Different Types of Circulatory Inflammatory Biomarkers Associated with Cerebral Arterial Atherosclerosis and Dolichoectasia

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Abstract

Background: Although inflammation is found to be related to arteriopathy pathogenesis, it is yet to be determined the distinct correlations of specific inflammatory biomarker types contributing to different cerebral large vessel diseases. We aimed to investigate the association between multiple inflammatory biomarkers and cerebral atherosclerosis and dolichoectasia in a community-based sample. Methods: A total of 960 participants of the Shunyi study were included. A panel of 14 circulatory inflammatory biomarkers was assessed and then grouped in three sets as systemic, endothelial-related, and media-related inflammation, based on underlying different inflammatory cascades. Intracranial atherosclerotic stenosis (ICAS), dolichoectasia estimated by magnetic resonance angiography, and carotid plaques estimated by ultrasound were also performed. Results: Endothelial-related inflammatory group was related to the presence of ICAS ($R^2 = 0.215$, $p = 0.024$) and carotid plaques ($R^2 = 0.342$, $p = 0.013$). Backward stepwise elimination showed that E-selectin was prominent ($\beta = 0.67$, 95% CI: 0.54–0.85, $p = 0.001$; $\beta = 0.79$, 95% CI: 0.68–0.93, $p = 0.005$). Systemic inflammatory group was associated with an increased basilar artery diameter ($R^2 = 0.051$, $p < 0.001$), and backward stepwise elimination showed that IL-6 was prominent ($\beta = 0.07$, 95% CI: 0.03–0.11, $p < 0.001$). Conclusion: Different types of inflammatory biomarkers were associated with atherosclerosis and dolichoectasia, respectively, implying dissimilar inflammatory processes. Further confirming of their distinct anti-inflammatory roles as potential therapeutic targets is warranted.

Keywords

Inflammatory biomarkers · Intracranial atherosclerotic stenosis · Carotid plaques · Arterial dolichoectasia · Community-based cohort
Introduction

Cerebral arterial atherosclerosis and dolichoectasia are common age-related cerebral large-vessel neuroimaging burdens with different underlying pathomechanisms. Previous studies indicated that atherosclerosis primarily involves endothelial dysfunction and dolichoectasia primarily involves elastolysis of the tunica media and smooth muscle cell migration [1, 2].

In the last 2 decades, dozens of biomarkers of inflammation have been found to be related to cardiovascular and cerebrovascular events. Elevated C-reactive protein (CRP) levels may relate to a higher atherosclerotic burden or higher risk for plaque rupture and thrombosis [3]. The increased activity of cell adhesion molecules such as E-selectin, P-selectin, intracellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1) contributes to endothelial dysfunction [4, 5]. Activation of matrix metalloproteinases (MMPs), mediated by increased inflammatory biomarkers, enables degradation of elastin and collagen, mainly resulting in tunica media dysfunction, and influences arterial remodeling, leading to various pathological disorders, such as aneurysm formation and excessive venous dilation [6].

Based on their distinctive mechanisms involved in different inflammatory cascade, biomarkers of inflammation can be roughly classified as systemic inflammation (e.g., CRP, interleukin-6 [IL-6], and tumor necrosis factor-α [TNF-α], etc.), endothelial-related inflammation (ICAM-1, VCAM-1, P-selectin, endothelial-leukocyte adhesion molecule [E-selectin], lipoprotein-associated phospholipase A2 [Lp-PLA2], chitinase-3-like-1 protein [YKL-40], and CD40 ligand [CD40L], etc.), and media-related inflammation (MMP-2, 3, 9, and osteopontin [OPN], etc.). Therefore, different types of biomarkers implicated in endothelial and tunica media dysfunction are believed to contribute to different vascular disease pathogenesis.

Given the complexity of inflammatory pathways, it is likely that a comprehensive panel of inflammatory biomarkers is concurrently involved in the development of age-related cerebral large vessel disease rather than act alone. However, prior studies usually have investigated the associations between arteriopathy and single rather than multiple inflammatory biomarkers, leaving the weights of interaction unassessed and the power of specific type biomarkers underestimated. Furthermore, findings on the association of various inflammatory biomarkers in disease cohorts and general populations have been inconsistent, and exploring inflammation impacts on cerebral arterial atherosclerosis and dolichoectasia simultaneously in a single community-based population might help to further elucidate the different pathogenesis to reduce interference. In the present study, we aimed to investigate and compare the association of a comprehensive panel of inflammatory biomarkers with carotid and intracranial arterial atherosclerosis, or dolichoectasia in a community-based sample, which might add new evidence for different inflammatory spectrum act in various cerebral large vessel diseases.

Materials and Methods

Population

The present study was a cross-sectional analysis of an ongoing community-based Shunyi Study in China, as described elsewhere [7]. Briefly, 1,586 inhabitants ≥35 years of age who lived independently were recruited from June 2013 to April 2016 in Shunyi, a suburban district of Beijing. All participants were invited to undergo baseline brain magnetic resonance imaging (MRI) and carotid ultrasound examinations. Among the participants, 329 refused or had contradictions for MRI examination. This current study was performed based on 1,257 participants who underwent baseline MRI. We further excluded subjects without inflammatory biomarker tests (n = 257), and with stroke, fever, or other active inflammatory or neoplastic disease (n = 40). Finally, a total of 960 participants were included in the present analysis (online suppl. Table 1).

The study was approved by the Ethics Committee at Peking Union Medical College Hospital (reference number: B-160) and conformed to the ethical guidelines of the 1975 Declaration of Helsinki. Written informed consent was issued by all participants.

Clinical Characteristics

Demographic and clinical information including age, sex, smoking status, body mass index, blood pressure, history of hypertension, diabetes mellitus, hyperlipidemia, and current medication were collected using a structured questionnaire and anthropometric measurements were conducted by trained staff. Venous blood samples, routinely drawn after an overnight fast, were analyzed for lipid spectrum and glucose. Body mass index was calculated as the ratio of weight (kilograms) to the square of height (meters squared). Blood pressure was measured three times, and the mean value was utilized. Smoking status was classified into current smoking (at least within the preceding month) and noncurrent smoking. Hypertension was defined as self-reported hypertension, the use of oral anti-hypertension drugs, or systolic blood pressure ≥140 mm Hg, or diastolic blood pressure ≥90 mm Hg. Diabetes mellitus was defined as fasting serum glucose ≥7.0 mmol/L, self-reported diabetes mellitus, or use of oral antidiabetic drugs or insulin. Hyperlipidemia was defined as self-reported hyperlipidemia, treatment with antihyperlipidemic medication, total cholesterol >5.2 mmol/L, or low-density lipoprotein >3.36 mmol/L.
Inflammation, Atherosclerosis, and Dolichoectasia

Venous overnight fasting blood samples were drawn and centrifuged. In addition, serum was stored at −80°C. We investigated a group of fourteen serum inflammatory biomarkers including systemic inflammation (hsCRP, IL-6, TNF-α), endothelial-related inflammation (E-selectin, P-selectin, ICAM-1, VCAM-1, CD40L, Lp-PLA2, and YKL-40), and media-related inflammation (MMP-2, MMP-3, MMP-9, and OPN). hsCRP was detected using immunetric analysis by an automatic biochemistry analyzer (AU5800; Beckman Coulter, Brea, CA, USA). All other thirteen biomarkers were measured by the Luminex LiquiChip method (LUMINEX 100/200 TM System) using commercially available kits from R&D Systems (one kit numbered LXSARH-02 for MMP-2 and MMP-9 and another kit numbered LXSARH-11 for the other eleven biomarkers). The LiquiChip integrates flow cytometry, laser technology, digital signal processing, and traditional chemical technology and has the greatest features of high throughput and high flexibility. Intra-assay coefficients of variation for the thirteen biomarkers measured using LiquiChip method (all <10%) are listed as follows: 4% for IL-6, 10.4% for TNF-α, 9.4% for E-selectin, 6.3% for P-selectin, 3.1% for ICAM-1, 2.2% for VCAM-1, 3.9% for Lp-PLA2, 3.7% for CD40L, 5.2% for YKL-40, 5.4% for MMP-2, 5.6% for MMP-3, 3.6% for MMP-9, and 5.8% for OPN.

Assessment of Arterial Atherosclerosis and Dolichoectasia

Intracranial atherosclerotic stenosis (ICAS) was assessed at the site of the most severe degree of stenosis by time-of-flight magnetic resonance angiography (TOF-MRA) using established criteria [8]. The definition of ICAS was any degree of stenosis in at least one of the following arteries: internal carotid artery, middle cerebral artery, anterior cerebral artery, intracranial segment of vertebral artery, basilar artery (BA), and posterior cerebral artery. The presence of carotid plaque was determined in bilateral common, internal, and bifurcation sites of the carotid arteries in a supine position with a color Doppler ultrasound diagnostic system (Esaote, Firenze, Italy) using a 5–13 MHz vascular probe LAS523 according to a standardized scanning protocol. Plaques were identified as focal structures encroaching into the arterial lumen by at least 1.5 mm [9].

The maximum diameter of BA was measured by TOF-MRA. The minor axis in the cross-section was recorded as the diameter for arteries extending obliquely. In addition to the diameter of the BA, the lateral displacement of the BA, and the height of the BA bifurcation were evaluated by TOF-MRA as described elsewhere [10]. BA dolichoectasia (BADE) was defined as a BA diameter >4.5 mm, laterality score >2, or height of bifurcation score >2 by the use of criteria proposed by Smoker et al. [11] (online suppl. Fig. 2).

Trained physicians who were blind to all clinical data rated ICAS, carotid plaques, BA diameters, and BADE independently. The intrarater agreements were assessed on a random sample of 50 individuals with a more than 1-month interval between the first and second readings. The results of the intrarater agreement were as follows: the intraclass correlation coefficient was 0.93 for the presence of ICAS and 0.95 for BA diameter, and the weighted κ coefficient was 0.63 for laterality of BA and 0.73 for height of BA bifurcation.

Inflammatory Biomarkers

All fourteen biomarkers were standardized to eliminate the influence of unit and extreme observations. Descriptive analyses were conducted using the mean and standard deviation or median and 25–75th percentiles for continuous variables as appropriate and frequency and percentage for categorical variables. Aiming to limit the number of comparisons and to decrease the risk of admitting type 1 error, we divided the biomarkers into three sets: systemic inflammation (hsCRP, IL-6, TNF-α), endothelial-related inflammation (E-selectin, P-selectin, ICAM-1, VCAM-1, CD40L, Lp-PLA2, YKL-40), and media-related inflammation (MMP-2, MMP-3, MMP-9, OPN). In the primary analyses, a global omnibus test based on the regression model assessed whether at least one inflammatory biomarker of the three individual sets was significantly related to the arteries. We fitted general linear or logistic regression models for continuous or categorical artery variables, respectively. We used a partial F test for general linear models and a likelihood ratio test for logistic models to assess the significance of adding a set of inflammatory biomarkers to an existing regression model, with the degrees of freedom equal to the number of inflammatory biomarkers in the given set [12, 13]. To be concerned about the confounding bias in the observational study, we adjusted covariates step by step based on clinical knowledge and previous studies. In the primary analysis, no covariate was adjusted; in the secondary analysis, age, sex, current smoking, hypertension, diabetes mellitus, and hyperlipidemia were adjusted. For the omnibus test which was significant at the 0.05 level in fully adjusted models, we additionally conducted regression analyses forcing in all covariates and a given set of inflammatory biomarkers; inflammatory biomarkers were then selected to remain in the model according to covariate-adjusted significance levels of 0.01 using the backward elimination method. To account for multiple testing in the combinations of artery and inflammatory biomarker set analyses, we used a two-tailed p < 0.01 statistical significance threshold for primary and secondary analyses. This was a compromise for avoiding type 1 and type 2 errors due to our small sample size. All statistical analyses were conducted using SAS 9.4 (SAS Institute, Inc., Cary, NC, USA).

Results

A total of 960 participants were included in the present analysis, and the mean age was 56.02 ± 9.18 years, and 64.17% were women. Table 1 shows the demographic and clinical characteristics of the present study.

Association between Inflammatory Biomarkers and Arterial Atherosclerosis

ICAS and carotid plaques were present in 15.26% (146/957) and 49.13% (454/924) of the sample, respectively (Table 1). Systemic and endothelial-related inflammatory biomarkers were associated with the presence of ICAS (Table 2, $R^2 = 0.031, p = 0.001$; $R^2 = 0.056, p < 0.001$, respectively), whereas media-related biomarkers showed no significant correlation ($R^2 = 0.013, p = 0.130$). For ex-
tracranial atherosclerosis, all groups of biomarkers were related to the presence of carotid plaques ($R^2 = 0.035, p < 0.001$ for systemic biomarkers; $R^2 = 0.119, p < 0.001$ for endothelial-related biomarkers; $R^2 = 0.091, p < 0.001$ for media-related biomarkers). Additional adjustment for classical cardiovascular risk factors revealed that only endothelial-related biomarkers were still prominent in persons with ICAS and carotid plaques ($R^2 = 0.215, p = 0.024$; $R^2 = 0.342, p = 0.013$, respectively). To screen out the greatest contributor of endothelial-related biomarkers based on the fully adjusted model, backward stepwise elimination showed that higher levels of serum E-selectin ($\beta = 0.67, 95\% CI: 0.54–0.85, p = 0.001; \hat{\beta} = 0.79, 95\% CI: 0.68–0.93, p = 0.005$) predicted a higher presence of ICAS and carotid plaques, respectively.

### Intracranial Arterial Dolichoectasia and Inflammatory Biomarkers

The average BA diameter was 3.06 mm ($n = 955$). All groups of biomarkers were related to a larger BA diameter ($R^2 = 0.026, p < 0.001$ for systemic biomarkers; $R^2 = 0.020, p = 0.034$ for endothelial-related biomarkers; $R^2 = 0.018, p = 0.002$ for media-related biomarkers). Additional adjustment for classical cardiovascular risk factors did not

### Table 1. Characteristics of the study population

| Clinical characteristics [continuous, mean ± SD; categorical, n (%)] | All participants, n = 960 |
|---------------------------------------------------------------|-------------------------|
| Age, years                                                   | 56.02±9.18              |
| Female                                                       | 616 (64.17)             |
| Body mass index, kg/m²                                       | 26.55±3.80              |
| SBP, mm Hg                                                   | 133.63±19.27            |
| DBP, mm Hg                                                   | 78.72±10.82             |
| Current smoker                                               | 210 (22.41)             |
| Hypertension                                                 | 512 (53.39)             |
| Diabetes mellitus                                            | 157 (16.35)             |
| Hyperlipemia                                                 | 483 (50.31)             |
| Assessment of arterial atherosclerosis, n (%)                 |                          |
| ICAS                                                         | 146 (15.26)             |
| Carotid plaque                                               | 454 (49.13)             |
| Assessment of dolichoectasia [continuous, mean ± SD; categorical, n (%)] |               |
| BADE                                                        | 36 (3.77)               |
| Diameter of BA, mm                                           | 3.06±0.56               |
| Biomarkers of inflammation (25th, 75th percentile)            |                          |
| hsCRP, mg/L                                                  | 1.21 (0.61, 2.45)       |
| IL-6, pg/mL                                                  | 15.84 (14.22, 17.71)    |
| TNF-α, pg/mL                                                 | 39.95 (33.19, 46.45)    |
| E-selectin, ng/mL                                            | 25.91 (14.99, 39.86)    |
| P-selectin, ng/mL                                            | 43.41 (35.50, 52.14)    |
| ICAM-1, ng/mL                                                | 294.64 (193.77, 544.98) |
| VCAM-1, ng/mL                                                | 752.54 (585.54, 1,005.20) |
| Lp-PLA2, ng/mL                                               | 75.43 (57.77, 96.95)    |
| YKL-40, ng/mL                                                | 28.58 (14.48, 59.39)    |
| CD40L, ng/mL                                                 | 10.22 (8.41, 12.12)     |
| MMP-2, ng/mL                                                 | 166.58 (142.92, 189.61) |
| MMP-3, ng/mL                                                 | 9.79 (6.20, 14.94)      |
| MMP-9, ng/mL                                                 | 91.12 (62.34, 135.87)   |
| OPN, ng/mL                                                   | 19.09 (14.93, 24.78)    |

Missing data: body mass index, 24; SBP, 4; DBP, 4; current smoker, 23; hypertension, 1; ICAS, 3; carotid plaque, 36; BADE, 5; BA, diameter, 5. SBP, systolic blood pressure; DBP, diastolic blood pressure; ICAS, intracranial atherosclerotic stenosis; BADE, basilar artery dolichoectasia; BA, basilar artery; hsCRP, high-sensitivity C-reactive protein; IL, Interleukin; TNF-α, tumor necrosis factor α; ICAM-1, intracellular adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1; Lp-PLA2, lipoprotein-associated phospholipase A2; YKL-40, chitinase-3-like-1, protein; CD40L, CD40 ligand; MMP, matrix metalloproteinases; OPN, osteopontin.
alter the relation of systemic biomarkers and BA diameter ($R^2 = 0.051$, $p < 0.001$). Based on the fully adjusted model, the greatest contributor of systemic biomarkers by backward stepwise elimination of individual systemic-related inflammatory biomarkers was IL-6 ($\beta = 0.07$, 95% CI: 0.03–0.11, $p < 0.001$). As a multiple index, BADE occurred in 3.77% (36/955) of the sample. We observed borderline significant correlations between systemic biomarkers and BADE ($R^2 = 0.032$, $p = 0.038$). Adjustment analysis revealed that association was not prominent in all biomarker groupings.

Discussion

This study provides multiple assessments of the association of a panel of fourteen inflammatory biomarkers with cerebral arterial atherosclerosis as well as dolichoectasia in a community-based sample. Our results suggest that higher levels of endothelial-related inflammatory biomarkers are significantly related to both ICAS and carotid plaques independent of traditional cardiovascular risk factors, and no correlation was found between inflammatory biomarkers and BADE ($R^2 = 0.032$, $p = 0.038$). Further examination of individual biomarkers revealed that E-selectin was positively related to artery atherosclerosis, and IL-6 was positively associated with enlarged BA.

Many previous studies have shown that arterial atherosclerosis is frequently related to chronic low-grade inflammation [14–16], which is indicated by elevated circulating levels of proinflammatory cytokines and chemokines, including some sensitively general but nonspecific biomarkers of systemic inflammation, such as CRP, IL-6, and TNF-α [17]. P-selectin, E-selectin, ICAM-1, and VCAM-1 are part of the large family of cellular adhesion molecules, which are enhanced by endothelial cells and stimulated by the binding of CD40L to CD40 [18]. In addition, observational studies have generally suggested a role of Lp-PLA2 and YKL-40 in endothelial dysfunction [19–21]. MMPs, a family of zinc-dependent endoproteases secreted by vascular smooth muscle, along with OPN, a highly phosphorylated protein that can upregulate pro-MMP-9 activity and MMP-2 expression, were observed to have multiple roles in tunica media remodeling [22, 23]. Inflammatory biomarkers normally interact with each other in different phases of inflammatory pathways rather than act alone. However, due to the difficulty of testing and performing statistical analysis when multiple biomarkers are introduced, prior studies have usually investigated the association between atherosclerosis and one given inflammatory biomarker instead of multiple biomarkers, leaving the weights of each biomarker unassessed. To date, only the Framingham Offspring Cohort reported the association between cerebral vascular disease and a panel of 15 biomarkers tested using ELISA kits, colorimetric activity methods, or sandwich enzyme immunoassays. In addition, they divided 15 biomarkers into three groups according to their potential function to limit the times of comparisons and the risk of type 1 error [24]. Accordingly, based on the distinctive mechanism of biomarkers in vascular walls reported in the literature and results from clinical observation, while
also referring to the grouping method used in the Framingham Cohort, we tested 14 inflammatory biomarkers using the Luminex LiquiChip method, a more sensitive testing method, and grouped them into systemic inflammation biomarkers (hsCRP, IL-6, and TNF-α), endothelial-related inflammation biomarkers (E-selectin, P-selectin, ICAM-1, VCAM-1, CD40L, Lp-PLA2, and YKL-40), and media-related inflammation biomarkers (MMP-2, MMP-3, MMP-9, and OPN) in our statistical analysis.

Our study found that the presence of ICAS and carotid plaques were correlated with endothelial-related inflammatory biomarkers and had no association with media-related biomarkers, which supports our hypothesis that endothelial-related biomarkers contribute more predominantly than medial-related biomarkers in the atherosclerotic process. In the Offspring Cohort of the Framingham Heart Study, a significant association was observed between multibiomarker panels and internal carotid artery intima-media thickness and aortic atherosclerosis, suggesting a potential role of endothelial-related biomarkers in atherosclerosis, further in line with our findings that endothelial-related inflammation might have more predominant impacts on artery atherosclerosis than medial-related or systemic-related biomarkers [13, 25]. Plenty of evidence has documented the critical role of endothelial dysfunction in the atherosclerotic process, which can be verified by our results. Our results suggested that E-selectin likely had the most profound correlation with arterial atherosclerosis among the seven endothelial-related biomarkers tested in the present study. E-selectin is shed from damaged endothelial cells, and due to its exclusive endothelial source, it is considered to be one of the most specific measures of endothelial damage [26]. Accordingly, the associations demonstrated between E-selectin and the presence of ICAS and carotid plaques provide support that endothelial-related inflammation plays a key role in the development of arterial atherosclerosis.

Dolichoectasia is another common age-related vascular disease that mainly relates to the rarefaction of elastic tissue in the tunica media [1]. Previous studies of stroke patients (n = 510) found that intracranial arterial dilatation was associated with MMP concentrations and the MMP-3/5A allele, suggesting the involvement of MMPs in the enlargement of the intracranial artery due to a specific role in extracellular matrix degradation and remodeling [27]. However, such a correlation was not observed in our study, which may be explained by the different subjects in our community population, who have a relatively lower frequency of dolichoectasia (3.77%) compared with patients with brain infarction (12–17%) [27, 28]. In our study, we found that systemic biomarkers, especially IL-6, were positively associated with an increased BA diameter. To date, studies on BA ectasia and inflammation are still very limited, while the results from aortic wall biopsies have revealed that IL-6 levels in abdominal aortic aneurysms are significantly higher than those in atherosclerotic disease [29, 30]. A preclinical study suggested that the important role of IL-6 in abdominal aortic aneurysm pathogenesis primarily involves immune cell migration and infiltration [31], and IL-6 might also be involved in human BADE, as both aortic aneurysm and BADE are dilatation artery diseases. Therefore, the role of inflammation in cerebral dolichoectasia warrants further verification.

The main strengths of the current study include a comprehensive panel of inflammatory biomarkers assessed simultaneously in a single large community-based population sample size, and the sensitive evaluation method of multiple inflammatory biomarkers using Luminex LiquiChip. There are also several limitations to be addressed. First, the present study is a cross-sectional analysis, which does not allow for causal inference, and we cannot exclude the possibility of residual confounding. Second, the arterial wall is not visualized in MRA, and lumen-based measurement may therefore underestimate both the atherosclerotic burden and plaque-related outward remodeling. Third, the present study sample was relatively young with a lower burden of cardiovascular risk factors, which made selection bias possible and reduced the observed frequency of arterial atherosclerosis and dolichoectasia. Fourth, levels of circulating biomarkers were measured only once, and intra-individual variation and insidious infectious diseases cannot be taken into account.

**Conclusion**

Our findings may improve the current understanding of the complex relationship between different inflammatory pathways and cerebral large vessel disease. Specific pathways should be given priority in intervention studies targeting inflammation for treating atherosclerotic diseases.

**Statement of Ethics**

This study protocol was reviewed and approved by the Ethics Committee at Peking Union Medical College Hospital, approval number B-160. The study was conducted ethically in accordance
Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Yi-Cheng Zhu, Shu-Yang Zhang, Li-Ying Cui, and Yong Sheng contributed to study conceptualization and hypothesis formation. Yuan Cao, Ding-Ding Zhang, and Yi-Cheng Zhu contributed to formal analysis, methodology, writing, and work visualization. Yi-Cheng Zhu contributed to funding acquisition. Yuan Cao, Jing-Yu Mu, Yi-Ming Liu, Feng Gao, Fei Han, and Fei-Fei Zhai contributed to data curation. Li-Xin Zhou, Jun Ni, Ming Yao, Ming-Li Li, and Zheng-Yu Jin contributed to supervision and review of the original draft.

Data Availability Statement

All data generated or analyzed during this study are included in this article and its online supplementary material files. Further enquiries can be directed to the corresponding author.

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Inflammation, Atherosclerosis, and Dolichoectasia

with the World Medical Association Declaration of Helsinki. Written informed consent to participate in the study was obtained from all participants.
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