IL-33/ST2 signaling promotes TF expression by regulating NF-κB activation in coronary artery endothelial microparticles

Yujuan Yuan[1], Hui Cheng[1], Jing Tao[1], Nijiati Muyesai[2]

[1]Department of Cardiology, People's Hospital of Xinjiang Uygur Autonomous Region, China
[2]Xinjiang Emergency Center, People's Hospital of Xinjiang Uygur Autonomous Region, China

Submitted: 20 April 2021; **Accepted:** 15 May 2021
Online publication: 22 May 2021

Arch Med Sci 2022
DOI: https://doi.org/10.5114/aoms/137377

Corresponding author:
Nijiati Muyesai
People's Hospital of Xinjiang Uygur Autonomous Region
120 Longquan Street
Urumqi 830001, Xinjiang, China
Tel.: +86-13899955322
E-mail: muyassar11@aliyun.com

Abstract

Introduction: Interleukin 33 (IL-33) was previously shown to induce angiogenesis and inflammatory activation of endothelial microparticles (EMPs). Tissue factor (TF) plays a central role in hemostasis and thrombosis.
Material and methods: The study analyzed the coronary blood of level of CD31+EMPs, TF protein and IL-33 protein in acute myocardial infarction (AMI) and stable coronary artery disease (SCAD) patients. Human coronary artery endothelial cells (HCAECs) were treated with IL-33 to obtain EMPs. The TF activity of EMPs was tested by Thermo Fisher by adding the TF antibody. Furthermore, TF and tissue factor pathway inhibitor (TFPI) protein were tested by ELISA. Finally, NF-κB inhibitor dimethyl fumarate (DMF) and soluble extracellular domain of ST2 coupled to the Fc fragment of human IgG1 (sST2) were added to HCAECs which were treated with IL-33, and the TF protein level was also tested by ELISA.
Results: The AMI patients had higher levels of CD31+EMPs, TF protein and IL-33 protein than the SCAD patients in the coronary artery. In AMI patients (n = 27), the IL-33 protein positively correlated with CD31+EMPs (r = 0.794, p < 0.01). According to the ROC curve analysis, the AUC of CD31+EMPs, TF protein and IL-33 protein were 0.888, 0.962 and 0.778 respectively. In the cell culture, the TF activity and TF protein in EMPs increased gradually with time of intervention by the treatment of IL-33. IL-33 binding to the ST2 receptor promoted TF expression by regulating NF-κB activation in EMPs of HCAECs.
Conclusions: Activated endothelial cells and EMPs they release simultaneously express TF, which is a risk factor for cardiovascular disease.

Key words: IL-33, TF, EMPs, AMI.

Introduction

Atherosclerosis is a chronic inflammatory disease of atherosclerotic plaque [1] that remains the leading cause of death worldwide. It is the leading contributor to coronary vascular disease (CVD), and its treatment is an essential step towards appropriate management and prevention of CVD [2]. The Burden of Disease Study in China showed a 20.6% increase in ischemic heart disease mortality from 1990 to 2017 [3]. According to the statistics of the American Heart Association, approximate-

ly 2.5 million people were hospitalized for acute myocardial infarction (AMI) each year, among whom 18% of women and 23% of men in a population with an average age of over 40 years died within one year of being diagnosed with AMI [4].

Microparticles (MPs) are cell membrane phosphatidylserine ranging from 0.1 to 1.0 µm, which contain information such as mRNA, microRNAs (miRNAs), receptor and specific proteins of the parent cell [5, 6]. Microparticles from endothelial cells, erythrocytes and platelets play an important role in the process of atherosclerosis [7, 8]. MPs shed from activated or apoptotic cells containing complex procoagulant and proinflammatory properties [9, 10]. The first step in the development of atherosclerotic lesions is endothelial dysfunction, which is a key factor in the development of coronary atherosclerosis disease [11]. Stimulating endothelial cells (ECs) to release endothelial microparticles (EMPs) can not only be used as an early alternative to endothelial dysfunction, but also as a biological mediator to regulate inflammation and coagulation after early EC injury.

Tissue factor (TF), an integral cell-surface glycoprotein and the major in vivo initiator of coagulation, plays a central role in hemostasis and thrombosis [12, 13]. Plaque rupture reveals TF to flowing blood, resulting in coronary thrombosis and occlusion with consequent AMI. Thrombosis is linked to inflammation in many clinical conditions [14].

Interleukin 33 (IL-33) is released in the extracellular space following cell injury [15]. IL-33 and ST2 are found locally in human atherosclerotic plaques [16]. Related studies have shown that circulating IL-33 levels are associated with thrombotic complications after rupture of coronary and carotid atherosclerotic plaques [17], and are associated with ST segment elevation myocardial infarction (STEMI) mortality [18, 19]. These studies showed that IL-33, locally expressed in atherosclerotic plaques, activates ECs by up-regulating the inflammatory system, through which it promotes leukocyte adhesion to ECs and thereby regulates EC proteolysis and promotes angiogenesis, which ultimately accelerates the development of atherosclerotic plaques [16, 20].

Inflammation and coagulation are interdependent, and jointly determine the formation of atherogenic plaque lesions and the clinical progression of arterial thrombosis complications such as AMI, unstable angina and stroke [21, 22]. Therefore, we investigated the effect of IL-33 on TF release of EMPs, which may be a new link between inflammation and coagulation.

Material and methods

Study population

The study population consisted of the patients admitted to the Department of Cardiology of People's Hospital of Xinjiang Uygur Autonomous Region in the period from June 2018 to January 2020. According to the inclusion and exclusion criteria, a total of 27 patients with AMI and 30 patients with stable coronary artery disease (SCAD) were included in this study. The trial was conducted in accordance with the Declaration of Helsinki. The study was approved by the Ethics Committee of the People's Hospital of Xinjiang Uygur Autonomous Region (No. 2017041), and all patients provided a signed informed consent form.

Inclusion criteria were as follows:

- AMI: measurement of elevated cardiac biomarkers (troponin preferred) that exceed the 99th percentile of the reference upper limit and fulfil at least one of the following conditions: 1) symptoms of myocardial ischemia; 2) new ischemic ECG changes; 3) pathological Q wave appeared in electrocardiogram; 4) imaging evidence of new loss of viable myocardium or new regional wall motion abnormality in a pattern consistent with an ischemic etiology; 5) identification of a coronary thrombus by angiography or autopsy;
- SCAD: a clinical syndrome of transient ischemic and hypoxia caused by increased myocardial load on the basis of fixed and severe coronary artery stenosis. Patients undergoing coronary angiography for the diagnosis of atherosclerotic heart disease and stent implantation were included in the study (refer to guidelines for the Diagnosis and Treatment of Stable Coronary Artery Disease, Chinese Journal of Cardiovascular Diseases, 2018).

The exclusion criteria were as follows: 1) serious liver or kidney dysfunction; 2) cancer or other debilitating disease; 3) diseases of the hematopoietic system; 4) uncontrolled infection; 5) infarction in another location of the body, such as cerebral infarction or pulmonary embolism; and 6) coronary artery spasm.

Sample collection

Circulating blood: Venous blood was collected within 24 h after admission for a general biochemical test.

Coronary blood: During PCI, the study subjects' coronary artery was entered via the radial artery during the operation and the guide wire reached the lesion site. The balloon entered the lesion plaque to dilate the balloon, which was suitable for the lesion vessels. After the balloon was rapidly discharged, 10 ml of coronary blood was extracted, and the balloon was evacuated from the guide wire. The specimens were centrifuged at 3500 × g 15 min at 4°C, and the supernatant was stored in the EP tube with EDTA of three grades. One sample was to obtain MPs and stored at –80°C

for qualitative and quantitative determination of MPs, and the remaining two samples were used for IL-33 and TF protein content detection.

Quantitation of CD31+EMPs by flow cytometry

The samples were dissolved at room temperature, 500 µl samples were taken from the EP tube, which were centrifuged at 2700 × g for 15 min and then 20,000 × g for 20 min at 4°C. After centrifugation, the supernatant was gently removed, and 100 µl of PBS was added. The extracted MPs were added with endothelial cell-specific monoclonal antibody (CD31) (1 : 50 dilution) at room temperature and incubated at 4°C for 30 min. Immediately after adding 200 µl of PBS, the BD FACS AccuriC6 flow cytometer was used for qualitative and quantitative MP detection.

The number of cells in the portal was 10000wh each time, which was read at a flow rate of 35 µl/min for 30 s. The number of CD31+EMPs was counted and the fluorescence percentage of ECs labelled with specific monoclonal antibodies was analyzed to further characterize CD31+EMPs. The final EMPs were expressed as percentages.

Tissue factor and IL-33 protein assays

Tissue factor protein levels in cell lysates were determined using a specific ELISA (Human TF, cusabio CSB-E07913h). The IL-33 protein levels were determined with a specific ELISA (Human IL-33, cusabio CSB-E13000h).

Cell culture

Human coronary artery endothelial cells (HCAECs) were purchased from GuangZhou Jennio Biotech Co. Ltd and cultured in M199 medium (Hyclone, SH30025) containing 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 µg/ml streptomycin. Cells were grown in 5% CO_2, 95% air in a humidified incubator at 37°C.

Treatment of cells

Human coronary artery endothelial cells were treated with 100 ng/ml of recombinant human (rh) IL-33 (peprotech, 200-33) and blank control for 3, 6, 9, 24 h respectively. In the experiment, the cell supernatant was collected and centrifuged at 500 g for 20 min to remove residual cells and pellets, and then the supernatant was transferred into a centrifuge tube and centrifuged at 2000 g for 20 min. The cell supernatant was transferred to a new centrifuge tube and centrifuged at 20000 g after 50 min, then the supernatant was removed, the precipitate was resuspended by adding PBS, and centrifugation was repeated once to precipitate the MPs.

For blocking the transmembrane receptor ST2, 5 µg/ml soluble extracellular domain ST2 coupled to the Fc fragment of human IgG1 (sST2) (G-Bioscience, BAN1479) and 5 g/ml IgG (Beyotime, A7028) were added to the pre-incubated cultured cells and shaken evenly. In addition, the NF-κB inhibitor dimethyl fumarate (DMF) (Selleck S6192), 100 ng/ml rh IL-33 and DMF+IL-33 upon being shaken evenly were cultured in an incubator. Upon the collection of the cell supernatant, the MPs were obtained by centrifugation with the same method as above. TF protein level in cell lysates was determined using the specific ELISA.

Tissue factor activity assays

100 µl PBS was added to resuscitate MPs. Tissue factor antibody (Ab) (absolute antibody, ab00516-10.6) and 1 µg/ml IgG (Beyotime, A7028) were added to the two groups, incubated at room temperature for 2 h. Then RIPA protein was lysed for 30 min for subsequent activity detection. After incubation in a 37°C incubator for 30 min, 20 µl FVIIa reaction substrate were added. The OD value of absorbance was detected at 0 min at OD 405 nm wavelength by a Thermo Fisher device (Multiskan 51119000). OD value was detected at intervals of 30 min until 2 h.

Tissue factor and TFPI protein assays

Human coronary artery endothelial cells were treated with 100 ng/ml of rh IL-33 and blank control for 3, 6, 9, 24 h. Samples of treated rh IL-33 were removed from the –80°C refrigerator and placed at room temperature. Detection of TF and tissue factor pathway inhibitor (TFPI) protein used the human TF (cusabio, CSB-E07913h) and human TFPI sandwich ELISA Kit (R&D Systems, DTFP10), respectively.

Standards and samples were pipetted into the wells, and TF and TFPI present were bound by the immobilized antibody. After removing any unbound substances, the biotin-conjugated antibody specific avidin conjugated horseradish peroxidase (HRP) was added to the wells. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution was added to the wells and color developed in proportion to the amount of TF and TFPI bound in the initial step. Absorbance read at 450 nm was compared to the values obtained with recombinant TF and TFPI standard.

Statistical analysis

Continuous variables were expressed as mean ± standard deviation, and the data were compared in terms of Student's t-test or rank sum test in the SPSS 21.0 statistical package for Windows. The χ^2 test was adopted to analyze the associa-

tion between categorical variables. Pearson's correlation coefficient was calculated to determine significant correlations. The value of $p \leq 0.05$ was considered significant.

Results

The baseline characteristics of the patients included in this study are shown in Table I according to AMI and SCAD. Striking differences between the two groups were observed for low-density lipoprotein cholesterol (LDL), Gensini score and the usage of prior statin. No significant differences were noted, such as age, sex, body BMI, hypertension, diabetes, smoking, TG, TC, HDL, CRP, Cr, Hb_{A1c}, LVEF, LVEDD, LVESD and the usage of prior antiplatelet.

Patients with AMI showed significantly higher levels of CD31+EMPs, TF protein and IL-33 protein than SCAD patients (11.10 (8.27, 13.20) vs. 3.92 (2.80,7.02), $p < 0.001$ for CD31+EMPs; 303.80 ±42.04 vs. 197.12 ±38.05, $p < 0.001$ for TF protein; 138.29 ±47.64 vs. 96.93 ±28.87, $p < 0.001$ for IL-33 protein) (Table II, Figure 1).

To test whether the level of IL-33 is associated with the level of CD31+EMPs or TF in coronary blood, we assessed the levels of IL-33, CD31+EMPs and TF in 27 patients with AMI. The levels of IL-33 protein and CD31+EMPs showed a significant positive correlation ($r = 0.794$, $p < 0.001$) (Figure 2 A). The level of IL-33 protein did not correlate with TF protein ($r = 0.064$, $p = 0.752$) (Figure 2 B).

To further investigate the efficiency of CD31+EMPs, TF protein and IL-33 protein as potential biomarkers of AMI, we performed ROC curve analysis on the patients with AMI. According to the outcome of ROC curve analysis, we found that the areas under the curve (AUC) of CD31+EMPs, TF protein and IL-33 protein were 0.888, 0.962, and 0.778 (Figure 3).

Table I. Clinical characteristics of AMI and SACD groups

Parameters	AMI (n = 27)	SCAD (n = 30)	$Z/t/\chi^2$-value	P-value
Age	58 ±12	62 ±8	1.402	0.166
Male/female	21/6	20/10	0.869	0.351
BMI [kg/m^2]	27.70 (24.54, 31.14)	25.85 (23.88, 28.17)	–0.703	0.482
Hypertension, n (%)	14 (51.85)	16 (53.33)	0.013	0.911
Diabetes, n (%)	10 (37.04)	7 (23.33)	1.275	0.259
Smoking, n (%)	18 (66.67)	14 (46.67)	2.369	0.129
CRP [mg/l]	3.49 (2.50, 14.05)	2.85 (2.50, 3.95)	–1.565	0.118
Cr [μmol/l]	69.9 (60.00, 78.05)	58.90 (54.03, 73.58)	–1.730	0.084
TG [mmol/l]	1.69 ±0.98	1.45 ±0.99	–0.925	0.359
TC [mmol/l]	4.48 ±1.47	4.31 ±1.26	–0.458	0.649
HDL [mmol/l]	0.97 ±0.31	0.88 ±0.04	–1.409	0.167
LDL [mmol/l]	3.18 ±1.14	2.10 ±0.83	–4.071	< 0.001
Hb_{A1c} (%)	6.58 ±1.56	6.35 ±1.27	0.617	0.540
LVEF (%)	50 (50, 56)	55 (49, 59)	–1.210	0.226
LVEDD [mm]	47 (45, 52)	48 (45, 51)	0.731	0.660
LVESD [mm]	29.81 ±3.98	27.41 ±7.90	1.419	0.162
Gensini score	62.13 ±38.29	21.45 ±18.33	5.198	< 0.001
Prior medication:				
Antiplatelet, n (%)	13 (48.15)	18 (60.00)	0.805	0.370
Statin, n (%)	6 (22.22)	17 (56.67)	7.005	0.008

AMI – acute myocardial infarction, SCAD – stable coronary artery disease, BMI – body mass index, CRP – C-reactive protein, Cr – creatinine, TG – triglycerides, TC – total cholesterol, LDL – low-density lipoprotein cholesterol, HDL – high-density lipoprotein cholesterol, LVEF – left ventricular ejection fraction, Hb_{A1C} – hemoglobin A1C, LVEDD – left ventricular end-diastolic diameter, LVESD – left ventricular end-systolic diameter

Table II. Levels of CD31+ EMPs, TF and IL-33 protein in patients with AMI and SCAD

Parameters	AMI	SCAD	Z/t-value	P-value
CD31+EMPs (%)	11.10 (8.27, 13.20)	3.92 (2.80, 7.02)	–5.019	< 0.001
TF [pg/ml]	303.80 ±42.04	197.12 ±38.05	–10.057	< 0.001
IL-33 [pg/ml]	138.29 ±47.64	96.93 ±28.87	–3.911	< 0.001

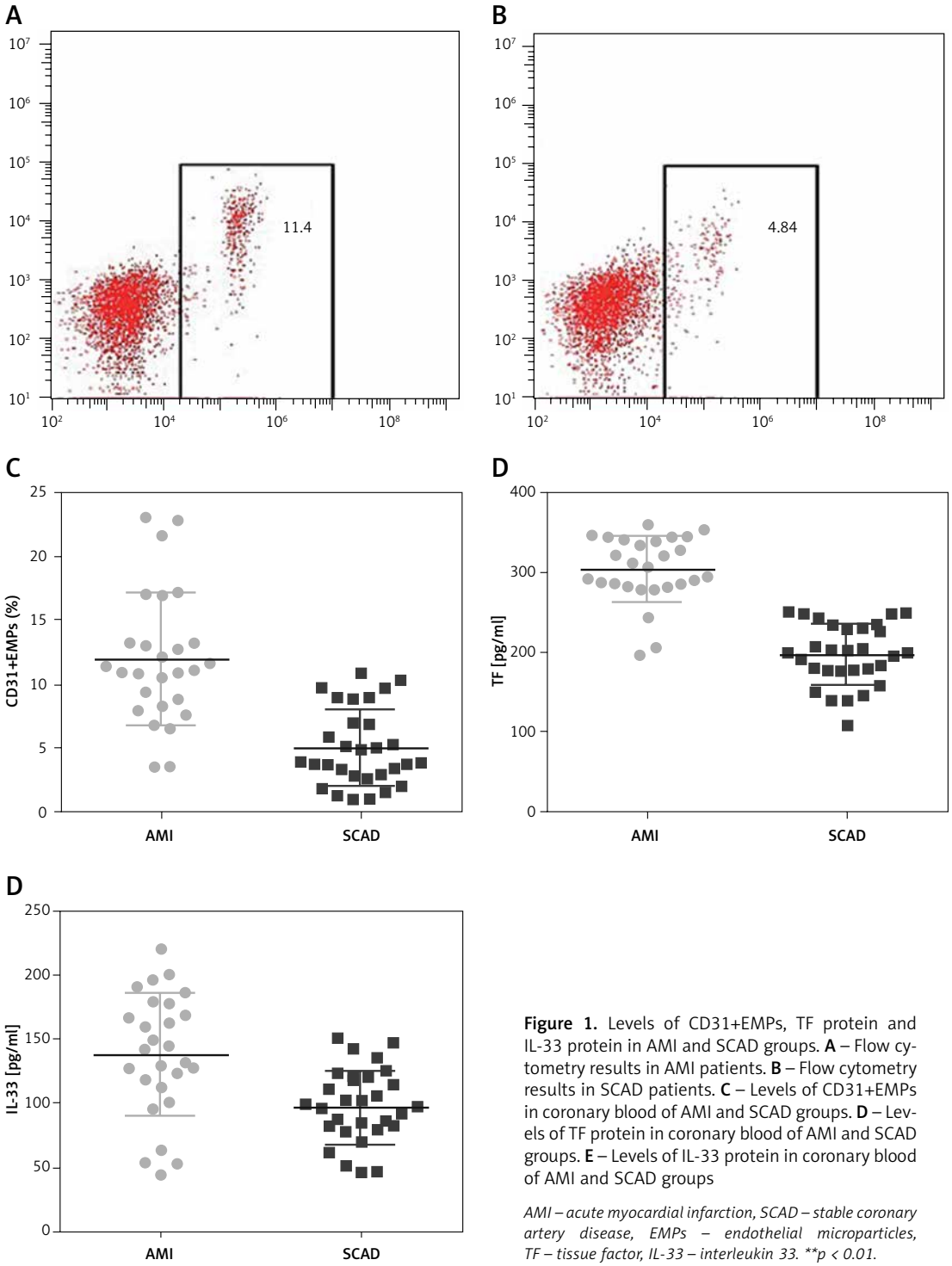

Figure 1. Levels of CD31+EMPs, TF protein and IL-33 protein in AMI and SCAD groups. **A** – Flow cytometry results in AMI patients. **B** – Flow cytometry results in SCAD patients. **C** – Levels of CD31+EMPs in coronary blood of AMI and SCAD groups. **D** – Levels of TF protein in coronary blood of AMI and SCAD groups. **E** – Levels of IL-33 protein in coronary blood of AMI and SCAD groups

*AMI – acute myocardial infarction, SCAD – stable coronary artery disease, EMPs – endothelial microparticles, TF – tissue factor, IL-33 – interleukin 33. **p < 0.01.*

Interleukin 33 increased TF activity of HCAEC derived MPs

Human coronary artery endothelial cells were treated with 100 ng/ml of rh IL-33 and blank control for 3, 6, 9, 24 h respectively. Microparticles were isolated from cell culture supernatants. Tissue factor Ab and IgG were added to the MPs of the two groups at each time point to test TF expression, which reflected the developmental stages that the TF was expressed. The results showed that the TF activity increased gradually with time of intervention (Figure 4). TF activity was significantly higher compared to controls ($p < 0.05$), but the time point of 24 h was not statistically significant.

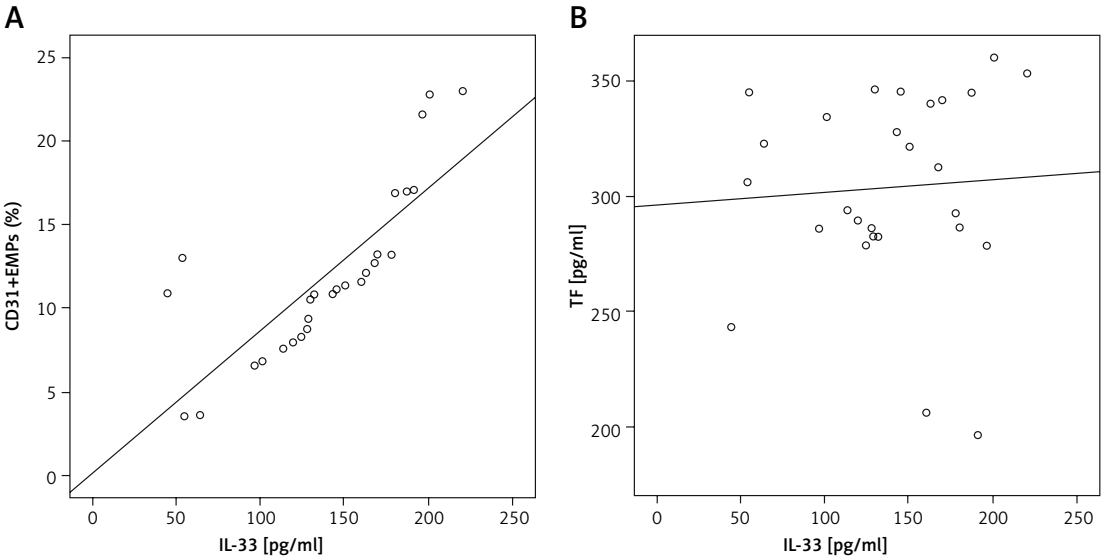

Figure 2. IL-33 is positively correlated with CD31+EMPs in patients with AMI. CD31+EMPs, IL-33 protein and TF protein were determined in coronary blood of patients with AMI. **A** – The level of IL-33 correlated with CD31+EMPs. **B** – In contrast, IL-33 did not correlate with TF protein. Pearson's correlation coefficient was calculated to determine significant correlations, $p < 0.05$ is considered significant

AMI – acute myocardial infarction, EMPs – endothelial microparticles, TF – tissue factor, IL-33 – interleukin 33.

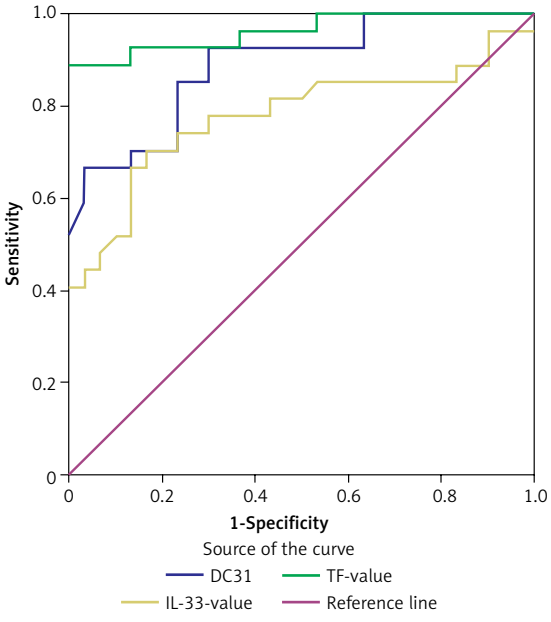

Figure 3. ROC curve analyses of CD31+EMPs, TF protein and IL-33 protein in AMI patients. We investigated the efficiency of CD31+EMPs, TF protein and IL-33 protein as potential biomarkers of AMI through ROC curve analysis. Areas under the curve (AUC) of EMPs, TF protein and IL-33 protein are 0.888, 0.962, and 0.778

AMI – acute myocardial infarction, EMPs – endothelial microparticles, TF – tissue factor, IL-33 – interleukin 33.

Interleukin 33 upregulated TF protein and downregulated TFPI protein in HCAEC derived MPs

Human coronary artery endothelial cells were treated with the presence or absence of rh IL-33. Microparticles were isolated from cell culture supernatants. TF and TFPI protein were tested by ELISA, and the expression was presented by the histogram. As Figure 5 shows, the viability of TF was significantly increased upon stimulation with rh IL-33 and the TFPI protein levels decreased conversely ($p < 0.05$).

Interleukin 33-induced TF expression ST2 and NF-κB

100 ng/ml DMF or 5 μg/ml sST2 was added to HCAECs which were treated with rh IL-33 and blank control. The TF protein level after adding DMF and sST2 was significantly lower compared to controls ($p < 0.05$) (Figure 6).

Discussion

Our results suggested that the levels of EMPs, TF protein and IL-33 protein in AMI were higher in patients with SCAD. We examined the diagnostic value of EMPs, TF protein and IL-33 protein in discriminating patients with AMI from patients with SCAD. We also found that IL-33 positively correlated with the level of CD31+EMPs in patients with AMI. No such correlation was found for the levels of IL-33 and TF. Furthermore, this evidence suggests that the higher levels of TF protein, EMPs and IL-33 protein could make them more likely to be potential biomarkers to distinguish patients with AMI from patients with SCAD.

In this study, we found that the pro-inflammatory cytokine IL-33 induced TF expression and TF activity in MPs of HCAECs as well as the release of

procoagulant EMPs. We found that IL-33 upregulated TF protein level and downregulated TFPI protein level in HCAEC MPs. Total cellular TF protein was increased in HCAECs after 3, 6, 9 and 24 h of treatment with IL-33 as compared to the control. TFPI protein levels slightly but significantly declined in MPs of HUVECs after 3, 6, 9 and 24 h of treatment with rh IL-33.

Other studies showed previously that IL-33 exerts its effects via binding to its cell surface receptor ST2 [16, 20]. In order to investigate whether the TF of EC-derived MPs induction by IL-33 was also ST2-mediated, we incubated EMPs with a specific anti-ST2 antibody in the presence or absence of rh IL-33, with the stimulatory effect of IL-33 on TF protein level inhibited. This indicated that the increased TF production was a specific effect of IL-33 on HCAECs, which could be blocked by sST2. In addition, the NF-κB inhibitor DMF was added to the HCAECs, which showed that IL-33/ST2 signaling promoted TF expression by regulating NF-κB activation.

High concentrations of EMPs may cause vascular damage and aggravate endothelial dysfunction. ECs and EMPs are the predominant sources of circulating blood-borne TF and contribute to the formation of a prothrombotic environment in patients with cardiovascular disease through the propagation of coagulation upon plaque rupture [23, 24].

Tissue factor expressed on the surface of MPs is the main activator of the blood coagulation pathway. More and more studies have found that TF plays an important role in the process of thrombosis on the basis of atherosclerosis. It is found that the expression of TF in coronary plaques of patients with acute coronary syndrome (ACS) is

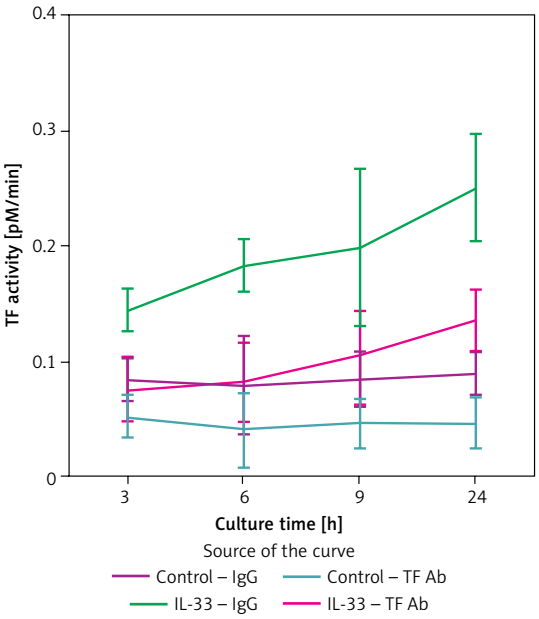

Figure 4. Tissue factor activity by treated TF antibody (Ab) and control. HCAECs were treated with 100 ng/ml of rh IL-33 and blank control to obtain MPs, and then TF Ab and IgG were added. The number of time-points examined for each TF reflects the developmental stages that the TF is expressed for 3, 6, 9, 24 h. The TF activity increased gradually with time of intervention for 3, 6, 9 h, but the time point of 24 h was not statistically significant

TF – tissue factor, HCAECs – human coronary artery endothelial cells, IL-33 – interleukin 33.

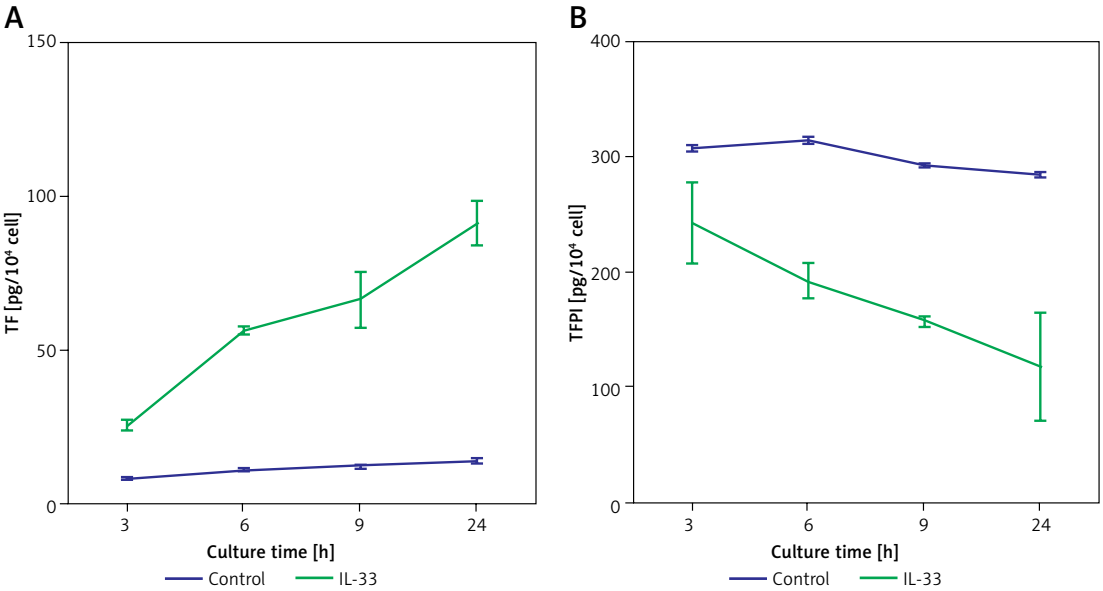

Figure 5. Human coronary artery endothelial cells incubated for 3, 6, 9, 24 h in the absence (Co) or presence of rh IL-33 (100 ng/ml). **A** – rh IL-33 induced TF expression and release of TF-positive MPs in HCAECs. **B** – rh IL-33 induced TFPI expression and release of TFPI-negative MPs in HCAECs

IL-33 – interleukin 33, TF – tissue factor, MPs – endothelial microparticles, HCAECs – human coronary artery endothelial cells, TFPI – tissue factor pathway inhibitor.

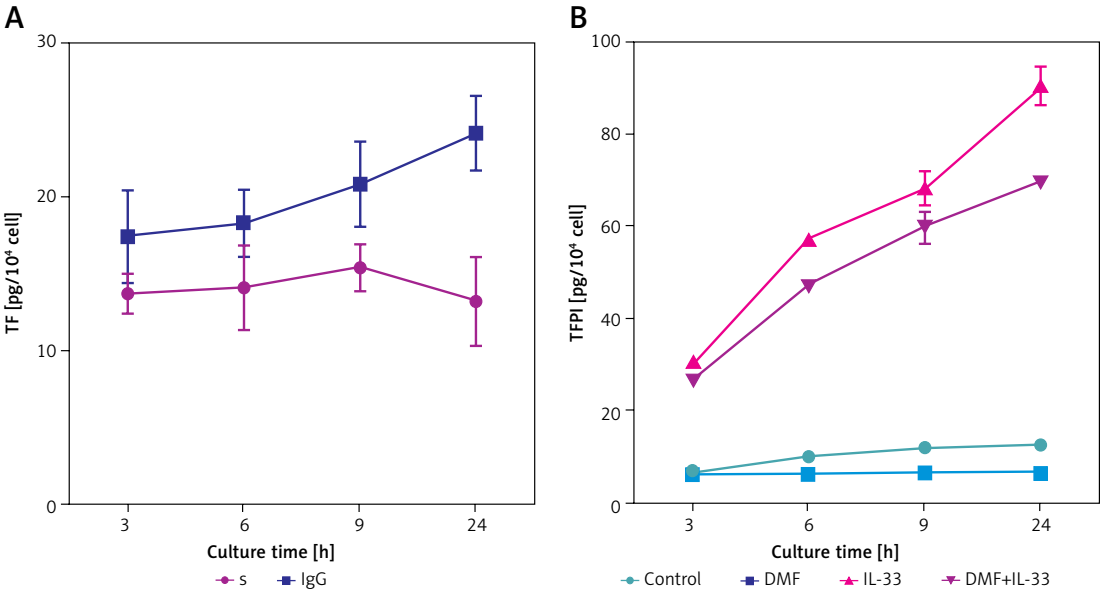

Figure 6. Human coronary artery endothelial cells incubated for 3 h, 6 h, 9 h, 24 h in the absence (Co)/presence of DMF (100 ng/ml), 5 µg/ml sST2 or rh IL-33 (100 ng/ml). **A** – Isolated MPs were incubated in the absence (Co) or presence of sST2 (5 µg/ml). **B** – Isolated MPs were incubated in the absence (Co) or presence of DMF (100 ng/ml) and rh IL-33 (100 ng/ml)

DMF – dimethyl fumarate, MPs – endothelial microparticles, IL-33 – interleukin 33.

higher than that of patients with stable angina [25]. Tissue factor not only promotes the generation of thrombin and the formation of fibrin, but also causes instability of atherosclerotic plaques without depending on the coagulation mechanism. The process includes TF, which leads to vascular smooth muscle migration, vascular hyperplasia, activation of protease receptor and an inflammatory response [26, 27].

A prospective study suggested that the levels of TF and MPs could serve as biomarkers for thrombosis risk [28]. Activated ECs and their released MPs simultaneously express TF, which is a risk factor for cardiovascular disease [3].

Studies have confirmed that IL-33 binds to the ST2 receptor and activates the NF-κB pathway [16, 20], which causes the expression of TF on the surface of coronary artery endothelial cells and umbilical vein endothelial cells and their source MPs. Researchers found that the expression level of TF mRNA was positively correlated with the expression of IL-33 mRNA in carotid atherosclerotic plaques [29], which proves that IL-33 acts on the TF activity at the endothelial cell surface, enhances the coagulation function of endothelial cell and mediates the formation of thrombus in atherosclerotic plaques [29].

In summary, in this study we observed that the levels of EMPs, TF protein and IL-33 were raised in the AMI patients and IL-33 was positively correlated with the circulating levels of CD31+EMPs in the AMI patients. In addition, it presents evidence of ST2/NF-κB mediated up-regulation of TF protein expression and activity in HCAEC MPs after treatment with IL-33. Furthermore, it suggests that IL-33 treatment increased the release of procoagulant HCAECs-derived MPs. The above findings provide a possible pathophysiologic explanation for a clinical association between IL-33 and atherosclerosis thrombotic events in patients with cardiovascular disease.

In our study the levels of EMPs, TF protein and IL-33 were raised and IL-33 was positively correlated with the circulating levels of CD31+EMPs in the AMI patients. In addition, we present evidence of ST2/NF-κB mediated up-regulation of TF protein expression and activity in HCAECs MPs after treatment with IL-33. Furthermore, it suggests that IL-33 treatment increased the release of procoagulant HCAEC-derived MPs.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (No: 82060076). Project of People's Hospital of Xinjiang Uygur Autonomous Region (No: 20190207).

Conflict of interest

The authors declare no conflict of interest

References

1. Hansson GK, Hermansson A. The immune system in atherosclerosis. Nat Immunol 2011; 12: 204-12.
2. Hirata T, Arai Y, Takayama M, Abe Y, Ohkuma K, Takebayashi T. Carotid plaque score and risk of cardiovascular mortality in the oldest old: results from the TOOTH study. J Atheroscler Thromb 2018; 25: 55-64.

3. Zhou M, Wang H, Zeng X, et al. Mortality, morbidity, and risk factors in China and its provinces, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet 2019; 394: 1145-58.
4. WHO The Atlas of Heart Disease and Stroke [Internet] WHO. Availablefrom: http://www.who.int/cardiovascular_diseases/resources/atlas/en.
5. Xu R, Greening DW, Zhu HJ, Takahashi N, Simpson RJ. Extracellular vesicle isolation and characterization:toward clinical application. J Clin Invest 2016; 126: 1152-62.
6. Nomura S, Shimizu M. Clinical significance of procoagulant microparticles. J Intensive Care 2015; 3: 2.
7. Robinson A, McCarty D, Douglas J. Novel oral anticoagulants for acute coronary syndrome. Ther Adv Cardiovasc Dis 2016; 11: 4-11.
8. Ferraris VA. Microparticles: the good, the bad, and the ugly. Thorac Cardiovasc Surg 2015; 149: 312-3.
9. VanWijk MJ, VanBavel E, Sturk A, Nieuwland R. Microparticles in cardiovascular diseases. Cardiovasc Res 2003; 59: 277-87.
10. Puddu P, Puddu GM, Cravero E, Muscari S, Muscari A. The involvement of circulating microparticles in inflammation, coagulation and cardiovascular diseases. Can J Cardiol 2010; 26: 140-5.
11. Gutiérrez E, Flammer AJ, Lerman LO, Elízaga J, Lerman A, Fernández-Avilés F. Endothelial dysfunction over the course of coronary artery disease. Eur Heart J 2013; 34: 3175-81.
12. Darbousset R, Thomas GM, Mezouar S, et al. Tissue factor-positive neutrophils bind to injured endothelial wall and initiate thrombus formation. Blood 2012; 120: 2133-43.
13. Chu AJ. Tissue factor upregulation drives a thrombosis-inflammation circuit in relation to cardiovascular complications. Cell Biochem Funct 2006; 24: 173-92.
14. Esmon CT. The interactions between inflammation and coagulation. Br J Haematol 2005; 131: 417-30.
15. Lin W, Zhou Q, Liu C, Ying M, Xu S. Increased plasma IL-17, IL-31, and IL-33 levels in chronic spontaneous urticarial. Sci Rep 2017; 7: 17797.
16. Demyanets S, Konya V, Kastl SP, et al. Interleukin-33 induces expression of adhesion molecules and inflmmatory activation in human endothelial cells and in human atherosclerotic plaques. Arterioscler Thromb Vasc Biol 2011; 31: 2080-9.
17. Stojkovic S, Thulin A, Hell L, et al. IL-33 stimulates the release of procoagulant microvesicles from human monocytes and differentially increases tissue factor in human monocyte subsets. Thromb Haemost 2017; 117: 1379-90.
18. Demyanets S, Speidl WS, Tentzeris I, et al. Soluble ST2 and interleukin-33 levels in coronary artery disease: relation to disease activity and adverse outcome. PLoS One 2014; 9: e95055.
19. Dhillon OS, Narayan HK, Khan SQ, et al. Pre-discharge risk stratifiation in unselected STEMI: is there a role for ST2 or its natural ligand IL-33 when compared with contemporary risk markers? Int J Cardiol 2013; 167: 2182-8.
20. Stojkovic S, Kaun C, Heinz M, et al. Interleukin-33 induces urokinase in human endothelial cells-possible impact on angiogenesis. J Thromb Haemost 2014; 12: 948-957.
21. De Caterina R, Husted S, Wallentin L, et al. General mechanisms of coagulation and targets of anticoagulants (Section I). Position Paper of the ESC Working Group on Thrombosis – Task Force on Anticoagulants in Heart Disease. Thromb Haemost 2013; 109: 569-79.
22. Stumpf C, Sheriff A, Zimmermann S, et al. C-reactive protein levels predict systolic heart failure and outcome in patients with first ST-elevation myocardial infarction treated with coronary angioplasty. Arch Med Sci 2017; 13: 1086-93.
23. Giesen PL, Rauch U, Bohrmann B, et al. Blood-borne tissue factor: another view of thrombosis. Proc Natl Acad Sci USA 1999; 96: 2311-5.
24. Mallat Z, Benamer H, Hugel B, et al. Elevated levels of shed membrane microparticles with procoagulant potential in the peripheral circulating blood of patients with acute coronary syndromes. Circulation 2000; 101: 841-3.
25. Annex BH, Denning SM, Channon KM, et al. Differential expression of tissue factor protein in directional atherectomy specimens from patients with stable and unstable coronary syndromes. Circulation 1995; 91: 619-22.
26. Breitenstein A, Tanner FC, Lüscher TF. Tissue factor and cardiovascular disease: quo vadis? Circ J 2010; 74: 3-12.
27. Badimon L, Vilahur G. Thombosis formation on atherosclerotic lesions and plaque rupture. J Intern Med 2014; 276: 618-32.
28. Owens AP, Mackman N. Microparticles in hemostasis and thrombosis. Circ Res 2011; 108: 1284-97.
29. Stojkovic S, Kaun C, Basilio J, et al. Tissue factor is induced by interleukin-33 in human endothelial cells: a new link between coagulation and inflammation. Sci Rep 2016; 6: 25171.