Effect of Smoking Status on Monocyte Tissue Factor Activity, Carotid Atherosclerosis and Long-Term Prognosis in Metabolic Syndrome

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Background: Smoking increases the risk of atherothrombotic events. Tissue factor (TF) mainly expressed on monocytes plays an important role in thrombosis and atherosclerosis. Metabolic syndrome (MetS) is being increasingly recognized as a major atherothrombotic risk factor, but the effects of smoking on monocyte TF activity (MTFA), carotid atherosclerosis estimated on carotid intima-media thickness (CIMT), and long-term prognosis in MetS remain unclear.

Methods and Results: A total of 301 MetS patients lacking any known cardiovascular disease were prospectively investigated and classified into 4 groups according to smoking status at entry and at 12 months as follows: never smokers, past smokers, quitters, and persistent smokers. Peripheral blood mononuclear cells (PBMC) were isolated, and MTFA was measured using a coagulation assay. Linear trends for higher baseline MTFA and CIMT were observed among persistent smokers, quitters, and past smokers compared with never smokers. At 12 months, MTFA and CIMT decreased in never and past smokers and quitters but increased in persistent smokers. Six acute myocardial infarctions and 8 strokes occurred during a median follow-up of 66.0 months. Persistent smoking was associated with an increased risk of events (P<0.001).

Conclusions: Smoking is associated with upregulated MTFA and progression of CIMT, which may be related to the risk of atherothrombotic events in MetS patients.

Key Words: Carotid intima-media thickness; Metabolic syndrome; Monocyte tissue factor activity; Smoking status
atherothrombotic events. For these reasons, CIMT is widely used in many clinical studies as a surrogate marker for carotid and coronary atherosclerosis.19,20

We previously found that monocyte TF activity (MTFA) is significantly upregulated in MetS compared with normal subjects, and the upregulation of MTFA is significantly associated with CIMT in MetS.13,17 No previous studies, however, have investigated the effects of smoking status on MTFA, progression of carotid atherosclerosis on CIMT, or the long-term prognosis in patients with MetS.

### Methods

### Subjects
A total of 301 selected (non-consecutive) patients with MetS (209 men and 92 women; mean age, 68.3±9.8 years) were enrolled in the present study. This study was a prospective cohort study, and all of the patients were recruited from Tama-Nagayama Hospital, Nippon Medical School, at an outpatient clinic between January 2005 and June 2013. None of the patients had received valacicline, a nicotinic receptor partial agonist and the most effective medication for tobacco cessation, before enrollment in the present study or during the follow-up period.

This study included patients with MetS who were ≥20 years old, and in whom MetS was diagnosed in accordance with the current Japanese criteria,21 including the presence of visceral obesity defined as waist circumference (WC) ≥85 cm in men or ≥90 cm in women as an essential component, combined with at least 2 of the following components: serum TG ≥150 mg/dL, HDL-C <40 mg/dL in either men or women, blood pressure ≥130/85 mmHg or having received anti-hypertensive medication, and fasting plasma glucose (FPG) ≥110 mg/dL.

Subjects with clinical signs of acute infection, autoimmune disorder, severe renal (serum creatinine >1.5 mg/dL) or hepatic disease, or with suspected malignancy were excluded from the present study. In addition, patients with a history of cardiovascular disease, including coronary artery disease, cardiomyopathy, heart failure and valvular heart disease, and stroke or peripheral artery disease were also excluded from the present study.

The investigation protocol was approved by the institutional ethics committee of Tama-Nagayama Hospital, Nippon Medical School. All of the subjects provided written consent before enrollment in this study.

### Table 1. Baseline MetS Clinical Characteristics and Blood Chemistry vs. Smoking Status

|                    | Never (n=108) | Past (n=74) | Quitters (n=56) | Persistent (n=63) | P-value |
|--------------------|--------------|-------------|----------------|------------------|---------|
| Age (years)        | 69.6±9.8     | 68.6±8.4    | 67.9±8.2       | 66.2±12.2        | 0.185   |
| Men                | 52 (48.1)    | 49 (66.2)   | 50 (89.3)      | 58 (92.1)        | <0.001  |
| BMI (kg/m²)        | 26.8±3.2     | 26.5±2.4    | 25.4±2.6       | 25.8±2.2         | 0.011   |
| WC (cm)            | 97.3±6.7     | 96.6±6.8    | 96.4±7.6       | 95.2±4.2         | 0.218   |
| SBP (mmHg)         | 136±14       | 130±5       | 131±6          | 133±6            | <0.001  |
| Heart rate (beats/min) | 72±7       | 71±4        | 70±4           | 73±3             | 0.021   |
| Hypertension       | 97 (89.8)    | 70 (94.6)   | 47 (83.9)      | 59 (93.7)        | 0.163   |
| Diabetes           | 82 (75.9)    | 54 (73.0)   | 33 (58.9)      | 32 (50.8)        | 0.003   |
| Dyslipidemia       | 84 (77.8)    | 61 (82.4)   | 48 (85.7)      | 61 (96.8)        | 0.010   |
| WBC (10³/mm³)      | 5,499±984    | 6,018±975   | 5,813±1,059    | 6,771±1,792      | <0.001  |
| BMI (kg/m²)        | 26.8±3.2     | 26.5±2.4    | 25.4±2.6       | 25.8±2.2         | 0.011   |
| WC (cm)            | 97.3±6.7     | 96.6±6.8    | 96.4±7.6       | 95.2±4.2         | 0.218   |
| SBP (mmHg)         | 136±14       | 130±5       | 131±6          | 133±6            | <0.001  |
| Heart rate (beats/min) | 72±7       | 71±4        | 70±4           | 73±3             | 0.021   |
| Hypertension       | 97 (89.8)    | 70 (94.6)   | 47 (83.9)      | 59 (93.7)        | 0.163   |
| Diabetes           | 82 (75.9)    | 54 (73.0)   | 33 (58.9)      | 32 (50.8)        | 0.003   |
| Dyslipidemia       | 84 (77.8)    | 61 (82.4)   | 48 (85.7)      | 61 (96.8)        | 0.010   |
| WBC (10³/mm³)      | 5,499±984    | 6,018±975   | 5,813±1,059    | 6,771±1,792      | <0.001  |

Data given as n (%), mean±SD or median (IQR). ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; CCB, calcium channel blockers; eGFR, estimated glomerular filtration rate; FPG, fasting plasma glucose; GI, glucosidase inhibitor; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; hsCRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; mCIMT, mean carotid intima-media thickness; MetS, metabolic syndrome; MTFA, monocyte tissue factor activity; NGSP, National Glycohemoglobin Standardization Program; PBMC, peripheral blood mononuclear cells; SBP, systolic blood pressure; WBC, white blood cells; WC, waist circumference.
Smoking Status

Information on smoking status was collected at entry and again at 12 months using a standardized questionnaire administered by each doctor at an outpatient clinic of Tama-Nagayama Hospital, Nippon Medical School. The patients were classified into 4 groups based on smoking status response to both questionnaires: never smokers, defined as patients who had never smoked a cigarette before entry; past smokers, defined as those who had smoked in the past but had stopped smoking before entry; quitters, defined as those who had smoked at entry but stopped smoking within 12 months after entry; and persistent smokers, defined as those who were smokers both at entry and at 12 months.

Monocyte Isolation and MTFA

Citrated venous peripheral blood was collected from MetS patients. Peripheral blood mononuclear cells (PBMC; monocytes and lymphocytes) were isolated as described previously.9,13,17 Briefly, PBMC were obtained by gradient centrifugation on a Lymphoprep (Nycomed, Oslo, Norway). PBMC in serum-free Roswell Park Memorial Institute (RPMI) media were incubated at 37°C and 5% CO2. The viability of the PBMC was >99%, as determined on trypan blue exclusion. PBMC were pipetted into 96-well plates, and 10⁶ PBMC were seeded per well. For all of the experiments, the isolated PBMC were used because other blood cells, such as lymphocytes, are not capable of expressing TF.9,13,17

We have previously shown that the TF activity is similar between PBMC and purified monocytes,13,17 indicating monocytes to be the principal cells capable of TF synthesis, and TF activity in PBMC to be equivalent to MTFA. Therefore, PBMC were routinely cultured for this study as previously described.9,13,17 TF activity (equivalent MTFA) in PBMC was observed after 4 h and reached an optimum level between 8 and 24 h, declining slightly thereafter.9 Therefore, PBMC were routinely cultured in 96-well plates for 16 h, washed twice with RPMI to remove lymphocytes, and tested. MTFA was measured using a 1-stage coagulation assay with an automatic coagulometer (Diagnostica Stago) as previously described,13,17 and MTFA was calculated from a standard curve using serial dilutions of human tissue thromboplastin (Thromborel-S, Behring) as a standard. The results are expressed as milliunits (mU) TF/10⁶ PBMC.

Table 2. Multivariate Indicators of Percent Change in mCIMT

| Indicator                                      | β     | P-value |
|-----------------------------------------------|-------|---------|
| Age                                          | −0.009| 0.830   |
| Gender (male)                                 | 0.102 | 0.044   |
| Smoking status at 12 months                   | 0.194 | <0.001  |
| Statins                                       | −0.168| <0.001  |
| CCB                                          | 0.107 | 0.015   |
| ACEi or ARB                                   | 0.045 | 0.239   |
| Pioglitazone                                  | −0.241| 0.005   |
| Sulfonylurea                                  | −0.034| 0.411   |
| α-Gl                                         | 0.045 | 0.250   |
| Percent change in SBP                         | −0.037| 0.520   |
| Percent change in heart rate                  | −0.002| 0.975   |
| Percent change in BMI                         | 0.178 | <0.001  |
| Percent change in WC                          | −0.005| 0.915   |
| Percent change in eGFR                        | −0.046| 0.272   |
| Percent change in TG                          | 0.009 | 0.875   |
| Percent change in HDL-C                       | −0.139| 0.005   |
| Percent change in LDL-C                       | 0.026 | 0.588   |
| Percent change in FPG                         | 0.014 | 0.787   |
| Percent change in HbA1c                       | −0.016| 0.721   |
| Percent change in HOMA-IR                     | 0.179 | <0.001  |
| Percent change in hsCRP                       | 0.035 | 0.424   |
| Percent change in MTFA                        | 0.266 | <0.001  |

Model adjusted R²=0.634. β, regression coefficient; TF, tissue factor; TG, triglycerides. Other abbreviations as in Table 1.

CIMT

The right and left carotid arteries were examined according to a standardized protocol by 1 blinded trained sonographer using B-mode ultrasound with a 10-MHz linear probe (LOGIQ 7; GE, Milwaukee, WI, USA). CIMT was measured at 3 points in each common carotid artery 10 mm proximal to the site of trypan blue exclusion. PBMC were pipetted into 96-well plates, and 10⁶ PBMC were seeded per well. For all of the experiments, the isolated PBMC were used because other blood cells, such as lymphocytes, are not capable of expressing TF.9,13,17

Follow-up and Outcome

The outcome data were collected through serial direct contact with the patients (every 4–8 weeks) at an outpatient clinic of Tama-Nagayama Hospital, Nippon Medical School, until December 2015. We were able to follow up all of the patients for a median period of 66.0 months (range, 26–105 months) to determine the incidence of atherothrombotic events, including AMI and stroke. None of the patients died from atherothrombotic events or dropped out of the present study.

Stroke was diagnosed in the event of any of the following: cerebral infarction or cerebral bleeding; or evidence of an acute disturbance in the focal neurological function with symptoms lasting >24 h and diagnosed by either or both computed tomography or magnetic resonance imaging. AMI was diagnosed in the event of the following: hospitalization due to chest pain lasting >20 min diagnosed on both electrocardiogram changes and biomarkers for myocardial necrosis, including an increase in creatine kinase and...
followed by the Bonferroni correction. Bivariate correlations between the parameters were assessed using Pearson’s or Spearman’s correlation (r) coefficient for normal or skewed distributions, respectively.

Percent change was defined as (12-month follow-up−baseline)/baseline × 100. The association between percent change in mCIMT and other variables was explored using a multiple linear regression with a forward stepwise selection of covariates. The examined variables included age; gender and smoking status at 12 months; percent change from baseline to 12 months in body mass index (BMI), WC, systolic blood pressure (SBP), heart rate, LDL-C and HDL-C, FPG, HbA1c, HOMA-IR, eGFR, hsCRP, and MTFA; and the use of statins, calcium channel blockers (CCB), angiotensin-converting enzyme inhibitors (ACEI), or angiotensin receptor blockers (ARB), sulfonylurea, pioglitazone, or α-glucosidase inhibitors (α-GI).

Kaplan-Meier survival curves were used to assess the positivity for cardiac troponin T.

### Statistical Analysis
The present study was originally designed to enroll more than 1,600 patients, because 400 patients were required in each group in order to achieve a power of 80% to detect a 20% difference in percent change in MTFA from baseline to 12 months. The results are presented as mean±SD for the continuous variables and as n (%) for the categorical variables. Student’s t-test for independent samples and chi-squared test were used to compare the continuous and categorical variables, respectively. Distributions of MTFA, hsCRP, and TG were skewed, and the Mann-Whitney test was used for unpaired comparisons between 2 groups, while Wilcoxon’s signed rank test was used for paired comparisons within groups, with the data expressed as median (IQR). Differences between the 4 groups were analyzed using 2-way analysis of variance (ANOVA)
incidence of atherothrombotic events such as AMI and stroke, according to smoking status, and log-rank test was used to compare the results between the 4 groups.

Univariate and multivariate Cox regression analyses for atherothrombotic events were used to calculate the estimated hazard ratio (HR) and 95% CI, where appropriate. Variables with P≤0.05 on univariate analysis were entered into a multivariate model. Model 1 consisted of age, gender, and smoking status at 12 months, and percent change from baseline to 12 months in BMI, HDL-C, HOMA-IR (a marker of insulin resistance) and MTFA; and the use of statins, CCB, or sulfonylureas. In addition, 

SPSS for Windows, version 22.0 (IBM, Tokyo, Japan), was used for all statistical analyses. P<0.05 was considered to indicate statistical significance.

**Results**

Of the 301 patients with MetS at entry, 108 (35.9%) were never smokers, 74 (24.6%) were past smokers, and 119 (39.5%) were current smokers. Of the 119 current smokers, 56 (47.1%) quit smoking within 12 months after entry, while the remaining 63 patients (52.9%) were persistent smokers.

Baseline clinical characteristics are listed in Table 1. The persistent smokers had significantly higher hsCRP and TG and MTFA, and had a higher prevalence of male gender and dyslipidemia, a lower prevalence of diabetes, and were less likely to be treated with pioglitazone than the never and past smokers and quitters. In addition, we noted linear trends in higher hsCRP and MTFA among persistent smokers, quitters, and past smokers vs. never smokers. No significant differences were observed, however, in HDL-C and HOMA-IR between the 4 groups.

On multivariate linear regression analysis, male gender and smoking status at 12 months; change from baseline to 12 months in BMI, HDL-C, HOMA-IR (a marker of insulin resistance) and MTFA; and the use of statins, CCB, or pioglitazone were each significantly and independently associated with the percent change in mCIMT from baseline to 12 months (Table 2).

**Change in Clinical Characteristics and Blood Chemistry After 12 Months**

Table 3 lists the clinical characteristics and blood chemistry at baseline and at 12 months, and the percent change from baseline to 12 months. Figure 1 shows the percent changes in MTFA (Figure 1A) and mCIMT (Figure 1B) from baseline to after 12 months.

After 12 months, BMI increased by 0.49%, 0.19%, and 0.39% from baseline in the never and past smokers and quitters, respectively, but decreased by 0.67% from baseline in persistent smokers (ANOVA; P=0.011). WC decreased by 2.65%, 1.77%, 1.21%, and 0.82% from baseline in never and past smokers, quitters, and persistent smokers, respectively (ANOVA; P=0.218). SBP decreased by 4.17%, 0.80%, and 0.87%, and 0.99%, from baseline in never and past smokers, quitters, and persistent smokers, respectively (ANOVA; P=0.01). LDL-C decreased by 2.95%, 1.07%, and 2.69% from baseline in never and past smokers, and quitters, respectively, but increased by 4.32% from baseline in persistent smokers (ANOVA; P=0.006, Table 3). In addition, HDL-C increased by 7.89%, 1.99%, and 5.10% from baseline in never and past smokers and quitters, respectively, but decreased by 3.77% from baseline in persistent smokers (ANOVA; P<0.001). HOMA-IR, a marker of insulin resistance, decreased by 28.2%, 11.3%, and 15.2%, from baseline in never and past smokers and quitters, respectively, but was almost unchanged in persistent smokers (down 1.07%, ANOVA; P<0.001, Table 3).

Interestingly, hsCRP markedly decreased in never smokers (by 28.2%) and past smokers and quitters (by 2.71% and 1.46%, respectively), but markedly increased in persistent smokers (by 21.3%; ANOVA, P<0.001). MTFA decreased by 6.12%, 5.91%, and 11.59% from baseline in never and past smokers and quitters, respectively, but markedly increased from baseline in persistent smokers (by 33.3%; ANOVA, P<0.001, Table 3, Figure 1A). Furthermore, mCIMT regressed by 2.78%, 0.33%, and 1.16% from baseline in never and past smokers and quitters, respectively, but progressed by 33.3% from baseline in persistent smokers (ANOVA; P<0.001, Table 3, Figure 1B).

**Clinical Characteristics, Smoking Status and Atherothrombosis Status**

With regard to atherothrombotic events, a total of 6 AMI
and 8 strokes occurred during a median follow-up of 66.0 months (range, 26–105 months). Clinical characteristics and smoking status according to atherothrombotic event status are given in Table 4.

Four AMI and 7 strokes occurred in 11 of the 63 persistent smokers (17.5%), 2 AMI were observed in 2 of the 56 quitters (1 each; 3.6%), and 1 stroke occurred among the 74 past smokers (1.4%). None of the never smokers had any atherothrombotic events during the follow-up period.

The patients with atherothrombotic events had higher white blood cell count, plasma hsCRP, and MTFA and a higher prevalence of persistent smoking than those who did not (Table 4). The overall clinical characteristics and blood chemistry, however, were similar between the groups (Table 4).

On Kaplan-Meier survival curves, persistent smoking was significantly related to an increased incidence of atherothrombotic events during follow-up (log-rank 28.76, P<0.001, Figure 2).

| Table 4. MetS Subject Characteristics vs. Atherothrombosis Status |
|---------------------------------------------------------------|
| **Atherothrombotic event (n=14)** | **No atherothrombotic event (n=287)** | **P-value** |
| --- | --- | --- |
| Age (years) | 71.4±12.2 | 68.1±9.7 | 0.233 |
| Men | 12 (85.7) | 197 (68.6) | 0.176 |
| Smoking status | | | <0.001 |
| Never smokers (%) | 0 (0) | 108 (37.6) | |
| Past smokers (%) | 1 (7.1) | 73 (25.4) | |
| Quitters (%) | 2 (14.3) | 54 (18.8) | |
| Persistent smokers (%) | 11 (78.6) | 52 (18.2) | |
| BMI (kg/m²) | 25.8±2.9 | 26.3±2.7 | 0.561 |
| WC (cm) | 94.5±5.9 | 96.6±6.5 | 0.232 |
| SBP (mmHg) | 132±4 | 133±10 | 0.695 |
| Heart rate (beats/min) | 73±3 | 71±5 | 0.171 |
| Hypertension (%) | 13 (92.9) | 260 (90.6) | 0.776 |
| Diabetes (%) | 8 (57.1) | 193 (67.2) | 0.562 |
| Dyslipidemia (%) | 14 (100) | 240 (83.6) | 0.138 |
| WBC (/m³) | 7,021±1,686 | 5,899±1,249 | 0.001 |
| Hemoglobin (g/dL) | 13.5±1.5 | 14.0±1.2 | 0.124 |
| hsCRP (mg/L) | 3.40 (3.20–3.63) | 1.80 (1.00–2.90) | <0.001 |
| LDL-C (mg/dL) | 121±31 | 120±24 | 0.858 |
| HDL-C (mg/dL) | 58±9 | 54±10 | 0.201 |
| Triglycerides (mg/dL) | 139 (131–251) | 152 (104–183) | 0.754 |
| FPG (mg/dL) | 121±22 | 131±32 | 0.270 |
| HbA1c (NGSP; %) | 5.9±0.4 | 6.0±0.8 | 0.583 |
| HOMA-IR | 3.1±1.5 | 5.1±3.3 | 0.186 |
| eGFR (mL/min/1.73m²) | 63.1±14.4 | 68.0±12.3 | 0.155 |
| MTFA (mU TF/10⁶ PBMC) | 90.4 (86.0–111.4) | 66.2 (47.2–99.4) | 0.012 |
| Statin | 7 (41.7) | 181 (62.1) | 0.399 |
| ACEI or ARB | 9 (75.0) | 182 (64.0) | 0.947 |
| CCB | 10 (58.3) | 224 (69.7) | 0.522 |
| Pioglitazone | 2 (16.7) | 99 (34.1) | 0.152 |
| Metformin | 2 (16.7) | 39 (13.4) | 0.941 |
| Sulfonylurea | 1 (8.3) | 19 (6.5) | 0.939 |
| α-GI | 7 (58.3) | 95 (32.6) | 0.247 |
| mCIMT (mm) | 1.07±0.14 | 1.03±0.17 | 0.282 |

Data given as n (%), mean±SD or median (IQR). Abbreviations as in Tables 1,2.
Smoking in Metabolic Syndrome

Predictors of Atherothrombotic Events

On multivariate Cox hazard analysis (model 1), smoking status at 12 months (HR, 3.20; 95% CI: 1.02–10.12, P=0.048) and MTFA (HR, 1.02; 95% CI: 1.00–1.04, P=0.046) were each significant and independent predictors of atherothrombotic events in patients with MetS (Table 5).

In addition, on multivariate Cox hazard analysis using model 2, smoking status at 12 months (HR, 2.47; 95% CI: 1.01–6.04, P=0.048), hsCRP at 12 months (HR, 11.74; 95% CI: 1.13–121.82, P=0.044) and MTFA at 12 months (HR, 1.02; 95% CI: 1.00–1.04, P=0.046) were each significant and independent predictors of atherothrombotic events in patients with MetS (Table 6).

### Table 5. Predictors of Atherothrombotic Events in MetS Patients (Model 1)

|                   | Univariate |                |          |          |          |          |          |
|-------------------|------------|----------------|----------|----------|----------|----------|----------|
|                   | HR         | 95% CI          | P-value  | HR       | 95% CI   | P-value  |
| Age               | 1.04       | 0.98–1.10       | 0.228    |          |          |          |
| Gender (male)     | 2.61       | 0.58–11.66      | 0.209    |          |          |          |
| Smoking status at 12 months | 4.51       | 1.93–10.53      | <0.001   | 3.20     | 1.02–10.12 | 0.048   |
| Statin use        | 0.63       | 0.22–1.79       | 0.381    |          |          |          |
| CCB               | 0.82       | 0.27–2.44       | 0.716    |          |          |          |
| ACEI or ARB       | 1.09       | 0.36–3.24       | 0.883    |          |          |          |
| Pioglitazone      | 0.37       | 0.08–1.67       | 0.198    |          |          |          |
| Sulfonylurea      | 0.99       | 0.13–7.63       | 0.997    |          |          |          |
| α-Gl              | 1.88       | 0.66–5.36       | 0.238    |          |          |          |
| Percent change in SBP | 1.08       | 0.93–1.25       | 0.346    |          |          |          |
| Percent change in heart rate | 1.00     | 0.89–1.13       | 0.973    |          |          |          |
| Percent change in WC | 1.04       | 0.92–1.19       | 0.520    |          |          |          |
| Percent change in eGFR | 1.11       | 0.92–1.33       | 0.270    |          |          |          |
| Percent change in HDL-C | 0.90       | 0.87–0.94       | <0.001   | 0.97     | 0.91–1.03 | 0.263   |
| Percent change in LDL-C | 1.04       | 1.01–1.07       | 0.010    | 1.02     | 0.97–1.07 | 0.570   |
| Percent change in FPG | 1.04       | 0.99–1.09       | 0.055    |          |          |          |
| Percent change in HbA1c | 1.08       | 0.98–1.18       | 0.107    |          |          |          |
| Percent change in HOMA-IR | 1.02       | 1.00–1.03       | 0.023    | 1.01     | 0.98–1.03 | 0.904   |
| Percent change in hsCRP | 1.01       | 1.01–1.02       | 0.008    | 1.01     | 0.99–1.02 | 0.775   |
| Percent change in MTFA | 1.03       | 1.01–1.04       | <0.001   | 1.02     | 1.00–1.04 | 0.046   |
| Percent change in mCIMT | 1.14       | 1.07–1.21       | <0.001   | 1.08     | 0.97–1.21 | 0.142   |

Abbreviations as in Tables 1, 2.

### Table 6. Predictors of Atherothrombotic Events in MetS Patients at 12 Months (Model 2)

|                   | Univariate |                |          |          |          |          |          |
|-------------------|------------|----------------|----------|----------|----------|----------|----------|
|                   | HR         | 95% CI          | P-value  | HR       | 95% CI   | P-value  |
| Age               | 1.04       | 0.98–1.10       | 0.228    |          |          |          |
| Gender (male)     | 2.61       | 0.58–11.66      | 0.209    |          |          |          |
| Smoking status at 12 months | 4.51       | 1.93–10.53      | <0.001   | 2.47     | 1.01–6.04 | 0.048   |
| Statin use        | 0.63       | 0.22–1.79       | 0.381    |          |          |          |
| CCB               | 0.82       | 0.27–2.44       | 0.716    |          |          |          |
| ACEI or ARB       | 1.09       | 0.36–3.24       | 0.883    |          |          |          |
| Pioglitazone      | 0.37       | 0.08–1.67       | 0.198    |          |          |          |
| Sulfonylurea      | 0.99       | 0.13–7.63       | 0.997    |          |          |          |
| α-Gl              | 1.88       | 0.66–5.36       | 0.238    |          |          |          |
| SBP at 12 months  | 1.03       | 0.96–1.10       | 0.490    |          |          |          |
| Heart rate at 12 months | 1.17       | 1.02–1.34       | 0.024    | 1.01     | 0.83–70.99 | 0.939   |
| WC at 12 months   | 0.97       | 0.90–1.05       | 0.492    |          |          |          |
| eGFR at 12 months | 0.97       | 0.93–1.02       | 0.194    |          |          |          |
| HDL-C at 12 months | 0.94       | 0.88–1.00       | 0.053    |          |          |          |
| LDL-C at 12 months | 1.02       | 0.99–1.04       | 0.149    |          |          |          |
| FPG at 12 months  | 0.99       | 0.98–1.02       | 0.878    |          |          |          |
| HbA1c at 12 months | 1.06       | 0.52–2.13       | 0.882    |          |          |          |
| HOMA-IR at 12 months | 0.94       | 0.79–1.13       | 0.471    |          |          |          |
| hsCRP at 12 months | 38.45      | 3.36–440.3      | <0.001   | 11.74    | 1.13–121.82 | 0.044  |
| MTFA at 12 months | 1.04       | 1.02–1.05       | <0.001   | 1.02     | 1.01–1.04 | 0.039   |
| mCIMT at 12 months | 22.09      | 2.08–235.1      | 0.010    | 1.68     | 0.04–78.38 | 0.793   |

Abbreviations as in Tables 1, 2.
Cigarette smoking is a major preventable risk factor for acute coronary thrombosis leading to AMI, stroke, and sudden cardiac death. Homeostasis maintains the integrity of the circulatory system after vascular injury and prevents the sequelae of uncontrolled hemorrhaging. This process regulates the intravascular balance of anti-thrombotic/prothrombotic factors and profibrinolytic/anti-fibrinolytic factors via interrelated functions of EC, platelets, and coagulation factors. Cigarette smoke exposure seems to alter the balance of anti-thrombotic/prothrombotic factors by affecting the functions of EC, platelets, and coagulation factors.

The mechanisms by which smoking increases the risk of atherothrombotic events are not fully understood. Cigarette smoking confers a hypercoagulable state. TF is expressed in EC and SMC and it is highly expressed in peripheral blood monocytes and macrophages in atherosclerotic plaque, forming a high-affinity complex with factors VII and VIIa. These complexes lead to thrombin formation, which in turn activates the clotting cascade and platelets. In addition, TF overexpression in a rat model accelerated neointimal development and thrombus formation. Therefore, TF plays a significant role in both thrombus formation and in the progression of atherosclerosis.

There are contradictory reports about the synthesis and expression of TF antigen/activity in the surrounding environment of EC. EC are well documented to synthesize TF by several agonists such as thrombin, lipopolysaccharide (LPS) and tumor necrosis factor (TNF)-α in vitro. A recent report by Pawlinski and Mackman, however, failed to show that TF on the endothelium may play any role in thrombin generation in a murine endotoxemia model.

In contrast to EC, SMC constitutively express TF in vitro and in vivo, providing the vessel with a hemostatic barrier. SMC have been implicated in the pathophysiology of unstable angina; and macrophages, as well as intimal SMC from human plaque from coronary and carotid endarterectomy specimens, express TF mRNA and protein. In normal arteries, little or no TF is found in the intima and media. TF is rapidly induced in the SMC, however, by a variety of agonists, including LPS, TNF-α and platelet-derived growth factor (PDGF). Scheter et al showed that human SMC synthesized TF after stimulation with PDGF.

Ostergard and Bjorklid showed that monocytes are the only cell type that can be induced to synthesize TF de novo, and that the only active TF in blood may be associated with circulating CD14-positive monocytes in 1–2% of healthy subjects. In addition, they also showed that human granulocytes acquired TF activity but did not synthesize it themselves, and found no detectable TF mRNA in resting or stimulated eosinophils. These data suggest that the main sources of TF may be peripheral blood monocytes and macrophages in atherosclerotic plaque, and that TF may also be expressed in SMC and EC.

MetS is associated with abdominal obesity, blood lipid disorders, inflammation, insulin resistance, hypercoagulable state, and an increased risk of developing atherothrombotic events, including AMI and stroke. In addition, low-grade inflammation associated with MetS leads to the onset of insulin resistance and type 2 diabetes mellitus.

Arterial wall thickening may be assessed in vivo on ultrasound measurement of CIMT, which is correlated with coronary and carotid atherosclerosis and is a significant predictor of future atherothrombotic events. For these reasons, CIMT has been widely used in many clinical studies as a surrogate marker for carotid and coronary atherosclerosis.

Although smoking is involved in atherothrombotic events, the pathophysiological mechanisms of smoking in the development of atherothrombotic diseases are complicated and not clearly understood. Furthermore, the pathophysiological mechanisms of smoking status in the development of atherothrombotic disease in patients additionally complicated with MetS are even more confusing and complicated.

In the present study, baseline hsCRP and MTAFA were significantly higher in persistent smokers than in quitters and past and never smokers. We also observed linear trends in higher baseline hsCRP, mCIMT and MTAFA in persistent smokers, quitters, and past smokers than in never smokers. After 12 months, hsCRP was decreased in never and past smokers as well as in quitters but markedly increased in persistent smokers. mCIMT and MTAFA decreased in never and past smokers and quitters, but markedly increased from baseline in persistent smokers.

In addition, on multivariate linear regression analysis, smoking status at 12 months, HDL-C, HOMA-IR, and MTAFA as well as the use of statins were each significantly and independently associated with percent change in mCIMT from baseline to 12 months in patients with MetS. This suggests that smoking status at 12 months and a persistent hypercoagulable state on MTAFA are significantly related to the pathogenesis and development of carotid atherosclerosis as determined by mCIMT, which was a surrogate marker of atherothrombotic disease in patients with MetS.

On Kaplan-Meier curve analysis, persistent smoking was significantly associated with an increased incidence of atherothrombotic events during the follow-up period. Furthermore, multivariate Cox hazard analysis (model 1) showed that smoking status at 12 months and percent increase in MTAFA from baseline to after 12 months were each significantly and independently correlated with the incidence of atherothrombotic events. In addition, a multivariate Cox hazard analysis (model 2) also showed that smoking status and MTAFA at 12 months were independently associated with events in patients with MetS. This indicates that a persistent hypercoagulable state according to MTAFA at 12 months and percent increase in MTAFA from baseline are significantly associated with the pathogenesis and development of carotid atherosclerosis in smokers, which may be related to an increased risk of atherothrombotic events in patients with MetS who smoke.

**Endothelial Dysfunction**

Cigarette smoke contains high levels of free radicals, and the scavenging activity of cigarette smoke-derived free radicals leads to a reduction in nitric oxide (NO) bioavailability. Therefore, smoking impairs endothelium-dependent vasodilation of coronary arteries, reduces coronary flow reserve, and induces coronary spasm.

The assessment of endothelium-dependent vasodilatation is widely used to evaluate endothelial function, and the
measurement of flow-mediated dilatation (FMD) of the brachial artery is most frequently used in clinical settings. Celermajer et al showed that continuous smoking impairs the FMD of the brachial artery in a dose-dependent manner.

**Thrombosis and Inflammation**

Exposure to cigarette smoke results in systemic inflammation, and smoking increases the levels of inflammatory markers, including hsCRP and TNF-α. Systemic inflammation has been linked to the process of plaque rupture and acute coronary syndrome and sudden cardiac death, highlighting the relevance of inflammation in mediating plaque instability.

Thrombosis and inflammation are interrelated and mutually reinforcing processes. Thrombosis promotes inflammation, and inflammation promotes thrombosis. An inflammatory response is involved in TF expression on monocytes.

In the present study, smoking status was significantly related to hsCRP level and to MTFA. On multivariate Cox hazard modeling, hsCRP at 12 months was a risk factor of atherothrombotic events. Furthermore, hsCRP was significantly and positively related to MTFA. In our previous report on MetS patients with carotid atherosclerosis based on CIMT ≥1.1 mm, hsCRP was significantly higher in patients with carotid atherosclerosis than in those without.

Therefore, a hypercoagulable state (upregulation of MTFA) associated with inflammation may play a pivotal role in the pathogenesis and development of carotid atherosclerosis and atherothrombotic disease in patients with MetS who smoke.

**Insulin Resistance in MetS**

Insulin resistance is more prevalent in smokers than in non-smokers. Smoking was found to be a risk factor for diabetes and incident MetS by exacerbating insulin resistance and central obesity. In the present study, HOMA-IR, a marker of insulin resistance, decreased from baseline to 12 months in never and past smokers and quitters but was almost unchanged in persistent smokers. In addition, the percent change in mCIMT and the progression of carotid atherosclerosis were significantly and independently related to HOMA-IR, suggesting that insulin resistance is associated with carotid atherosclerosis in MetS patients who smoke.

**Effect on Lipid Profiles**

Craig et al observed a statistically significant correlation between smoking and increased LDL-C but decreased HDL-C. Cigarette smoking enhances the oxidative modification of LDL-C, which has been shown to impair endothelial function. In the present study, LDL-C decreased from baseline in never and past smokers and quitters but increased in persistent smokers. In addition, HDL-C increased from baseline in never and past smokers and quitters but decreased in persistent smokers. These changes in the lipid profiles from baseline may play a major role in the development of atherothrombotic events in MetS patients who smoke.

Taken together, these present and previous findings indicate that smoking upregulates MTFA and advances carotid atherosclerosis and increases the risk of atherothrombotic events such as AMI and stroke in patients with MetS.

**Study Limitations**

Several limitations associated with the present study warrant mention. First, this study had a small subject group and a cross-sectional design. Future studies will be required to confirm these findings using large-scale clinical trials. Second, we did not measure endothelial function using reactive hyperemia peripheral arterial tonometry. Third, we did not estimate smoking status after 12 months.

**Conclusions**

Smoking upregulates MTFA and advances carotid atherosclerosis as estimated on CIMT and increases the risk of atherothrombotic events such as AMI and stroke in patients with MetS. Further studies are needed to confirm these findings.

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**Disclosures**

The authors declare no conflicts of interest.

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