothelial layer. The median layer of the artery is also involved in the pathophysiology of atherosclerosis. In the median layer, vascular smooth muscle cells switch phenotype and become pro-inflammatory and macrophage-like cells that are non-contractile. Endothelial dysfunction allows inflammatory cells to access the median layer of the artery. Macrophages in the median layer accumulate, consume lipids, and become foam cells that form the core of the atherosclerotic plaque.

Inflammatory cells introduced to the median layer acquire pro-inflammatory state. Current understanding is that several genetic and extrinsic factors together, rather than one alone, can trigger the inflammation in artery walls. For example, poor dental health and inflammatory disorders of the skin have been associated with atherosclerosis.[1–6]

Atherosclerosis presents in multiple artery beds. Lesions can be found solitarily in a single artery segment, or it can be a multilevel disease comprising small and large arteries. Even though the disease process and preventive methods are shared, there are subtle differences in the pathophysiology of different vascular beds in atherosclerosis. Different risk factors might contribute unevenly to atherosclerotic changes in different locations, and inflammatory and histological details are differently emphasized in different vascular beds.[7–9] Aortic calcification is a manifestation of atherosclerosis in the aorta. Merely atherosclerotic plaques in the abdominal aorta have little immediate clinical significance as they are often observed in computed tomography studies conducted for other indications, but it has been linked to clinical risks and manifestations of cardiovascular disease. In the big picture, different phenotypes respective to different vascular beds atherosclerosis are not yet definitively established.[10–13] Inflammation that drives atherosclerosis cannot be directly visualized in current clinical practice, and the disease in a certain arterial bed becomes apparent only when an event occurs or a diseased artery bed is detected during imaging or invasive investigation. Therefore, associations between risk factors, inflammatory markers, and diseased arterial beds should be established. Current understanding of atherosclerotic inflammation is mainly based on studies investigating atherosclerosis in carotid and coronary arteries and peripheral artery disease (PAD), but to the best of our knowledge, inflammatory profile and/or phenotype of aortic atherosclerosis is not established yet.

The present study's primary aim is to explore associations between large artery atherosclerosis as measured by aortic calcification and certain circulating cytokines in a cohort of patients with PAD. This establishes hypotheses for future studies investigating large artery atherosclerosis. Previous publications have reported the association of cytokine levels with different risk factors within the same cohort.[9] Based on this, our hypothesis is that certain cytokine levels would be elevated in individuals with greater aortic calcification.

METHODS

Study population

PURE ASO (The Role of Purinergic Signaling in Atherosclerosis) is a prospective cohort including 226

17528062, 2024, 10, Downloaded from https://ascpt.onlinelibrary.wiley.com/doi/10.1111/cts.70036 by Duodecim Medical Publications Ltd, Wiley Online Library on [02/10/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

patients with invasively treated PAD enrolled between February 2012 and March 2013 in Turku University Hospitals Department of Vascular Surgery. Cohort data consists of demographic factors, relevant medical history, prescribed medication, previous invasive procedures regarding cardiovascular diseases, and circulating cytokine levels. Circulating cytokine levels were determined from serum samples using a multiplex assay kit (Bio-Plex Pro Human Cytokine 21- and 27-plex panels, Bio-Rad Laboratories). Samples were obtained prior to the index invasive treatment. This method is described in detail in our previous article about this same cohort.[14,15] Cytokines in these panels represent well cytokine profile associated with atherosclerosis.[14,15] All cohort patients who had no missing values ($n = 156$) regarding cytokines, aortic calcification index (ACI) measurements or significant clinical variables compose a subgroup of patients for this study. Categorical demographic factors are recorded as binary variables, and this categorization is also utilized in the multivariate analysis. No composite or sum variables were used.

Measurements

ACI measurement method is elaborated in our recent publications.[16] Briefly, calcification of aorta is determined using enhanced or unenhanced computed tomography studies showing the entire abdominal aorta. The degree of calcification in cross-sections (axial plane) of the aorta 5 mm apart from each other was recorded on a scale from 0 to 12. The index was calculated using the following formula:

$$\text{ACI} = (\text{Total sum of calcification in all slices}) / \left(12^{*}n\right) * 100.$$

ACI is measured from imaging studies that were available in our institutions' Picture archiving and communication system (PACS). We did not perform additional imaging due to this study protocol. Many of the patients had recent imaging studies available due to the diagnostic workup of PAD and the rest had available imaging studies related to other visits at our institution. Ankle-brachial index (ABI) and Toe-brachial index (TBI) were measured at our department's vascular laboratory.[17] Lowest ABI and TBI measurements were noted. ABI and TBI are reported as indices and they do not have a unit.

Statistical methods

Categorical variables are reported as n (%). Continuous variables are reported as mean (standard error, SE, p-value) and regression coefficients are reported as β (standard error, SE, p-value). Continuous variables fit for formal distribution were tested visually and using the Shapiro-Wilks test. A p-value of less than 0.05 is considered statistically significant. Logarithmic transformations of cytokine levels were used in univariate regressions because none of the cytokine levels were normally distributed. Simple linear regression was used to analyze associations between continuous variables. Clinically relevant risk factors were selected as variables for multivariate linear regression analysis. Logarithmic transformation of cytokine values was used in regression analyses. Univariate binary regression was conducted to determine each cytokine's association with higher ACI. Patients were further categorized in tertiles according to ACI, and also in relation to ACI over or under 50. Key cytokines were analyzed in these groups and binary regressions were run with ACI over 50 and ACI higher than lowest tertile as response variables. ACI over 50 was selected as a threshold level because the mean and median of ACI are both close to 50 (mean ACI = 51.27, median ACI is 52.26). Each binary regression ROC curves AUC value with 95% confidence intervals was analyzed and plotted. Statistical analysis including AUC/ROC analyses and plots were conducted using JMP®, Version Pro 17. SAS Institute, Cary, NC, 1989–2021, and for visualization, we used GraphPad Prism version 9.5.1 for Windows, GraphPad Software, San Diego, California USA, www.graphpad.com.

New written informed consent was waived based on the study design. This study protocol is covered by the informed consent which was acquired from patients included in the cohort at the time of the enrollment. All patients have signed an informed consent upon recruitment into the cohort. The study protocol adheres to the ethical guidelines of the 1975 Declaration of Helsinki. The study protocol has been approved by the Institute's ethics committee (institutional review board). The patient data of the cohort will not be made available because the data contains information that could compromise the privacy of the cohort patients. All authors had full access to all the data and are responsible for data integrity and the data analysis.

RESULTS

Clinical characteristics

Mean (SE) age of 156 cohort subgroup patients was 70.7 (9.5) years. 64 (41.0%) were women. 100 (64.1%) of patients were ex- or current smokers. The mean ABI was 0.64 (0.5) 69 (46.3%) patients had ABI 0.5–0.9 and 64 (42.2%) had ABI <0.5. The mean ACI was 52.3 (25.3). The mean LDL

17528062, 2024, 10, Downloaded from https://ascpt.onlinelibrary.wiley.com/doi/10.1111/cts.70036 by Duodecim Medical Publications Ltd, Wiley Online Library on [02/10/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

TABLE 1 Baseline clinical characteristics.

	n (%) or Mean (SD)
Age	70.7 (9.5)
Women	64 (41.0)
Hypertension	110 (70.5)
Dyslipidemia	50 (32.0)
Type 2 diabetes	43 (27.6)
Type 1 diabetes	10 (0.06)
No diabetes	103 (66.0%)
Coronary artery disease	45 (28.8)
Congestive heart failure	26 (16.7)
Chronic kidney failure	33 (21.1)
Previous stroke	19 (12.2)
Atrial fibrillation	26 (16.7)
COPD	38 (24.4)
Smoking (current and post-cessation)	100 (64.1)
Serum	
LDL	2.24 (0.97)
HDL	1.44 (0.54)
Creatinine	93.8 (72.7)
ACI	51.3 (25.3)
ABI (lower)	0.64 (0.48)
TBI	0.31 (0.17)

was 2.24 mmol/L (1.0) and the mean HDL was 1.4 mmol/L (0.5). The mean serum creatinine was 93.8 μmol/L (72.7). Clinical characteristics are presented in Table 1. Patients were categorized also into tertiles according to ACI, see baseline characteristics in Table S1.

Cytokines and ACI

Compared in ACI tertiles, logCTACK was significantly higher in the highest tertile compared with the lowest or middle tertiles, logCTACK (SE) 7.29 (0.05) in the first tertile, 7.35 (0.05) in the second tertile and 7.53 (0.05) in the third tertile, ANOVA *p*-value of 0.002, Tukey's HSD *p*-value of 0.002 for the third versus first tertile and 0.023 for the third tertile versus second tertile. LogMIG was significantly higher in the highest tertile compared with the lowest tertile, 7.65 versus 7.30, Tukey's HSD *p*-value of 0.028.

Out of the 39 cytokines screened in univariate analysis, only Log MIP 1a (β 12.54, SE 5.81, p 0.033), log CTACK (β 23.08, SE 5.22, $p < 0.001$) and log MIG (β 9.40, SE 2.82, p 0.001) were statistically significantly associated with higher ACI. Of clinical characteristics, only age was associated with ACI (β 0.88 per year, SE 0.20, $p < 0.001$) in univariate linear regression. Cytokines are presented in Tables 2 and 3. Univariate regression analyses are presented in Table 4.

TABLE 2 Cytokines in multiplex assay.

IL-1b	Interleukin-1-beta
IL-1ra	Interleukin-1 receptor antagonist
IL-2	Interleukin-2
IL-2Rα	Interleukin-2 receptor alpha
IL-4	Interleukin-4
IL-5	Interleukin-5
IL-6	Interleukin-6
IL-7	Interleukin-7
IL-8	Interleukin-8
IL-9	Interleukin-9
IL-10	Interleukin-10
IL-12	Interleukin-12
IL-13	Interleukin-13
IL-16	Interleukin-16
IL-17	Interleukin-17
IL-18	Interleukin-18
Eotaxin	Eotaxin
TNF-a	Tumor necrosis factor-alpha
IFN-g	Interferon-gamma
IP-10	Interferon-gamma-induced protein-10
CTACK	Cutaneous T-cell-attracting chemokine
MCP-1	Monocyte chemotactic protein-1
MIP-1a	Macrophage inflammatory protein-1 alpha
MIP-1b	Macrophage inflammatory protein-1 beta
MIF	Macrophage migration inhibitory factor
MIG	Monokine induced by interferon-gamma
M-CSF	Macrophage colony-stimulating factor
GM-CSF	Granulocyte-macrophage colony-stimulating factor
G-CSF	Granulocyte colony-stimulating factor
FGF	Basic fibroblast growth factor
PDGF	Platelet-derived growth factor
HGF	Hepatocyte growth factor
VEGF	Vascular endothelial growth factor
SCF	Stem cell factor
SCGF-b	Stem cell growth factor beta
SDF-1a	Stromal cell-derived factor-1 alpha
b-NGF	Beta nerve growth factor
GROa	Growth-regulated oncogene alpha
TRAIL	Tumor necrosis factor-related apoptosis-inducing ligand

Note: MCP-3, IFN-α2, LIF, IL-1α, IL-3, IL-15, and TNF-β were mostly below a detectable limit and therefore excluded from the analyses. Cytokine RANTES was constantly higher than the measurable range and therefore excluded from the analyses.

17528062, 2024, 10, Downloaded from https://ascpt.onlinelibrary.wiley.com/doi/10.1111/cts.70036 by Duodecim Medical Publications Ltd, Wiley Online Library on [02/10/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

TABLE 3 Key cytokines.

	Mean	Median	Min	Max	SE	SD	25% quartile	75% quartile
CTACK	1730.1	1634.0	664.98	3663.4	49.87	622.9	1219.3	2143.4
MIG	2222.2	1739.8	249.02	14,697.6	143.42	1791.3	1161.0	2732.5
MIP1a	45,027	45,566	32,203	35.67	0.35	11,414	8.18	12.56

TABLE 4 Univariate and multivariate linear and binary regressions of log ACI.

	Unadjusted β (SE)	p-Value
Univariate linear		
log MIP-1a	12.54 (5.81)	**0.033**
log CTACK	23.08 (5.22)	**<0.001**
log MIG	9.40 (2.82)	**0.001**
Age	0.88 (0.20)	**<0.001**
	Adjusted β (SE)[a]	**p-Value**
Multivariate linear		
log MIP-1a	5.22 (5.90)	0.377
log CTACK	17.90 (5.55)	**0.002**
CTACK[b]	0.11 (0.003)	**0.001**
100 units CTACK[b]	1.08 (0.32)	**0.001**
log MIG	6.80 (3.33)	**0.043**
Age	0.91 (0.22)	**<0.001**
Multivariate binary for ACI over 50		
log MIP-1a	0.21 (0.54)	0.693
log CTACK	0.97 (0.53)	0.068
log MIG	0.38 (0.31)	0.221
Multivariate binary for ACI over lowest tertile		
log MIP-1a	1.28 (0.62)	**0.038**
log CTACK	1.01 (0.55)	0.068
log MIG	0.63 (0.34)	0.063

Note: Bold = $p < 0.05$ indicating statistical significance.

Abbreviations: SE, standard error; β, regression coefficient.

[a]Adjusted for cardiovascular risk factors age, sex, smoking, hypertension, dyslipidemia, diabetes type 1 or type 2, chronic kidney disease.

[b]No logarithmic transformation.

In multivariate analysis, log CTACK was associated with ACI, when the analysis was adjusted for cardiovascular risk factors (hypertension, smoking, sex, diabetes type 1 or type 2, age, dyslipidemia, and chronic kidney disease, each as a separate binary categorical variable). Regression estimate β for log CTACK was 17.90 (SE 5.55, p 0.002). Age was associated with increased ACI in multivariate analysis, regression estimate β for age was 0.91 (SE 0.22, $p < 0.001$) per 1 year. Multivariate analysis with the same risk factor variables was run separately with MIP-1a and MIG instead of CTACK. MIP-1a was not significantly associated with ACI. MIG was associated with ACI, estimate β for MIG was 0.003 (SE 0.001, p 0.030) per one unit. The estimate β for logarithmic transformation of MIG was 6.80 (SE 3.33, p 0.043). Univariate and multivariate regression analyses are presented in Table 4.

Multivariate analysis was run for ACI as categorical variable, comparing the lowest tertile with two higher tertiles. Odds ratio (OR) for logCTACK for having ACI greater than the lowest tertile was 1.01 (SE 0.55), p-value of 0.067. Analysis was adjusted with the above-mentioned cardiovascular risk factors. LogMIG was associated with two higher tertiles of ACI, OR 0.63 (SE 0.34), p 0.063). When ACI was used as a categorical variable comparing patients with ACI < 50 with those who had ACI > 50 in the same model, logCTACK, logMIG, or logMIP-1a were not associated with higher ACI. These results are presented in Table 4.

Each cytokine's association with ACI was further established using AUC/ROC values; we ran a binary regression model with binary variable "ACI over 50" as a dichotomic outcome variable separately with each cytokine. Then, every model's ROC curve and AUC value with respective 95% confidence interval were determined. The results of the ROC-analysis are illustrated in Figure 1. CTACK, MIG, IFNg, IL5, and MIP1a were associated statistically significantly with ACI over 50. Further categorization in relation to ACI over 50 was not conducted.

DISCUSSION

CTACK, MIG, and MIP-1a demonstrated associations with ACI as univariates, while MIG and CTACK remained associated with ACI in the multivariate analysis. These three cytokines stood out from the other 39 cytokines in AUC/ROC analyses derived from regression models where CTACK, MIG, and MIP-1a showed association with higher ACI whereas other cytokines did not. Cutaneous T-cell-attracting chemokine (CTACK), also known as C-C motif chemokine ligand 27 or CCL27, is a chemotactic cytokine associated with the skin and other barrier tissues. It is predominantly expressed in the skin keratinocytes and it is induced by TNFα.[18] The primary function of CTACK is to recruit resident T-cells and regulate T-cell-related immune homeostasis in the skin. While the direct association of

17528062, 2024, 10, Downloaded from https://ascpt.onlinelibrary.wiley.com/doi/10.1111/cts.70036 by Duodecim Medical Publications Ltd, Wiley Online Library on [02/10/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

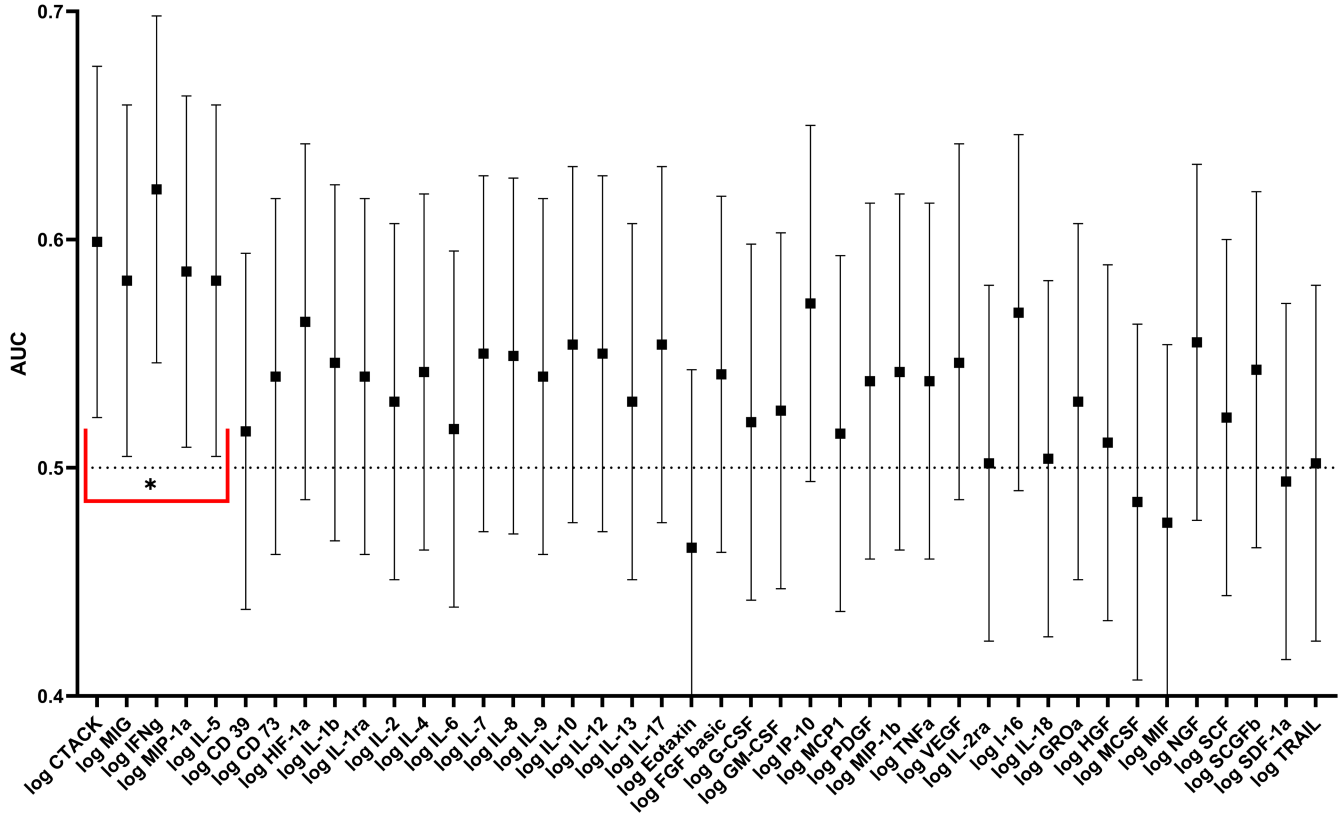

FIGURE 1 AUC values for each ROC curve derived from binary regression run for binary variable "ACI over 50" with each cytokine separately. AUC values for each cytokine tested for ACI over 50. Upper and lower bars represent AUC values 95% confidence intervals. AUC value was statistically significant for CTACK, MIG, IFN-γ, MIP-1a, and IL-5. AUC value is derived from binary univariate regression where the response variable was a binary variable of ACI over 50 was coded as 1, positive level. Each cytokine was tested separately against this binary variable and ROC curve was analyzed for each test and AUC value for each ROC curve was plotted here. *AUC values 95% confidence intervals do not cross 0.5.

CTACK with cardiovascular diseases has not been previously established, it has been utilized as a biomarker in the CHDRA model for predicting cardiovascular risk, which is comparable to the Framingham risk score. In that model, CTACK demonstrated a significant association with the risk of cardiovascular disease.[19] Previous analyses of the present PUREASO cohort revealed associations between CTACK and age, CTACK and proximal PAD, and CTACK and Th1-IFN-γ induced cytokines.[9]

Disturbances in the CTACK regulation have been linked with inflammatory skin diseases, such as atopic dermatitis and psoriasis.[18,20,21] Psoriasis, in turn, is associated with systemic inflammation and a higher risk of cardiovascular diseases, sharing similarities in inflammatory mechanisms.[4,22] In both atherosclerosis and psoriasis, T-helper cells type 1 and 17 (Th1, Th17) as well as interleukins 1 and 17 play crucial roles. Novel pharmacotherapy targeted at these cascades has shown a reduction in atherosclerotic signs in psoriasis patients.[4,23] Monokine induced by gamma-interferon (MIG; CXCL9) has been relatively less studied. MIG is a cytokine that serves as a chemoattractant for Th1-cells and it is induced by IFN-γ. MIG is known to be associated with atherosclerosis and cardiovascular diseases.[24,25] Excessive MIG expression is seen in skin inflammations, particularly in conditions other than psoriasis, although MIG is associated with psoriasis to some degree.[26,27]

Pathophysiology of atherosclerosis varies between the affected vascular beds (lower extremities, coronary, and carotid arteries). Clinically, PAD manifestations occur mainly due the thrombosis without plaque rupture, whereas coronary artery disease events occur mainly due to plaque rupture. The influence of the risk factors varies between the different vascular beds, and atherosclerotic inflammation is more pronounced in coronary and carotid arteries than in peripheral arteries. The atherosclerosis in the peripheral arteries presents with fibroproliferative pathology instead of lipid-rich cores and thin fibrous caps seen in clinically unstable coronary artery disease. Coronary, carotid, and peripheral arteries face distinct blood flow conditions and luminal shear stress, which may also affect pathological alterations. There are also differences in circulating miRNA profiles in different arterial beds of atherosclerosis. Aortic atherosclerosis biomarkers

17538062, 2024, 10, Downloaded from https://ascpt.onlinelibrary.wiley.com/doi/10.1111/cts.70036 by Duodecim Medical Publications Ltd, Wiley Online Library on [02/10/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

have been sought in relation to atherosclerosis-related aneurysms, and proteomics have revealed differences in aortic and coronary atherosclerosis, but the detailed soluble inflammatory profile of the aortic atherosclerosis is not yet established – even though aortic calcification is connected to cardiovascular disease burden.[7–9,28,29]

Atherosclerotic diseases in different vascular beds seem to present with distinct circulating cytokine profiles, even though evidence is still scarce. Coronary artery disease and carotid atherosclerosis are associated with several interleukins in slightly different proportions. Especially interleukin 6 (IL-6) and tumor necrosis factor-alpha (TNF-α) seem to be promoted in coronary artery disease.[30,31] A recent study suggests that slightly different sets of circulating cytokines are present in patients with PAD. For example, one of the inflammatory cytokines found by Ferreira et al. was MIP-1a, which also showed the association with ACI in this study.[32] Expression of IL-6 is also increased in PAD. Also, MCP-1 and TNF-alfa are associated with PAD.[33] IL-6 seems to be associated with atherosclerosis in large, coronary, and peripheral arteries.[34]

In this study, these conventional pro-inflammatory cytokines were not highlighted, presumably, we did not compare PAD patients to disease-free controls. However, previous studies on this same cohort showed that multiple cytokines are associated with more severe PAD. In this context, it is interesting that non-conventional cytokines CTACK and MIG presented an association with ACI.

We aimed to explore cytokines and aortic atherosclerosis relationship to establish a correlation, and further studies are needed to determine causative relationships underlying this finding. For this purpose, our results provide valuable insight into the potential link between dysregulation of inflammation in aortic atherosclerosis. Based on what is already known about these cytokines, one potential hypothesis to be explored is that the skin, as a barrier tissue, might act as an initial site of inflammation dysregulation that eventually contributes to the development of atherosclerosis and cardiovascular disease. The significant associations of CTACK and MIG with ACI support this concept, and we suggest that a systematic investigation of skin-related inflammatory mechanisms and their potential association with atherosclerotic cardiovascular diseases should be carried out. Understanding these associations can potentially shed light on the underlying mechanisms of cardiovascular diseases and open avenues for targeted therapeutic interventions in the future.

CONCLUSION

Cytokines CTACK and MIG are associated with increasing aortic calcification.

LIMITATIONS

There are limitations regarding this study. Our method is merely explorative, and validation of these findings is warranted in a controlled or comparative setting. Also, due to the explorative approach, careful consideration is warranted in the interpretation of results regarding causality and correlation, as the latter would require a comparative setting and different categorization of subjects. For the purposes of this study, control group or validation cohort is not available, and as we aim for exploration and hypothesis generation, we see that such is not needed. We did not find significant results in all conventional cytokines associated with atherosclerosis. This may be due to the lack of a comparison group or due to the fact that all our subjects suffered from symptomatic atherosclerotic disease and had heavy atherosclerotic burden, which might mean that all groups already had high values and therefore differences are not significantly noticeable. There are several comorbidities that might elevate cytokine levels, and our approach does not allow us to control all of them. The soluble cytokine profile of aortic atherosclerosis can be investigated in various settings, including patients with different degrees of clinical and subclinical atherosclerosis, but our cohort does not reach such categorization and therefore our results may not be generalizable to different patient categories. Moreover, multicollinearity might be present with multiple cytokines but they were analyzed individually in this study. Risk factor contribution and potential collinearity with these cytokines cannot be controlled in this study setting, but we see that multiple statistical methods that are applied can reduce the risk of significant collinearity.

Finally, age might contribute to the known risk factors of atherosclerosis and it is included as an individual risk factor in the multivariable analyses. Age might be a risk factor and a confounding factor in these analyses. Our approach does not allow us to control bias generated by age or other confounding factors, but for the purposes of this study, we see that such is not needed as we did not aim to study age in relation to aortic calcification.

AUTHOR CONTRIBUTIONS

V.R., V.N., and D.L. wrote the manuscript; H.H., J.J. T.K., J.G., and D.L. designed the research; J.J. and H.H. performed the research; V.R., D.L., and H.H. analyzed the data.

ACKNOWLEDGMENTS

The authors have nothing to report.

FUNDING INFORMATION

No funding was received for this work.

17528062, 2024, 10, Downloaded from https://ascpt.onlinelibrary.wiley.com/doi/10.1111/cts.70036 by Duodecim Medical Publications Ltd, Wiley Online Library on [02/10/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

CONFLICT OF INTEREST STATEMENT

Ville Rantasalo has received grants from Paavo Ilmari Ahvenainen Foundation, the Finnish Foundation for Cardiovascular Research, Aarne Koskelo Foundation, Maire Taponen Foundation, and Finnish Medical Foundation. There were no other financial or non-financial interests. Listed sponsors had no role in the study design, collection, analysis or interpretation of data, writing the manuscript, or decision to submit for publication. All other authors declared no competing interests in this work.

ORCID

Ville Rantasalo https://orcid.org/0000-0003-1385-2870
Dan Laukka https://orcid.org/0000-0002-5604-936X
Veikko Nikulainen https://orcid.org/0000-0001-9453-4254
Juho Jalkanen https://orcid.org/0000-0002-1693-8293
Jarmo Gunn https://orcid.org/0000-0002-6996-0726
Tuomas Kiviniemi https://orcid.org/0000-0002-0908-3741
Harri Hakovirta https://orcid.org/0000-0002-1518-045X

REFERENCES

1. Zieleniewska NA, Kazberuk M, Chlabicz M, Eljaszewicz A, Kamiński K. Trained immunity as a trigger for atherosclerotic cardiovascular disease—a literature review. *J Clin Med*. 2022;11:3369.
2. Wolf D, Ley K. Immunity and inflammation in atherosclerosis. *Circ Res*. 2019;124:315-327.
3. Moriya J. Critical roles of inflammation in atherosclerosis. *J Cardiol*. 2019;73:22-27.
4. Terui H, Asano Y. Biologics for reducing cardiovascular risk in psoriasis patients. *J Clin Med*. 2023;12:1162.
5. Kommoss KS, Enk A, Heikenwalder M, et al. Cardiovascular comorbidity in psoriasis – psoriatic inflammation is more than just skin deep. *J Dtsch Dermatol Ges*. 2023;21:718-725.
6. Czerniuk MR, Surma S, Romanczyk M, et al. Unexpected relationships: periodontal diseases: atherosclerosis-plaque destabilization? From the teeth to a coronary event. *Biology (Basel)*. 2022;11:272.
7. Narula N, Olin JW, Narula N. Pathologic disparities between peripheral artery disease and coronary artery disease. *Arterioscler Thromb Vasc Biol*. 2020;40:1982-1989.
8. Teixeira AR, Ferreira VV, Pereira-Da-Silva T, Ferreira RC. The role of miRNAs in the diagnosis of stable atherosclerosis of different arterial territories: a critical review. *Front Cardiovasc Med*. 2022;9:1-14.
9. Jalkanen J, Hollmen M, Maksimow M, Jalkanen S, Hakovirta H. Serum cytokine levels differ according to major cardiovascular risk factors in patients with lower limb atherosclerosis. *Cytokine*. 2019;114:74-80.
10. Lewis JR, Schousboe JT, Lim WH, et al. Long-term atherosclerotic vascular disease risk and prognosis in elderly women with abdominal aortic calcification on lateral spine images captured during bone density testing: a prospective study. *J Bone Miner Res*. 2018;33:1001-1010.
11. Oishi H, Horibe H, Yamase Y, et al. Predictive value of abdominal aortic calcification index for mid-term cardiovascular events in patients with acute coronary syndrome. *Heart Vessels*. 2020;35:620-629.
12. Herrington DM, Mao C, Parker SJ, et al. Proteomic architecture of human coronary and aortic atherosclerosis. *Circulation*. 2018;137:2741-2756.
13. Yagi H, Nishigori M, Murakami Y, et al. Discovery of novel biomarkers for atherosclerotic aortic aneurysm through proteomics-based assessment of disease progression. *Sci Rep*. 2020;10:6429.
14. Jalkanen J, Maksimow M, Hollmen M, Jalkanen S, Hakovirta H. Compared to intermittent claudication critical limb ischemia is associated with elevated levels of cytokines. *PLoS One*. 2016;11:e0162353.
15. Jalkanen J, Yegutkin GG, Hollmen M, et al. Aberrant circulating levels of purinergic signaling markers are associated with several key aspects of peripheral atherosclerosis and thrombosis. *Circ Res*. 2015;116:1206-1215.
16. Rantasalo V, Gunn J, Kiviniemi T, et al. Intracranial aneurysm is predicted by abdominal aortic calcification index: a retrospective case-control study. *Atherosclerosis*. 2021;334:30-38.
17. Wickström JE, Laivuori M, Aro E, et al. Toe pressure and toe brachial index are predictive of cardiovascular mortality, overall mortality, and amputation free survival in patients with peripheral artery disease. *Eur J Vasc Endovasc Surg*. 2017;53:696-703.
18. Vestergaard C, Johansen C, Otkjaer K, Deleuran M, Iversen L. Tumor necrosis factor-alpha-induced CTACK/CCL27 (cutaneous T-cell-attracting chemokine) production in keratinocytes is controlled by nuclear factor kappaB. *Cytokine*. 2005;29:49-55.
19. Cross DS, McCarty CA, Hytopoulos E, et al. Coronary risk assessment among intermediate risk patients using a clinical and biomarker based algorithm developed and validated in two population cohorts. *Curr Med Res Opin*. 2012;28:1819-1830.
20. Davila ML, Xu M, Huang C, et al. CCL27 is a crucial regulator of immune homeostasis of the skin and mucosal tissues. *iScience*. 2022;25:104426.
21. Garzorz-Stark N, Krause L, Lauffer F, et al. A novel molecular disease classifier for psoriasis and eczema. *Exp Dermatol*. 2016;25:767-774.
22. Teague HL, Li H, Berg AR, et al. The relationship between circulating APOA-1 and atherosclerosis initiation and progression in psoriasis. *J Invest Dermatol*. 2023;143:1947-1954.e1944.
23. Tsiogka A, Gregoriou S, Stratigos A, et al. The impact of treatment with IL-17/IL-23 inhibitors on subclinical atherosclerosis in patients with plaque psoriasis and/or psoriatic arthritis: a systematic review. *Biomedicine*. 2023;11:318.
24. Yu HT, Lee J, Shin E-C, Park S. Significant association between serum monokine induced by gamma interferon and carotid intima media thickness. *J Atheroscler Thromb*. 2015;22:816-822.
25. Liang Y, Yang C, Zhou Q, et al. Serum monokine induced by gamma interferon is associated with severity of coronary artery disease. *Int Heart J*. 2017;58:24-29.
26. Goebeler M, Toksoy A, Spandau U, Engelhardt E, Bröcker EB, Gillitzer R. The C-X-C chemokine Mig is highly expressed in the papillae of psoriatic lesions. *J Pathol*. 1998;184:89-95.
27. Flier J, Boorsma DM, van Beek PJ, et al. Differential expression of CXCR3 targeting chemokines CXCL10, CXCL9, and CXCL11 in different types of skin inflammation. *J Pathol*. 2001;194:398-405.

17528062, 2024, 10, Downloaded from https://ascpt.onlinelibrary.wiley.com/doi/10.1111/cts.70036 by Duodecim Medical Publications Ltd, Wiley Online Library on [02/10/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

28. Poredos P, Cevc M, Blinc A. Characteristics of atherosclerosis in femoropopliteal artery and its clinical relevance. *Atherosclerosis*. 2021;335:31-40.
29. Schousboe JT, Vo TN, Langsetmo L, et al. Abdominal aortic calcification (AAC) and ankle-brachial index (ABI) predict health care costs and utilization in older men, independent of prevalent clinical cardiovascular disease and each other. *Atherosclerosis*. 2020;295:31-37.
30. Ansari WM, Humphries SE, Naveed AK, Khan OJ, Khan DA, Khattak EH. Effect of coronary artery disease risk SNPs on serum cytokine levels and cytokine imbalance in premature coronary artery disease. *Cytokine*. 2019;122:154060. doi:10.1016/j.cyto.2017.05.013
31. Haybar H, Bandar B, Torfi E, Mohebbi A, Saki N. Cytokines and their role in cardiovascular diseases. *Cytokine*. 2023;169:156261. doi:10.1016/j.cyto.2023.156261
32. Ferreira J, Roque S, Longatto-Filho A, et al. Higher levels of cytokines in patients with chronic limb-threatening ischemia. *Ann Vasc Surg*. 2024;106:255-263. doi:10.1016/j.avsg.2024.02.033
33. Ueki Y, Miura T, Miyashita Y, et al. Inflammatory cytokine levels after endovascular therapy in patients with peripheral artery disease. *Angiology*. 2017;68(8):734-740. doi:10.1177/0003319716682122
34. Prapiadou S, Živković L, Thorand B, et al. Proteogenomic data integration reveals CXCL10 as a potentially downstream causal mediator for IL-6 signaling on atherosclerosis. *Circulation*. 2024;149(9):669-683. doi:10.1161/circulationaha.123.064974

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Rantasalo V, Laukka D, Nikulainen V, et al. Association between aortic calcification and cytokine levels in patients with peripheral artery disease. *Clin Transl Sci*. 2024;17:e70036. doi:10.1111/cts.70036

17528062, 2024, 10, Downloaded from https://ascpt.onlinelibrary.wiley.com/doi/10.1111/cts.70036 by Duodecim Medical Publications Ltd, Wiley Online Library on [02/10/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License