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Fibrosis-related biomarkers and large and small vessel disease: The Cardiovascular Health Study

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ABSTRACT

Objective: Fibrosis has been implicated in a number of pathological, organ-based conditions of the liver, kidney, heart, and lungs. The objective of this study was to determine whether biomarkers of fibrosis are associated with vascular disease in the large and/or small vessels.
Methods: We evaluated the associations of two circulating biomarkers of fibrosis, transforming growth factor-β (TGF-β) and procollagen type III N-terminal propeptide (PIIINP), with incident peripheral artery disease (PAD) and subclinical macrovascular (carotid intima-media thickness, flow-mediated vasodilation, ankle-brachial index, retinal vein diameter), and microvascular (retinal artery diameter and retinopathy) disease among older adults in the Cardiovascular Health Study. We measured TGF-β and PIIINP from samples collected in 1996 and ascertained clinical PAD through 2011. Measurements of large and small vessels were collected between 1996 and 1998.
Results: After adjustment for sociodemographic, clinical, and biochemical risk factors, TGF-β was associated with incident PAD (hazard ratio [HR] = 1.36 per doubling of TGF-β, 95% confidence interval [CI] = 1.04, 1.78) and retinal venular diameter (1.63 μm per doubling of TGF-β, CI = 0.23, 3.02). PIIINP was not associated with incident PAD, but was associated with carotid intima-media thickness (0.102 mm per doubling of PIIINP, CI = 0.029, 0.174) and impaired brachial artery reactivity (−0.20% change per doubling of PIIINP, CI = −0.39, −0.02). Neither TGF-β nor PIIINP were associated with retinal arteriolar diameter or retinopathy.

Abbreviations: ABI, ankle-brachial index; CHS, Cardiovascular Health Study; FMD, flow-mediated vasodilation; IMT, intima-media thickness; PAD, peripheral artery disease; PIIINP, procollagen type III N-terminal propeptide; TGF-β, transforming growth factor-β.

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Conclusions: Serum concentrations of fibrosis-related biomarkers were associated with several measures of large vessel disease, including incident PAD, but not with small vessel disease. Fibrosis may contribute to large vessel atherosclerosis in older adults.

1. Introduction

Reactive interstitial fibrosis is the pathological accumulation of excess collagen and extracellular matrix deposition in response to severe or repetitive tissue injury. Over time, fibrosis can lead to scar formation and organ dysfunction, as seen in end-stage liver disease, kidney disease, and idiopathic pulmonary fibrosis [1]. In older adults, fibrosis has been implicated in age-related ventricular stiffening and diastolic dysfunction [2]; for example, between the third and seventh decade of life, myocardial collagen content increases by almost 50% [3]. Whether fibrosis plays a role in vascular atherogenesis and function in older adults remains an attractive but still unproven hypothesis.

Transforming growth factor-β (TGF-β) is a pleiotropic cytokine and a central mediator of fibrosis. TGF-β activity promotes collagen biosynthesis and the release of collagen byproducts, including procollagen type III N-terminal propeptide (PIIINP). In addition to its pro-fibrotic activity, TGF-β has potent anti-inflammatory effects; it has been found to suppress the expression of pro-inflammatory adhesion molecules by the vascular endothelium, prevent leukocyte and macrophage recruitment, and de-activate T-cells enriched in rupture prone vascular plaques [4]. TGF-β's dual pro-fibrotic and anti-inflammatory effects complicate its ultimate relationship with vascular disease, as these effects are likely to have opposing effects on atherogenesis.

Although fibrosis is a plausible risk factor for vascular disease, evidence to support this hypothesis has been inconsistent. Previous studies have found an inverse association between TGF-β expression and the probability of aortic atherosclerosis [5], as well as lower circulating levels of TGF-β among individuals with advanced atherosclerosis [6]. In contrast, other studies have shown positive correlations between TGF-β expression and atherogenic stimuli such as shear stress [7], oxidized cholesterol [8], and angiotensin II [9], as well as lower circulating levels of TGF-β among individuals with advanced atherosclerosis [10]. Though relatively few studies of PIIINP have been conducted, one cross-sectional study found that serum PIIINP concentrations were higher among individuals with peripheral arterial disease (PAD) compared to individuals with normal vasculature [11]. A second study found a borderline association between PIIINP and carotid atherosclerosis [12]. Most of the previous studies that have been conducted have been small and cross-sectional. Larger studies, and studies with prospective endpoints, such as clinical PAD, could begin to address existing inconsistencies.

A key question that has not yet been investigated is whether fibrosis-related biomarkers are differentially associated with large vs. small vessel disease. Existing studies of fibrosis-related biomarkers have focused on large vessel disease in the carotid and brachial arteries [11,13,14]; no studies of microvascular outcomes have been conducted, despite the fact that these may represent distinct pathophysiologic processes. Large and small vessel disease have been shown to have different biological etiologies and risk factors [15]. For example, large vessel disease primarily results from atherosclerosis [16], while small vessel disease is thought be the result of multiple molecular and cellular mechanisms, including prolonged hyperglycemia, dysregulation of vascular tone, and oxidative stress [16,17]. These differences highlight the possibility that associations between fibrosis-related biomarkers and vascular parameters may differ in vascular beds of different size.

Herein, we sought to evaluate the associations of TGF-β and PIIINP with clinical PAD and a broad range of measures of vascular structure and function, in both large and small vessels, among participants in the Cardiovascular Health Study (CHS), a population-based study of older adults from four U.S. communities. Because these markers of fibrosis have already been measured in this large, prospective study with both central adjudication of PAD and detailed subclinical vascular phenotyping, we could address multiple dimensions of their association with vascular disease, including possible interactions with inflammation that we observed in studies of other cardiovascular outcomes.

2. Materials and methods

2.1. Study design

The design, rationale and examination details of CHS have been published elsewhere [18]. Briefly, 5201 participants were recruited from Medicare eligibility lists from Forsyth County, North Carolina; Sacramento County, California; Washington County, Maryland; and Pittsburgh, Pennsylvania in 1989–1990. A supplemental cohort of 687 mostly African-American participants was added in 1992–1993. Individuals were eligible to participate if they were at least 65 years old, living in the community, and expected to remain in that community for at least three years after baseline. Individuals under active cancer treatment, and those not able to provide written informed consent, were excluded. Follow-up interviews were conducted during annual visits through 1998–1999 and interim 6-month phone calls that are still ongoing. The present analysis was limited to follow-up through 2011. All participants in our study provided written informed consent, and the institutional review board at each center approved the study protocol.

2.2. Exposure assessment

Our analysis included 1384 individuals free from clinical PAD, myocardial infarction (MI), and stroke with measured levels of TGF-β and 2647 with measured levels of PIIINP. TGF-β and PIIINP were measured in 2011–2012 from stored EDTA plasma samples collected during the 1996–1997 CHS visit, which is baseline for these analyses. TFG-β was measured by ELISA (Quantikine Human TGF-β1 Immunoassay; R&D Systems, Minneapolis, MN). PIIINP was measured by the UniQ Intact N-terminal Propeptide of Type III Procollagen radioimmunoassay kit manufactured by Orion Diagnostics (Fountain Hills, AZ). Inter- and intra-assay coefficients of variation (CVs) were between 1.9–2.9% and 6.4–9.3%, respectively for TFG-β. For PIIINP, corresponding values were both less than 7.2%.

In pilot studies, we identified probable platelet contamination resulting in artificially elevated levels of TGF-β [19] at two of our four clinic sites. Hence, *a priori,* we only measured TGF-β at the two remaining sites. Of the two sites at which TGF-β was not measured, one site (Washington County, Maryland) did not enroll an African