**ORIGINAL ARTICLE**

**Soluble Tumor Necrosis Factor Receptors and Arterial Stiffness in Patients With Coronary Atherosclerosis**

Hack-Lyoung Kim,¹,²,* Jung Pyo Lee,¹,², Jung Nam An,¹,² Jin Hyuk Kim,¹ Woo-Hyun Lim,¹,² Jae-Bin Seo,¹,² Woo-Young Chung,¹,² Yoon Kyu Oh,¹,² Yon Su Kim,²–⁴ Chun Soo Lim,¹,² Joo-Hee Zo,¹,² Myung-A Kim,¹,² and Sang-Hyun Kim¹,²

**BACKGROUND**
Soluble forms of tumor necrosis factor receptors (sTNFRs) are emerging target molecules of inflammatory disease. However, their role in vascular biology is not well known. This study was performed to investigate the association between serum concentrations of sTNFRs and arterial stiffness.

**METHODS**
A total of 117 consecutive patients with suspected coronary artery disease (CAD) (63.6 ± 11.0 years; men, 65%) who were referred for invasive coronary angiography (ICA) were prospectively enrolled. Arterial blood sTNFR1 and sTNFR2 were measured using commercially available ELISA kits. Brachial-ankle pulse wave velocity (baPWV) measurements were made within 24 hours of blood sampling for sTNFRs measurement.

**RESULTS**
Most of the patients (86.3%) had significant CAD (stenosis ≥ 50%) in ICA. In simple linear regression analyses, there were significant positive correlations of baPWV with sTNFR1 (r = 0.483, P < 0.001) and sTNFR2 (r = 0.366, P < 0.001). In multiple linear regression analyses, sTNFR1 (β = 0.316, P < 0.001) and sTNFR2 (β = 0.235, P = 0.005) had independent association with baPWV even after controlling for potential confounders.

**CONCLUSION**
sTNFR1 and sTNFR2 were independently associated with baPWV in patients undergoing ICA. This result may extend previous knowledge on close interactions between inflammation and arterial stiffening.

**Keywords:** arterial stiffness; blood pressure; hypertension; inflammation; pulse wave velocity; soluble tumor necrosis factor receptor

doi:10.1093/ajh/hpw134

Arteries stiffen by aging and arteriosclerosis.¹,² Arterial stiffness is clinically important because it is highly predictive of cardiovascular morbidity and mortality.³–⁶ Measurement of pulse wave velocity (PWV) has been considered the most practical method for estimating arterial stiffness.⁷ A number of studies have advocated that PWV is well correlated with the parameters of arterial stiffness measured by invasive hemodynamic technique.⁸ In addition, the clinical utility of PWV has been proved in many prior studies³–⁶ and meta-analyses.⁹ Furthermore, simplicity of its measurement makes it possible to use in mass screening.¹

The concentration of tumor necrosis factor-α (TNF-α) is elevated in response to inflammatory process. Together with C-reactive protein (CRP) and interleukin-6 (IL-6), this pleiotropic cytokine has been used as a marker of systemic inflammation. In plasma, 2 soluble forms of TNF-α receptors (sTNFR1 and sTNFR2) are present, and they play a role in controlling TNF-α activity by binding to TNF-α.¹⁰ Importantly, sTNFRs are emerging molecules as useful markers and for immunologic therapy of the inflammatory process such as inflammatory bowel disease and rheumatoid arthritis.¹¹–¹³

A number of studies have identified abnormalities of arterial stiffness in subjects with chronic systemic inflammation.¹⁴–¹⁶ It has been suggested that the inflammatory process enhances arterial damage and atherosclerosis, subsequently increasing arterial stiffness. However, the contribution of sTNFRs to the development of structural vascular changes and arterial stiffening remains unclear.

Therefore, this study was performed to investigate potential correlations between sTNFRs and arterial stiffness measured by brachial-ankle PWV (baPWV).

**METHODS**

**Patients and study protocol**
This prospective and single center study was performed at Boramae Medical Center (Seoul, Korea). Between May 2013

Correspondence: Sang-Hyun Kim (shkimmd@snu.ac.kr).

Initially submitted June 28, 2016; date of first revision August 4, 2016; accepted for publication October 7, 2016; online publication December 7, 2016.

¹Department of Internal Medicine, Boramae Medical Center, Seoul, Korea; ²Seoul National University College of Medicine, Seoul, Korea; ³Seoul National University Kidney Research Institute, Seoul, Korea; ⁴Department of Medical Science, Seoul National University College of Medicine, Seoul, Korea

*The first 2 authors equally contributed to this work.

© American Journal of Hypertension, Ltd 2016. All rights reserved. For Permissions, please email: journals.permissions@oup.com

American Journal of Hypertension 30(3) March 2017 313
and November 2013, consecutive patients with suspected coronary artery disease (CAD) who were referred for invasive coronary angiography (ICA) were asked to participate in this study. All the study patients were clinically stable with optimal medical treatment, and ICA was electively performed. Patients with unstable features, including ongoing chest pain, dynamic electrocardiogram changes, cardiac enzyme elevations, and unstable hemodynamic parameters were excluded. The following patients were also excluded: those with history or clinical signs or symptoms suggestive of recent infection, malignancies, and chronic degenerative disease and those on medications with anti-inflammatory actions. We obtained information on demographic characteristics, including age and body mass index, as well as traditional risk factors including histories of hypertension, diabetes mellitus, dyslipidemia, and ischemic heart disease. Body mass index was calculated by dividing weight (kg) by height (m²). Blood pressure and heart rate were measured using oscillometric device by a trained nurse. Mean arterial pressure was calculated as (systolic blood pressure + 2 × diastolic blood pressure)/3. Hypertension was defined by a previous history of hypertension or antihypertensive medications. Diabetes mellitus was defined by a previous history of diabetes or anti-diabetic medications. Dyslipidemia was defined as a previous history of dyslipidemia or antidyplipemic medications. Current smoker was defined as subjects who had smoked regularly during previous 12 months. Venous blood samples for laboratory tests were collected after overnight 8-hour fasting, and absolute neutrophil count (ANC), CRP, total cholesterol, low-density lipoprotein and high-density lipoprotein, triglyceride, and serum creatinine were measured. The estimated glomerular filtration rate was calculated using the following formula: 175 × serum creatinine^{-1.154} × age^{-0.203} (×0.742, if woman). ICA and percutaneous coronary intervention were performed in accordance with current guidelines. More than 50% stenosis of the major epicardial coronary artery was considered significant coronary artery obstruction. The study protocol was approved by the Institutional Review Board of Boramae Medical Center (Seoul, Korea), and informed consents were obtained from all the study patients.

Measurement of sTNFRs

Arterial blood (20 ml) was drawn from the sheath of the femoral or radial artery in the supine position just before ICA. Blood samples were immediately cooled and centrifuged, and the plasma was frozen at −70 °C. Concentrations of plasma sTNFR1 and sTNFR2 were measured with commercially available kits (Quantikine; R&D Systems, Minneapolis, MN). The minimum detectable concentration was 0.43–1.20 pg/ml for sTNFR1 and 0.2–2.3 pg/ml for sTNFR2. The intraassay and interassay coefficients of variation were 5.0% and 8.8%, respectively.

Measurement of baPWV

All baPWV measurements were made within 24 hours of blood sampling for sTNFRs measurement. The baPWV measurement protocol has been previously described. Medications in current use were allowed at the day of examination. Caffeine and tobacco use were not allowed before the examination. Subjects were examined in the supine position after 5 or more minutes of rest. The baPWV were measured using a volume-plethysmographic apparatus (VP-1000; Colin Co. Ltd., Komaki, Japan) in accordance with the manufacturer’s recommendations. Cuffs were wrapped on both arms (brachial) and ankles. Phonograms, pulse volume waveforms, blood pressure, and heart rates were simultaneously recorded. PWV was calculated by measuring the time for the pulse wave to travel between the brachial and posterior tibial arteries (velocity = distance/time). The mean of left and right baPWV values was used for study analysis. All measurements were made by the same experienced operator who was blinded to subjects’ information. The intraobserver coefficient of variation of baPWV measurement was 5.1% in our laboratory.

Clinical events

Clinical follow-up was done by outpatient clinic visit or telephone contact at 1 month after discharge of index ICA, and every 3 months thereafter. During this follow-up, information on cardiovascular events including cardiac death, nonfatal myocardial infarction and coronary revascularization was obtained. Cardiac death was defined as the death after acute coronary syndrome, ventricular arrhythmia, heart failure, or sudden unexplained death.

Statistical analysis

Continuous variables were presented as mean ± SD, and categorical variables were expressed as percentages. Univariate associations between 2 variables were assessed using Pearson’s bivariate correlation analyses. Scatter plots were used for the demonstration of linear correlations between the 2 variables. The baPWV values according to sTNFR tertiles were compared using chi-square of linear by linear association. Multivariable linear regression analyses were performed to examine independent relationships between sTNFRs and baPWV. In addition, sTNFR1 and sTNFR2 were entered individually into separate multivariable models that included potential confounders, including age, gender, mean arterial pressure, heart rate, diabetes mellitus, and hypertension. Variance influence factor was used to overcome the multicollinearity problem during multivariable analysis. A P value of <0.05 was considered statistically significant. All statistical analyses were conducted using SPSS version 18.0 (IBM, Armonk, NY).

RESULTS

Baseline characteristics

A total of 117 patients were enrolled and analyzed. The baseline characteristics of the study patients are shown in Table 1. The mean age was 63.6 ± 11.0 years, and male were predominant (65%). Histories of hypertension, diabetes mellitus, dyslipidemia, and current smoking were identified in 68.4%, 22.2%, 71.8%, and 16.2% of the patients, respectively. Most of the patients (86.3%) had significant CAD in ICA. The proportions of patients with 1, 2, and 3 vessel diseases were 26.5%, 28.2%, and 31.6%, respectively. There were
Table 1. Baseline characteristics of study patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>n = 117</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>63.6 ± 11.0</td>
</tr>
<tr>
<td>Male sex, n (%)</td>
<td>76 (65.0)</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>24.8 ± 3.2</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>129 ± 22</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>77 ± 13</td>
</tr>
<tr>
<td>Mean arterial pressure, mm Hg</td>
<td>95 ± 15</td>
</tr>
<tr>
<td>Heart rate, per minute</td>
<td>70 ± 14</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>80 (68.4)</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>26 (22.2)</td>
</tr>
<tr>
<td>Dyslipidemia, n (%)</td>
<td>84 (71.8)</td>
</tr>
<tr>
<td>Current smoking, n (%)</td>
<td>19 (16.2)</td>
</tr>
</tbody>
</table>

no significant abnormalities in biochemical parameters. The mean value of baPWV was 1,560 ± 289 cm/s.

Associations between baPWV and sTNFRs

In simple linear regression analyses, there were significant positive correlations of baPWV with sTNFR1 (r = 0.483, P < 0.001) and sTNFR2 (r = 0.366, P < 0.001) (Figure 1). When the sTNFRs values were stratified into tertiles, the baPWV values increased proportionally with increasing sTNFR1 (P = 0.002) and sTNFR2 (P = 0.076) tertiles (Figure 2). In multiple linear regression analyses, sTNFR1 (β = 0.316, P < 0.001) and sTNFR2 (β = 0.235, P = 0.005) had independent associations with baPWV even after controlling for potential confounders, including age, gender, mean arterial pressure, heart rate, diabetes mellitus, and hypertension (Table 2).

Associations of baPWV with ANC and CRP

The associations of other inflammatory markers including ANC and CRP were tested. There were significant linear correlations of baPWV with ANC (r = 0.251, P = 0.010) and CRP (r = 0.407, P < 0.001) (Figure 3). Multiple linear regression analyses showed independent associations of baPWV with ANC (β = 0.166, P = 0.045) but not with CRP (P = 0.245) (Supplementary Table 1).

Clinical events

During median follow-up of 663 days (interquartile range, 654–761 days), clinical events including cardiovascular death (n = 2), nonfatal myocardial infarction (n = 2), and coronary revascularization (n = 6) occurred in 9 patients (7.7%). The serum levels of both sTNFR1 (1,263 ± 441 pg/ml vs. 2,995 ± 1,566 pg/ml, P = 0.456) and sTNFR2 (2,813 ± 1,215 pg/ml vs. 2,995 ± 1,566 pg/ml, P = 0.674) were not different between patients with and without events.

DISCUSSION

To the best of our knowledge, this is the first study showing that sTNFR1 and sTNFR2 are independently associated with arterial stiffness in patients undergoing ICA.

It has been well recognized that the inflammatory process is associated with structural vascular remodeling, endothelial dysfunction, oxidative stress, and atherosclerosis. All these pathologies are main contributors to arterial stiffening. Recent mechanistic studies in humans have focused on the relationship between inflammation and vascular function. Vlachopoulos et al. investigated 100 healthy subjects and showed that acute systemic inflammation provoked by Salmonella typhi vaccination leads to increased stiffness of large artery. A study by Zanoli et al. demonstrated that arterial stiffness is elevated in patients with inflammatory bowel disease than control patients, and reduced by immunomodulatory drugs. Sacre et al. also showed that PWV was significantly elevated in 40 patients with systemic lupus erythematosus than 35 control subjects. Similarly, Mäki-Petäjä et al. reported that aortic stiffness markedly increased in 17 patients with rheumatoid arthritis compared to 34 patients with stable cardiovascular disease, and anti-TNF-α therapy reduced aortic stiffness in those with rheumatoid arthritis.

On the other hand, there is a paucity of the literature regarding the relationship between sTNFRs and arterial stiffness. To our knowledge, there was only 1 study showing the relationship between sTNFRs and arterial stiffness. That study demonstrated that sTNFR2, but not sTNFR1, was independently and positively associated with aortic PWV in diabetic patients, which is in line with our result showing that both sTNFR1 and sTNFR2 had significant associations with baPWV even after controlling for powerful confounders, such as age and systolic blood pressure. Underlying pathophysiology explaining the association between sTNFRs and arterial stiffness is incompletely understood. However, considering that sTNFRs are potent inflammatory markers, the hypothesis can be reasonable that chronic inflammation, including the sTNFR1 and sTNFR2 associated system may contribute to increased arterial stiffness. Supporting this hypothesis, baPWV had significant linear correlations with ANC and CRP in our study. In addition, there is possibility that elevated concentrations of sTNFR1 and sTNFR2 are the manifestations of the disease process of stiffened arteries. Another possibility is that elevated...
concentrations of sTNFR1 and sTNFR2 contribute directly to arterial injury and arteriosclerosis. Exact mechanisms behind these associations will rest on further research.

The TNF-α system, including TNF ligands and its receptors, plays a pivotal role in immune homeostasis and inflammatory processes. It is highly upregulated in patients with inflammatory conditions, such as inflammatory bowel disease, rheumatoid arthritis, and cancers. Notably, anti-TNF-α therapy has been shown to be effective in the treatment of such diseases.11–13 In addition, sTNFRs have been considered the main regulator of TNF-α activity through neutralizing function of TNF-α. Recently, the role of sTNFRs in the cardiovascular system has been described in several studies.24 In our study, there were numerical differences in the levels of sTNFR1 and sTNFR2 according to the occurrence of cardiovascular events: both sTNFRs and sTNFR2 were higher in patients with cardiovascular events than those without. However, the differences did not reach statistical significance. This may be attributed to small number of study patients. Considering the difficulty in TNF-α measurement due to its limited half-life, sTNFRs may be more stable and useful markers than TNF-α itself in cardiovascular disease.

Our study has important diagnostic and therapeutic implications. Arterial stiffness is an independent predictor of adverse cardiovascular events. However, the markers for arterial stiffness remain limited. Our study provides the evidence for the important role of inflammation in the pathogenesis of heart failure.30
arterial stiffness. It has been shown that reductions in inflammation can reduce arterial stiffness. Therefore, specific inflammatory pathways involved in arterial stiffness may be good therapeutic targets. Such successful anti-inflammatory therapies can lead to improved patients' outcomes associated with increased arterial stiffness. In this point of view, our findings provide the evidence that sTNFR1 and sTNFR2 can be considered potential molecules for immunologic therapy as well as new makers of arterial stiffness.

This study has several limitations. First, other inflammatory markers, such as IL-6 were not investigated, which may support a correlation between arterial stiffness and inflammation. Second, our study population consisted of patients undergoing ICA, and most of them (86.3%) had significant CAD. Thus, the result could not be generalized to other populations. Third, the effects of concomitant medications were not considered. Lastly, the casual relationship between sTNFRs and arterial stiffness was not confirmed in our study because the association was observed in cross-sectional analysis. Additionally, direct evidence explaining the relationship between sTNFRs and arterial stiffness were lacking. Thus, further researches to elucidate the underlying mechanisms are warranted.

CONCLUSIONS

sTNFR1 and sTNFR2 may be independently associated with baPWV in patients undergoing ICA. Our data strengthen previous findings on the role of inflammation in the pathogenesis of arterial stiffening. Further longitudinal studies with larger sample size are needed to confirm our findings.

SUPPLEMENTARY MATERIAL

Supplementary materials are available at American Journal of Hypertension (http://ajh.oxfordjournals.org).

FUNDING

This study was supported by grant no. 04-2012-0640 from Seoul National University Hospital Research Fund.

DISCLOSURE

The authors declared no conflict of interest.

REFERENCES

bowel diseases is dependent upon inflammation and reduced by immuno-modulatory drugs. *Atherosclerosis* 2014; 234:346–351.


