IL-33 / ST2 Signaling Promotes TF Expression by Regulating NF-κB Activation in Coronary Artery Endothelial Microparticles of Acute Myocardial Infarction

Yujuan Yuan
People's Hospital of Xinjiang Uygur Autonomous Region
Hui Cheng
People's Hospital of Xinjiang Uygur Autonomous Region
Jing Tao
People's Hospital of Xinjiang Uygur Autonomous Region
Nijiati Muyesai (muyassar11@aliyun.com)
People's Hospital of Xinjiang Uygur Autonomous Region

Research

Keywords: IL-33, TF, EMPs, AMI

Posted Date: August 18th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-58732/v1

IL-33 / ST2 Signaling Promotes TF Expression by Regulating NF-κB Activation in Coronary Artery Endothelial Microparticles of Acute Myocardial Infarction

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

Author affiliations:

[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

*Correspondence: 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.

Contributions:

(I) Conception and design: All authors;

(II) Administrative support: All authors;

(III) Provision of study materials or patients: Yujuan Yuan, Nijiati Muyesai;

(IV) Collection and assembly of data: Yujuan Yuan, Jing Tao;

(V) Data analysis and interpretation: Yujuan Yuan, Hui Cheng, Nijiati Muyesai

(VI) Manuscript writing: Yujuan Yuan, Nijiati Muyesai

(VII) Final approval of manuscript: All authors.

Abstract

Background: Interleukin (IL)-33 was previously shown to induce angiogenesis and inflammatory activation of endothelial cells derived Microparticles（EMPs）. Tissue factor (TF) plays a central role in hemostasis and thrombosis.

Objective: The aim of this study was to investigate the effect of IL-33 on TF release of EMPs, which may be a new link between inflammation and coagulation.

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 MPs. The TF activity of EMPs was tested by thermo

fisher by adding the TF antibody. Furthermore, TF and 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 HCAECs, which were treated with IL-33, then the TF protein level also was tested by ELISA.

Results：The AMI patients have higher level of CD31+EMPs, TF protein and IL-33 protein than the SCAD patients in coronary blood. 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 areas under the curve (AUC) of CD31+EMPs, TF protein and IL-33 protein were 0.888, 0.962 and 0.778. In the cell culture, the TF activity and TF protein in ECs-derived MPs 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 ECs-derived MPs of HCAECs.

Conclusion：Activated endothelial cells and their released MPs simultaneously express TF, which is a risk factor for cardiovascular disease**.**

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

1. INTRODUCTION

Atherosclerosis(AS) remains the leading cause of death worldwide，which is a chronic inflammatory disease of atherosclerotic plaque[1]. As is the leading contributor to Coronary Vascular Disease(CVD), and treatment of atherosclerosis is an essential step towards appropriate management and prevention of CVD[2]. The Burden of disease study in China shows a 20.6% increase in ischemic heart disease mortality from 1990 to 2017[3]. Coronary heart disease is caused by coronary atherosclerosis that leads to occlusion and stenosis of the coronary arteries leading to myocardial ischemia and hypoxia in patients[4]. According to the Statistics of the American Heart Association, approximately 2.5 million people are hospitalized for Acute Myocardial Infarction (AMI) each year, in which 18% of women and 23% of men in a population with an average age of over 40 years die within one year of being diagnosed with AMI[5].

Microparticles (MPs) are cell membrane phosphatidylserine raging from 0.1 to 1.0μm，which are containing information like mRNA, microRNAs (miRNAs), receptor and specific proteins of parent cell[6,7]. MPs from endothelial cells, erythrocytes, monocytes, smooth muscle cells and platelets play an important role in the process of atherosclerosis[8,9]. MPs shed from activated or apoptotic cells contain complex procoagulant and proinflammatory properties[10,11]. Endothelial microparticles (EMPs) are complex vesicular structures shed from endothelial cells (ECs) to the circulation. The first step in the development of atherosclerotic lesions is endothelial dysfunction, which is a key factor in the development of coronary atherosclerosis disease[12]. There are many conditions that cause endothelial cell dysfunction, such as diabetes, dyslipidemia, hypertension, smoking, and aging. However, stimulating ECs to release 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 ECs 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[13]. Plaque rupture reveals TF to flowing blood, resulting in coronary thrombosis and occlusion with consequent AMI. TF, the major cellular initiator of the coagulation protease cascade, plays a important role in both thrombosis and inflammation[14]. Thrombosis and inflammation are linked in many clinical conditions[15].

Interleukin-33（IL-33）is released in the extracellular space following cell injury[16]. IL-33 and ST2 are found locally in human atherosclerotic plaques[17]. The binding of IL-33 to the ST2 receptor increases vascular permeability and promotes the production of inflammatory cytokines and vascular proliferation, which can activate the inflammatory response[18]. Related studies have shown that circulating IL-33 levels are associated with thrombotic complications after rupture of coronary and carotid atherosclerotic plaques[19], and are associated with STEMI mortality[20,21]. The level of IL-33 in circulating blood after Percutaneous Coronary Intervention (PCI) was associated with coronary stent stenosis[22]. These studies find that IL-33 is locally

expressed in atherosclerotic plaques, activates endothelial cells by up-regulating the inflammatory system, promotes leukocyte adhesion to endothelial cells, thereby regulating endothelial cell proteolysis and promoting angiogenesis, and ultimately accelerates the development of atherosclerotic plaques [17,23].

Inflammation and coagulation are interdependent, which jointly determine the formation of atherogenic plaque lesions and the clinical progress of arterial thrombosis complications such as AMI, unstable angina and stroke[24]. Therefore, we investigated the effect of IL-33 on TF release of EMPs, which may be a new link between inflammation and coagulation.

2. MATERIALS AND METHODS

2.1 Study population

The study population were that patients admitted to the Department of Cardiology of People's Hospital of Xinjiang Uygur Autonomous Region from June 2018 to January 2020. According to 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 People's Hospital of Xinjiang Uygur Autonomous Region (No.2017041), and all patients provided a signed informed consent form.

Inclusion criteria:

(1) AMI: Measurement of elevated cardiac biomarkers (troponin preferred) that exceed the 99th percentile of the reference upper limit and contain at least one of the following conditions: ①Symptoms of myocardial ischemia ②New or presumed significant ST-segment changes or new left bundle branch block ③Pathological Q wave appeared in electrocardiogram ④Coronary angiography revealed coronary thrombosis.

(2) 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: ①Serious liver or kidney dysfunction ② Cancer or other debilitating disease ③Diseases of the haematopoietic system ④ Uncontrolled infection ⑤Infarction in another location of the body, such as cerebral infarction or pulmonary embolism ⑥Coronary artery spasm.

2.2 Sample Collection

Circulating blood: venous blood was collected within 24 hours after admission for general biochemical test.

Coronary blood: During PCI, the study subjects entered the coronary artery 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, 10ml of coronary blood was extracted, and the balloon was evacuated from the guide wire. The specimens were stored in a container containing EDTA in three grades. One sample was centrifuged at 20,000×g 20 min at 4℃ to obtain MPs and stored at -80℃ for qualitative and quantitative determination of MPs, and the remaining two samples were used for IL-33 and TF protein content detection.

2.3 Quantitation of CD31+ EMPs by flow cytometry

The samples were dissolved at room temperature. 500ul samples were taken from the EP tube and centrifuged at 2700×g 4℃ for 15min, and the supernatant was transferred to the new EP tube. After centrifugation, the supernatant was gently removed, 100 ul 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℃ for 30min. Immediately after adding 200 ul PBS, the BD FACS AccuriC6 flow cytometer is used for qualitative and quantitative MPs detection.

The number of cells in the portal was 10000wh each time, reading at a flow rate of 35μl /min for 30 seconds, counting the number of CD31+EMPs and analyzing the fluorescence percentage of endothelial cells labeled with specific monoclonal antibodies to further characterize CD31+EMPs. The final EMPs are expressed as

percentages.

2.4 TF and IL-33 protein assays

TF 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).

2.5 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), 100U / mL penicillin and 100ug / mL streptomycin. Cells were grown in 5% CO2, 95% air humidified incubator at 37°C.

2.6 Treatment of cell

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

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

2.7 TF activity assays

Add 100ul PBS and resuscitate MP. TF Antibody (Ab) (Absolute antibody, ab00516-10.6) and 1ug/ml IgG (Beyotime, A7028) were added to the two groups, incubated at room temperature for 2h, then RIPA protein was lysed for 30min for subsequent activity detection. After incubation in a 37℃ incubator for 30min, add 20 leds FVIIa reaction substrate. The OD value of absorbance was detected at 0min at OD 405nm wave length by Thermo fisher (Multiskan 51119000). OD value was detected at intervals of every 30min until 2h.

2.8 TF and TFPI protein assays

HCAECs were treated with 100ng/ml of rh IL-33and blank control for 3,6,9,24h. Samples of rh IL-33 treating were removed from the -80℃ refrigerator and placed at room temperature. Detection of TF and TFPI protein used the Human TF（cusabio，CSB-E07913h ）and Human TFPI sandwich ELISA Kit（R&D Systems，DTFP10），respectively.

Antibody specific for TF and TFPI have been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any TF, TFPI present are bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for TF is added to the wells. After washing, avidin conjugated Horseradish Peroxidase (HRP) is added to the wells. An enzyme-linked polyclonal antibody specific for human TFPI is added to the wells. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of TF and TFPI bound in the initial step. The color development is stopped and the intensity of the color is measured，Absorbance read at 450nm was compared to those values obtained with recombinant TF and TFPI standard.

2.9 **Statistical analysis**: Continuous variables are expressed as mean ± standard deviation, and the data were compared Student's t-test or ANOVA in SPSS 21.0 statistical package for Windows. Values of $p \leq 0.05$ were considered significant.

3. RESULTS

3.1 The baseline characteristics of the patients including in this study were shown in

Table 1 according to AMI and SCAD. The striking difference between the two groups was observed in the low-density lipoprotein cholesterol (LDL). No significant differences were noted between the groups in other characteristics, such as age, sex, body mass index (BMI), hypertension, diabetes, smoking, triglycerides(TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL), C-reactive protein(CRP), creatinine(Cr) and left ventricular ejection fraction (LVEF).

Table1 The clinical characteristic of AMI and SACD group

	AMI(n=27)	SCAD(n=30)	*Z/t/* x^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	16	0.013	0.911
Diabetes (n)	10	7	1.275	0.259
Smoking (n)	18	14	2.369	0.129
CRP(mg/l)	3.49[2.50,14.05]	2.85[2.50,3.95]	-1.565	0.118
Cr(umol/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	0.000
LVEF%	50[50,56]	55[49,59]	-1.21	0.226

AMI: acute myocardial infarction，SCAD: stable coronary artery disease，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.

3.2 AMI patients showed significantly higher levels of CD31+EMPs, TF protein and IL-33 protein than SCAD patients (11.10[8.27, 13.20] versus 3.92[2.80,7.02], P < 0.001 for CD31+EMPs. 303.80.12±42.04 versus 197.12±38.05, P<0.001 for TF protein. 138.29.12±47.64 versus 96.93±28.87, P<0.001 for IL-33 protein [Table 2 and Figure1].

Table2 The levels of CD31+ EMPs, TF and IL-33 protein in patients with AMI and SCAD

	AMI	SCAD	*Z/t* Value	*P* Value
CD31+EMPs%	11.10[8.27,13.20]	3.92[2.80,7.02]	-5.019	0.000
TF(pg/ml)	303.80.12±42.04	197.12±38.05	-10.057	0.000
IL-33(pg/ml)	138.29.12±47.64	96.93±28.87	-3.911	0.000

3.3 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 significant positive correlation ($r = 0.794$, $p < 0.01$) [Figure2a]. The level of IL-33 protein did not correlate with TF protein ($r=0.064$, $p=0.752$) [Figure2b].

3.4 To further investigate the efficiency of CD31+EMPs, TF protein and IL-33 protein as potential biomarkers of AMI, we performed ROC curve analysis between patients with AMI and SCAD. 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].

3.5 IL-33 increased TF activity of HCAEC derived MPs

HCAECs were treated with 100ng/ml of rh IL-33 and blank control. MPs were isolated from cell culture supernatants. TF Ab and IgG were added to the MPs of two groups. The number of time-points examined for each TF reflects the developmental stages that the TF is expressed, totaling 4 conditions (TF and time point). 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 24h was not statistically significant.

3.6 IL-33 upregulates TF protein and downregulates TFPI protein in HCAEC derived MPs

HCAECs were treated with the presence or absence of rh IL-33. MPs were isolated from cell culture supernatants. TF and TFPI protein were tested by ELISA. Histogram representation of protein expression for TF and TFPI. The viability of TF was significantly increased upon stimulation with rh IL-33, Conversely, TFPI protein levels decreased [Figure 5].

3.7 IL-33-induced TF expression ST2 and NF-κB

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

4. DISCUSSION

Our results suggest that the level 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 could also show that IL-33 positively correlated with the level of CD31+ EMPs in patients with AMI. No such correlation was found for the level of IL-33 and TF. Furthermore, this evidence demonstrated that the high levels of TF protein, EMPs and IL-33 protein could more likely to be potential biomarkers to distinguish patients with AMI from patients with SCAD.

In our study, we demonstrate that the pro-inflammatory cytokine IL–33 induces TF expression and TF activity in MPs of HCAECs as well as the release of procoagulant EC-derived MPs. We find that IL-33 up regulates TF protein level and down regulates TFPI protein level in HCAECs MPs. Total cellular TF protein was increased in HCAECs after 3, 6, 9 and 24 hours (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.

The other studies showed previously that IL-33 exerts its effects via binding to its cell surface receptor ST2 [25,26]. In order to investigate if the TF of EC-derived MPs induction by IL-33 was also ST2-mediated, we incubated ECs MPs with a specific anti-ST2 antibody in the presence or absence of rh IL-33, the stimulatory effect of IL-33 on TF protein level was 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 were added 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. EC and EC-derived MPs 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 [27, 28].

TF expressed on the surface of MPs are the main activator of 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. Study has found that the expression of TF in coronary plaques of patients with acute coronary syndrome (ACS) is higher than that of patients with stable angina[29]. TF not only promotes the generation of thrombin and the formation of fibrin, but also causes instability of atherosclerotic plaques without dependence on the coagulation mechanism. The process includes TF causing vascular smooth muscle migration, vascular hyperplasia, activation of protease receptor and inflammatory response[30,31].

A prospective study suggested that the level of TF and MPs could serve as biomarkers for thrombosis risk [32]. Activated endothelial cells and their released MPs simultaneously express TF, which is a risk factor for cardiovascular disease [30].

Studies have confirmed that IL-33 binds to the ST2 receptor and activates the NF-κB pathway[25,33], which causes the expression of TF on the surface of coronary artery endothelial cells and umbilical vein endothelial cells and their source MPs. The coagulation time of circulation blood of normal people is shortened, and the study found that the expression level of TF mRNA and the expression of IL-33 mRNA in carotid atherosclerotic plaques are positively correlated[34], which proves that IL-33 acts on endothelial cells by the TF produced on the surface and enhances its coagulation function and mediates the formation of thrombus in atherosclerotic plaques[34].

In summary, we show here that AMI patients have high levels of EMPs, TF protein, IL–33 was also positively correlated with circulating levels of CD31+EMPs in patients with AMI. We present evidence of an ST2/NF-κB mediated up regulation of TF protein expression and activity in HCAECs MPs after treatment with IL-33. Furthermore, IL-33 treatment increased the release of procoagulant HCAECs-derived MPs. These results provide a possible pathophysiologic explanation for a clinical association between IL-33 and atherosclerosis thrombotic events in patients with cardiovascular disease.

Conflicts：

The authors do not have any possible conflicts of interest.

Acknowledgements

Funding：This work was supported by the National Natural Science Foundation of China (No:81760068). Project of People's Hospital of Xinjiang Uygur Autonomous Region (No:20190207).

Referrings

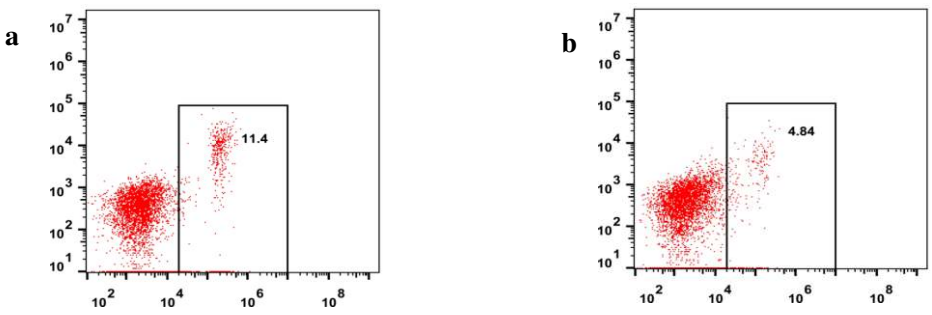
1. Hansson GK, Hermansson A. The immune system in atherosclerosis. Nat Immunol.2011;12:204–212.
2. Hirata T, et al. Carotid Plaque Score and Risk of Cardiovascular Mortality in the Oldest Old: Results from the TOOTH Study. Journal of atherosclerosis and thrombosis.2018;25:55–64.
3. Sankar NM, Ramani SS, Anantharaman R. Coronary artery disease in women. Indian Heart J.2017;69:807–808.
4. 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, Lancet 2019 09 28;394(10204)
5. WHO The Atlas of Heart Disease and Stroke [Internet] WHO [cited 2014Oct30.Availablefrom:http://www.who.int/cardiovascular_diseases/resources/atlas/en
6. Xu R, Greening DW, Simpson RJ, et al. Extracellular vesicle isolation and characterization:toward clinical application[J].J Clin Invest.2016;126 (4):1152–1162.
7. Nomura S, Shimizu M. Clinical significance of procoagulant microparticles[J].J Intensive Care.2015;3(1):2.
8. Robinson A, McCarty D, Douglas J. Novel oral anticoagulants for acute coronary syndrome. Ther Adv Cardiovasc Dis. 2016 Oct 7.
9. Ferraris VA1. Microparticles: the good, the bad, and the ugly. [J]Thorac Cardiovasc Surg.2015,149(1):312-313.
10. VanWijk MJ, VanBavel E, Sturk A, Nieuwland R. Microparticles in

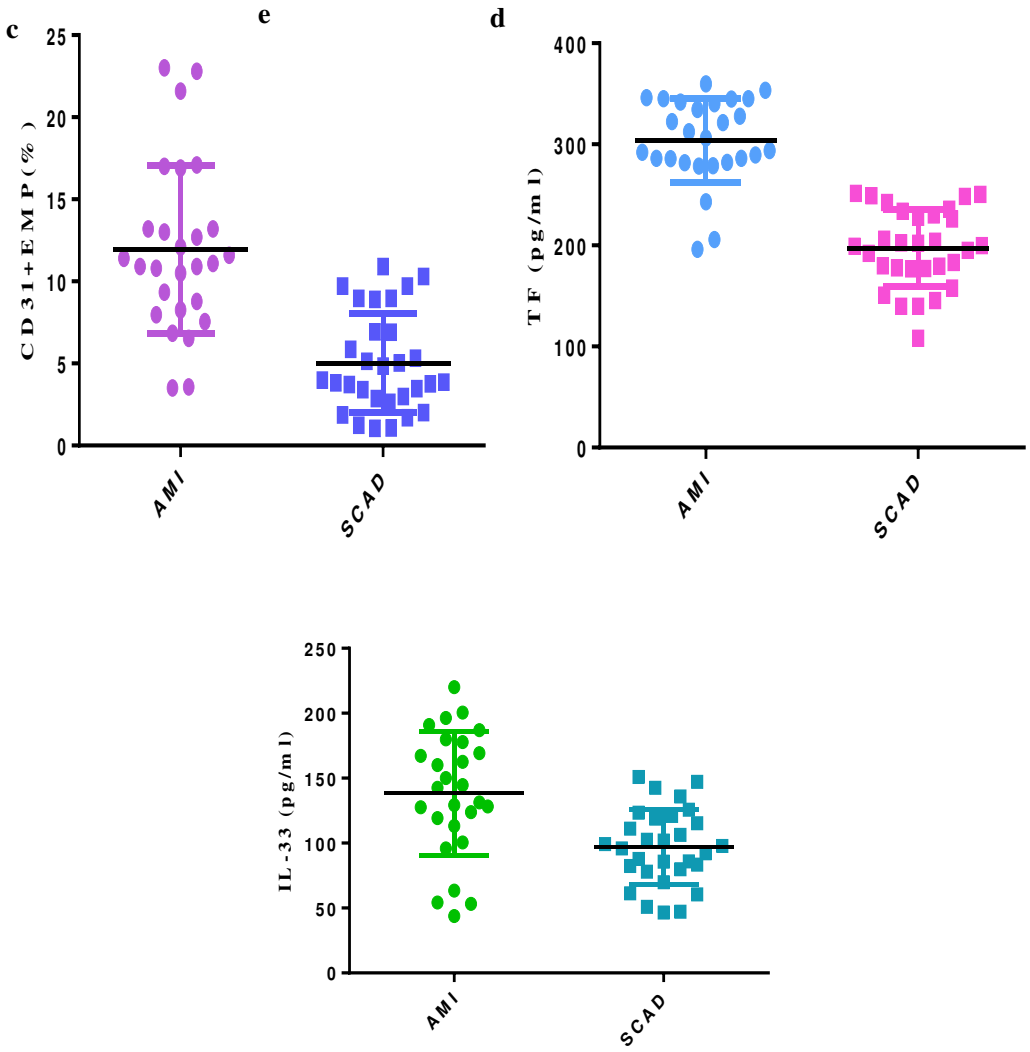
cardiovascular diseases. Cardiovasc Res.2003;59:277–287.

11. 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;26:140–145
12. Gutiérrez E, Flammer AJ, Lerman LO, et al.Endothelial dysfunction over the courseofcoronaryarterydisease[J].EurHeartJ. 2013,34(41):3175-3181.
13. Darbousset R, Thomas GM, Mezouar S, Frere C, Bonier R, Mackman N, Renne T, Dignat-George F, Dubois C, Panicot-Dubois L. Tissue factor-positive neutrophils bind to injured endothelial wall and initiate thrombus formation. Blood.2012;120:2133–2143
14. Chu AJ. Tissue factor upregulation drives a thrombosis-inflammation circuit in relation to cardiovascular complications. Cell Biochem Funct. 2006;24:173–192.
15. Esmon CT. The interactions between inflammation and coagulation. Br J Haematol.2005;131:417–430.
16. Wei Lin, Qiongyan Zhou, Chunbo Liu, Mengxia Ying and Suling Xu. Increased plasma IL-17, IL-31, and IL-33 levels in chronic spontaneous urticarial Sci Rep.2017; 7: 177-197.
17. 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[J].Arterioscler Thromb Vasc Biol.2011,31(9): 2080-2089.
18. Aoki S, Hayakawa M, Ozaki H, et al. ST2 gene expression is prolife ration dependent and its ligand, IL-33,induces inflammatory reaction in endothelial cells[J].Mol Cell Biochem.2010,335(1-2):75-81.
19. Stefan S, Åsa T; Lena H, Barbara T et al. IL-33 stimulates the release of procoagulant microvesicles from human monocytes and differentially increases tissue factor in human monocyte subsets. Thrombosis and Haemostasis. 2017;7:1379-1390.
20. Demyanets S, Speidl WS, Tentzeris ,et al. Soluble ST2 and Interleukin-33 Levels in Coronary Artery Disease: Relation to Disease Activity and Adverse

366 Outcome[J]. PlosOne.2014,9(4):e95055.
367 21. Dhillon OS, Narayan HK, Khan SQ, et al. Pre-discharge risk stratifiation in
368 unselected STEMI: is there a role for ST2 or its natural ligand IL-33 when
369 compared with contemporary risk markers?[J].IntJCardiol. 2013,167
370 (5):2182-2188.
371 22. Demyanets, S. et al. An increase of interleukin-33 serum levels after coronary
372 stent implantation is associated with coronary in-stent restenosis. Cytokine
373 2014,67, 65–70.
374 23. Stojkovic S, Kaun C, Heinz M, et al.Interleukin-33 induces urokinase in human
375 endothelial cells-possible impact on angiogenesis.J Thromb Haemost.2014,12(6):
376 948-957.
377 24. De Caterina, R. et al. General mechanisms of coagulation and targets of
378 anticoagulants (Section I). Position Paper of the ESC Working Group on
379 Thrombosis--Task Force on Anticoagulants in Heart Disease. Thromb Haemost
380 2013,109, 569–579.
381 25. Demyanets, S. et al. Interleukin-33 induces expression of adhesion molecules and
382 inflammatory activation in human endothelial cells and in human atherosclerotic
383 plaques. Arterioscler Thromb Vasc Biol.2011, 31, 2080–2089.
384 26. Stojkovic, S. et al. Interleukin-33 induces urokinase in human endothelial
385 cells-possible impact on angiogenesis. J Thromb Haemost.2014, 12, 948–957.
386 27. Giesen PL, Rauch U, Bohrmann B, et al. Blood-borne tissue factor: another view
387 of thrombosis. Proc Natl Acad Sci USA.1999; 96: 2311–2315.
388 28. Mallat Z, Benamer H, Hugel B, et al. Elevated levels of shed membrane
389 microparticles with procoagulant potential in the peripheral circulating blood of
390 patients with acute coronary syndromes. Circulation.2000; 101: 841–843.
391 29. Annex BH, Denning SM, Channon KM, et al. Differential expression of tissue
392 factor protein in directional atherectomy specimens from patients with stable and
393 unstable coronary syndromes[J].Circulation.1995,91(3):619-622.
394 30. Breitenstein A, Tanner FC, Lüscher TF. Tissue factor and cardiovascular disease:
395 quovadis?[J].CircJ.2010,74(1):3-12.

31. Badimon L, Vilahur G. Thombosis formation on atherosclerotic lesions and plaque rupture[J].J Intern Med. 2014,276(6):618-632.
32. Owens AP, Mackman N. Microparticles in hemostasis and thrombosis[J].Circ Res. 2011,108(10):1284-1297.
33. 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(6): 948-957.
34. 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 2016,05,04;6.





412

413

414 **Figure1 The levels of CD31+EMPs, TF protein and IL-33 protein in AMI and SCAD group.**

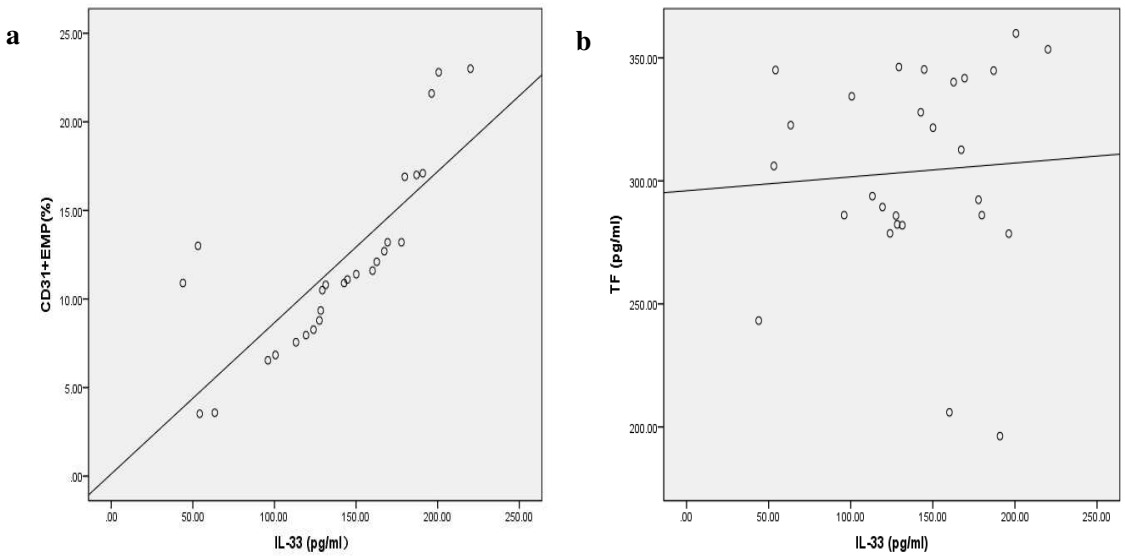
415 a) The flow cytometry result in AMI patient. b) The flow cytometry result in SCAD patient. c) The

416 level of CD31+EMPs in coronary blood of AMI and SCAD group. d) The level of TF protein in

417 coronary blood of AMI and SCAD group. e) The level of IL-33 protein in coronary blood of AMI

418 and SCAD group. **表示 P<0.01.

419



420

421 **Figure 2 IL-33 is positively correlated with CD31+EMP in patients with AMI.** CD31+EMPs,
422 IL-33 protein and TF protein were determined in coronary blood of patients with AMI. a) The
423 level of IL-33 was correlated with CD31+EMPs. b) In contrast to IL-33 did not correlate with TF
424 protein. Pearson's correlation coefficient was calculated to determine significant correlations.
425 $p<0.05$ was considered significant.

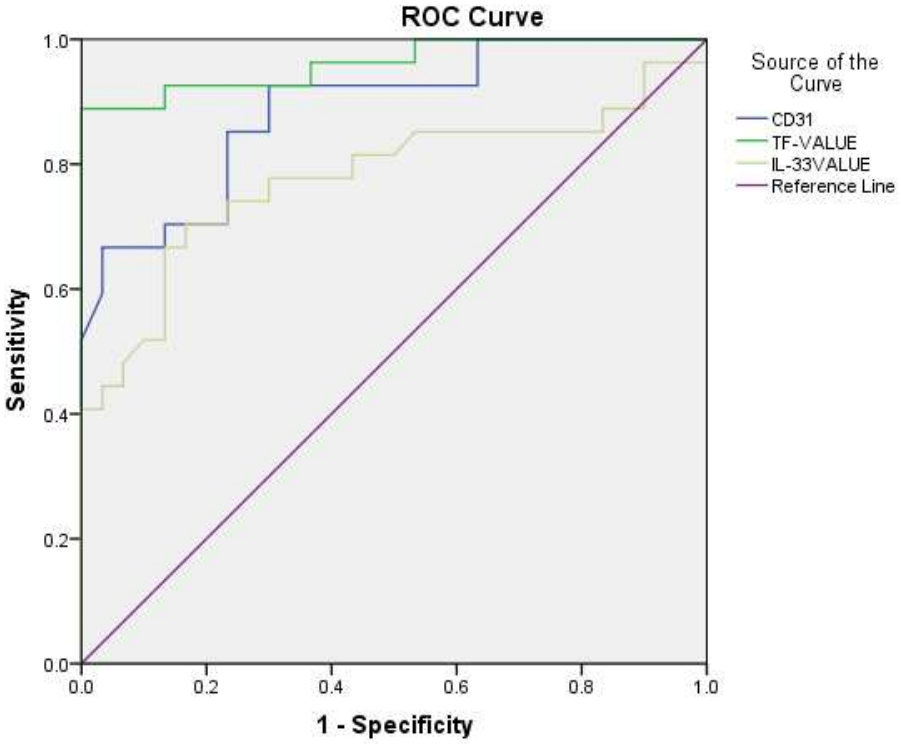


Figure 3 ROC curve analyses of CD31+EMPs, TF protein and IL-33 protein in AMI patients. To further investigate the efficiency of CD+31 EMPs, TF protein and IL-33 protein as potential biomarkers of AMI through ROC curve analysis between patients with AMI and SCAD. According to the areas under the curve (AUC) of EMPs, TF protein and IL-33 protein were 0.888, 0.962, and 0.778.

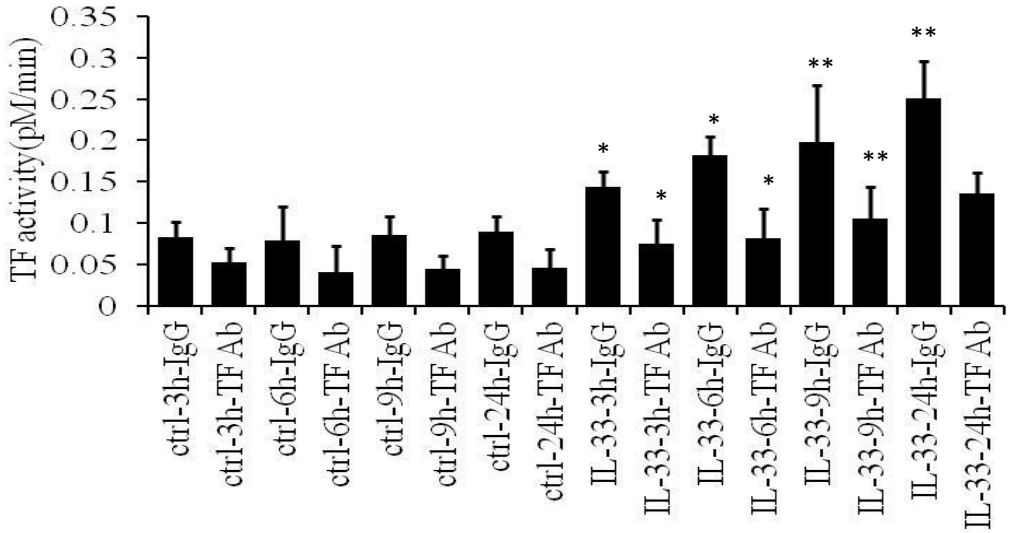


Figure 4 The TF activity by treated TF Antibody (Ab) and control. HCAECs were treated with 100ng/ml of rh IL-33 and blank control to obtain MPs， and then adding TF Ab and IgG. The number of time-points examined for each TF reflects the developmental stages that the TF is expressed for 3,6,9,24 hours. The TF activity increased gradually with time of intervention for 3,6,9 hours, but the time point of 24h was not statistically significant.* means P<0.05, ** means P<0.01.

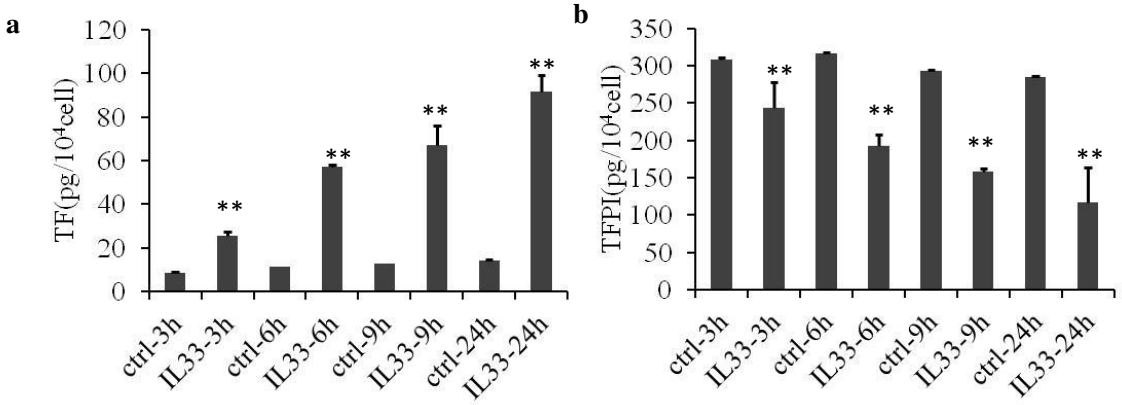


Figure 5: HCAECs were incubated for 3h,6h,9h,24h in the absence (Co) or presence of rh IL-33 (100 ng/ ml). a) rh IL-33 induces TF expression and the release of TF-positive MPs in HCAECs. b) rh IL-33 induces TFPI expression and the release of TFPI-negative MPs in HCAECs. ** means P<0.01

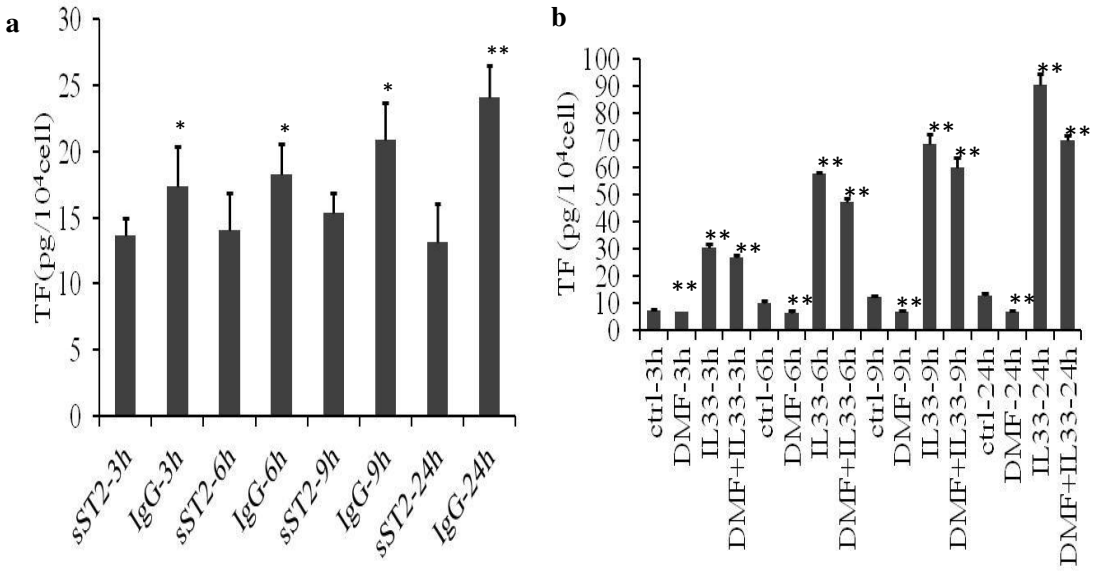


Figure 6: HCAECs were incubated for 3h,6h,9h,24h in the absence (Co), presence of DMF (100 ng/ ml), 5ug/ml sST2 or rh IL-33 (100 ng/ ml). a) Isolated MPs were incubated in the absence (Co) or presence of sST2 (5ug/ ml). b) Isolated MPs were incubated in the absence (Co) or presence of DMF (100 ng/ ml) and rh IL-33 (100 ng/ ml). * means $P<0.05$, ** means $P<0.01$

Figures

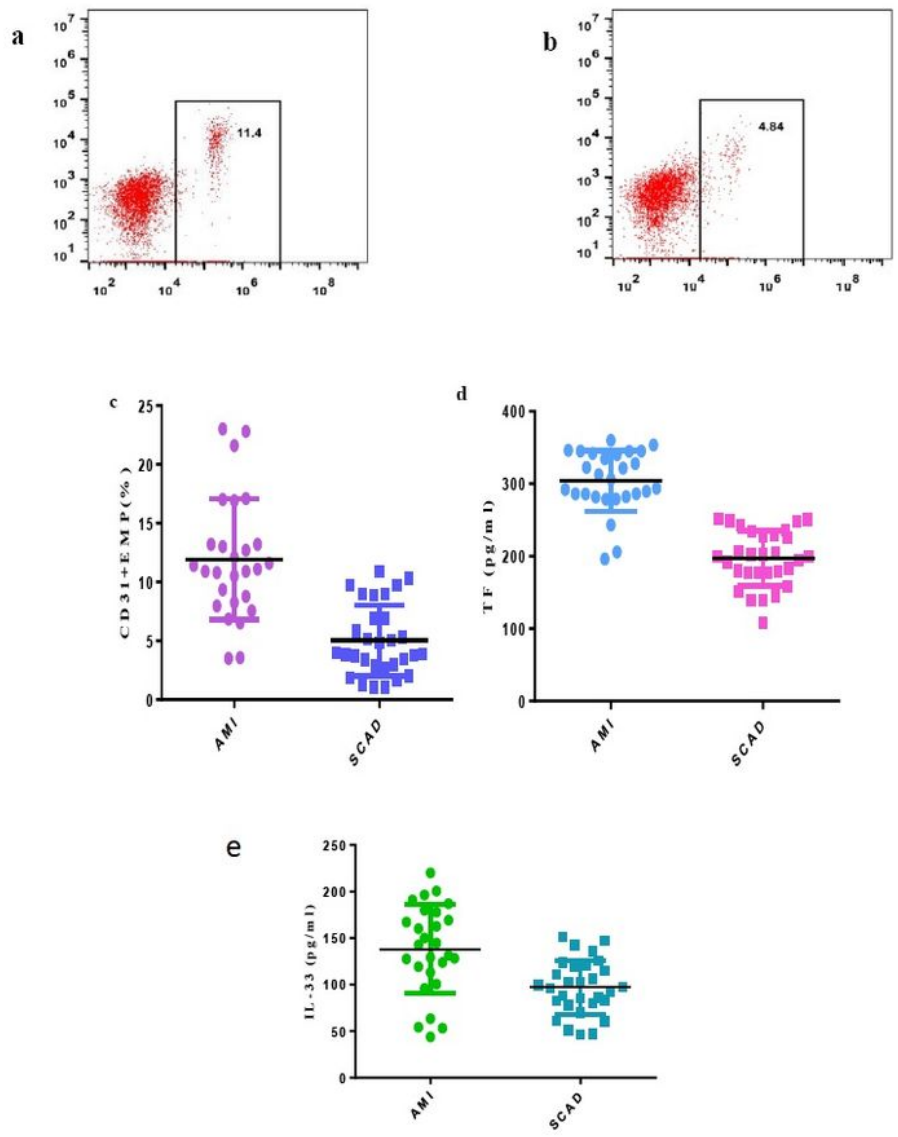


Figure1 The levels of CD31+EMPs, TF protein and IL-33 protein in AMI and SCAD group. a) The flow cytometry result in AMI patient. b) The flow cytometry result in SCAD patient. c) The level of CD31+EMPs in coronary blood of AMI and SCAD group. d) The level of TF protein in coronary blood of AMI and SCAD group. e) The level of IL-33 protein in coronary blood of AMI and SCAD group. **表示 $P<0.01$.

Figure 1

Figure 1

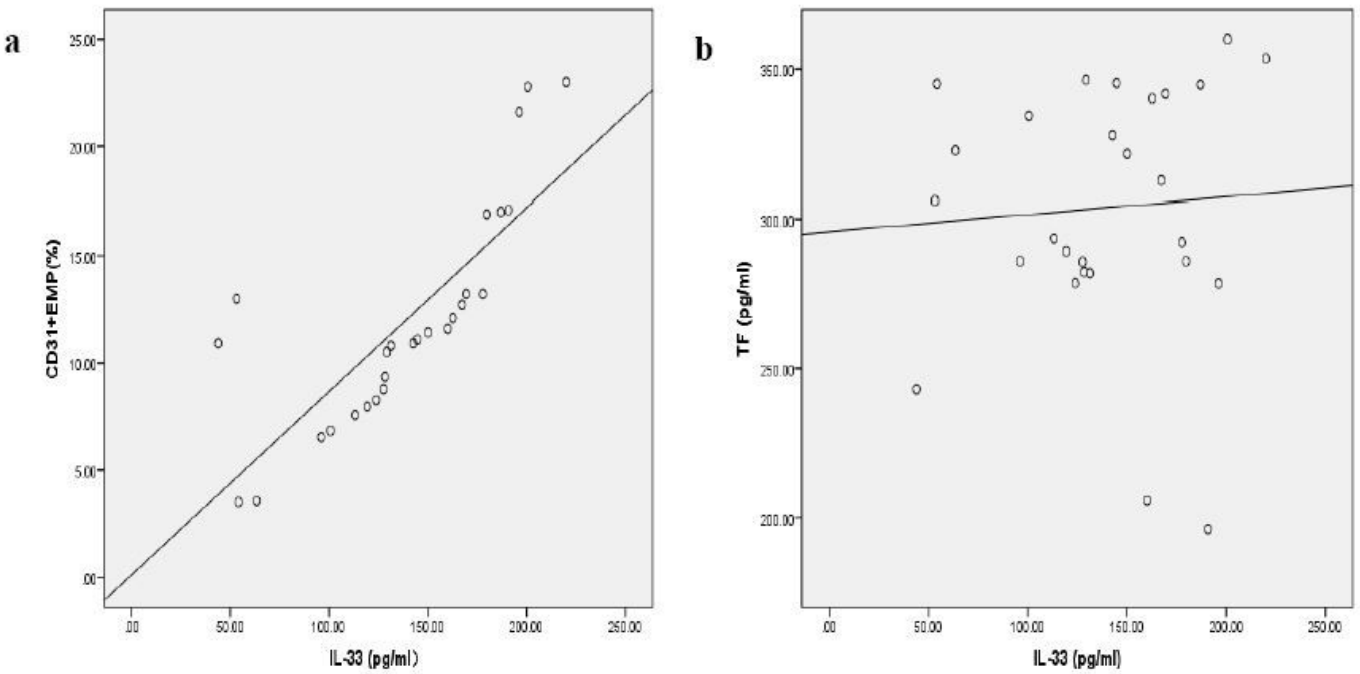


Figure 2 IL-33 is positively correlated with CD31+EMP in patients with AMI. CD31+EMPs, IL-33 protein and TF protein were determined in coronary blood of patients with AMI. a) The level of IL-33 was correlated with CD31+EMPs. b) In contrast to IL-33 did not correlate with TF protein. Pearson's correlation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

Figure 2

Figure 2

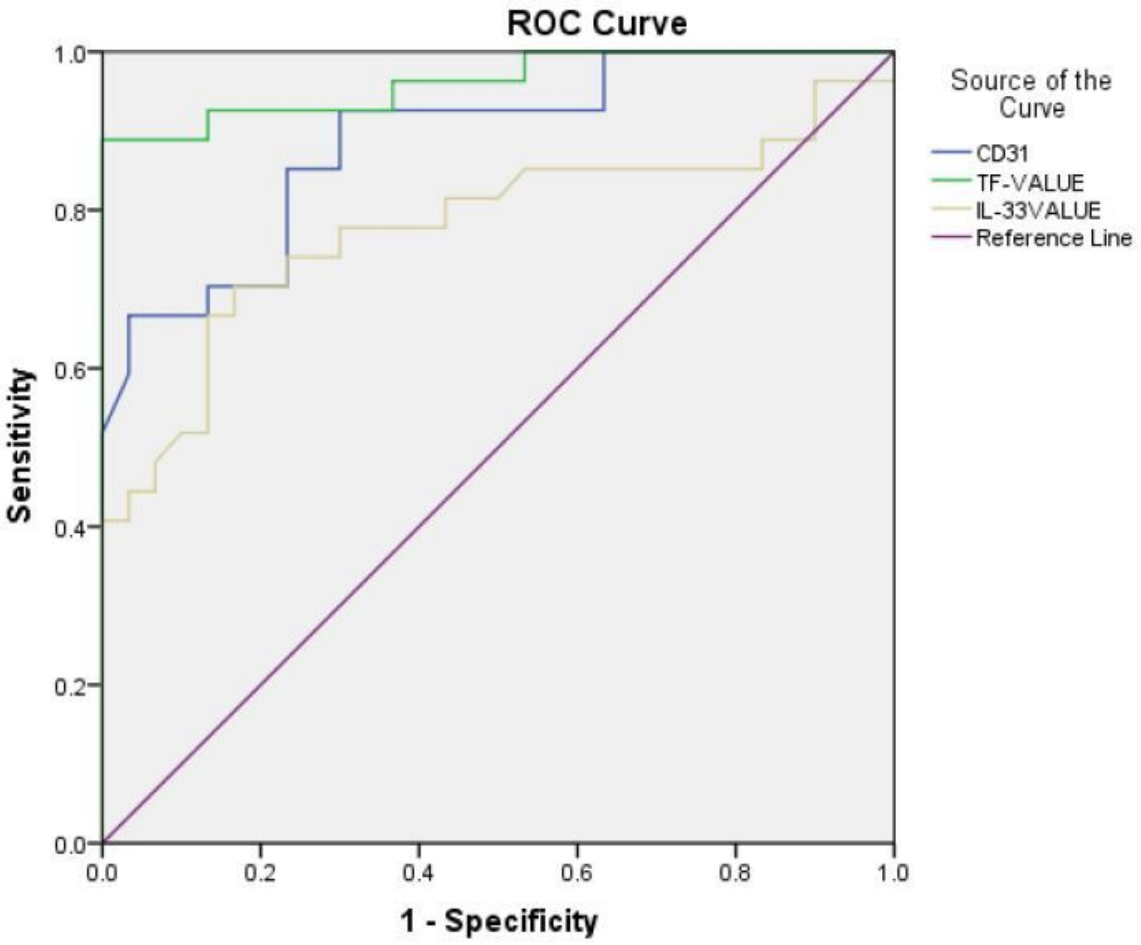


Figure 3 ROC curve analyses of CD31+EMPs, TF protein and IL-33 protein in AMI patients. To further investigate the efficiency of CD+31 EMPs, TF protein and IL-33 protein as potential biomarkers of AMI through ROC curve analysis between patients with AMI and SCAD. According to the areas under the curve (AUC) of EMPs, TF protein and IL-33 protein were 0.888, 0.962, and 0.778.

Figure 3

Figure 3

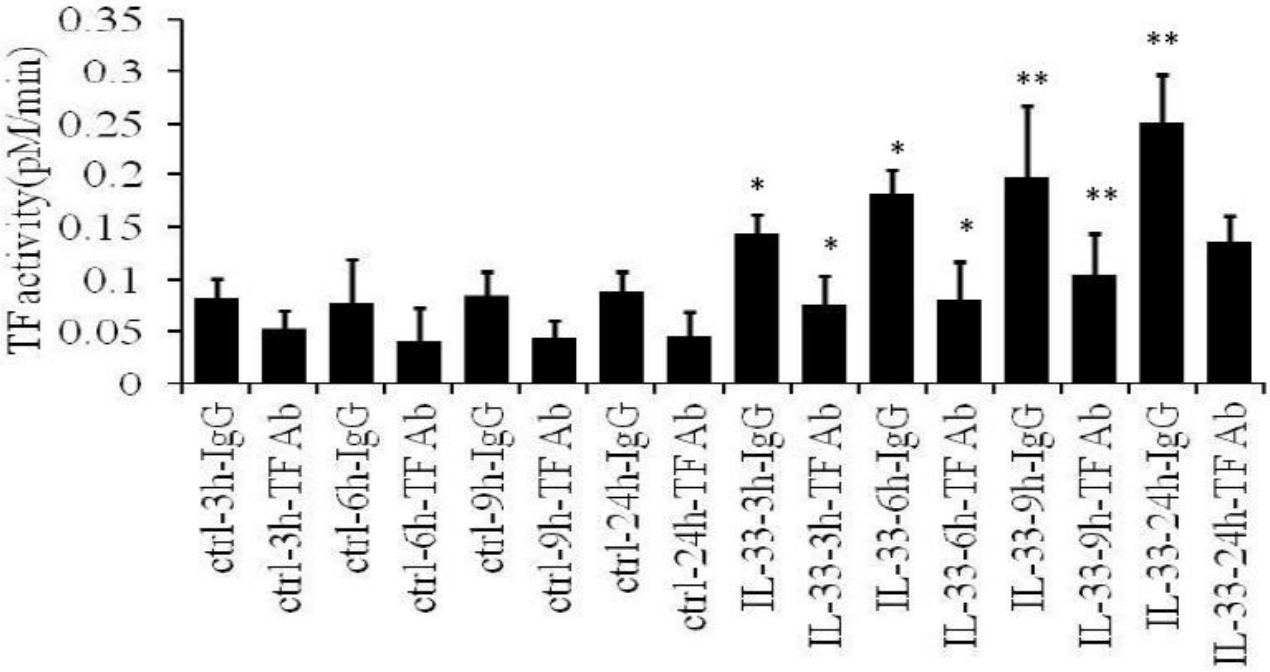


Figure 4 The TF activity by treated TF Antibody (Ab) and control. HCAECs were treated with 100ng/ml of rh IL-33 and blank control to obtain MPs， and then adding TF Ab and IgG. The number of time-points examined for each TF reflects the developmental stages that the TF is expressed for 3,6,9,24 hours. The TF activity increased gradually with time of intervention for 3,6,9 hours, but the time point of 24h was not statistically significant.* means $P<0.05$, ** means $P<0.01$.

Figure 4

Figure 4

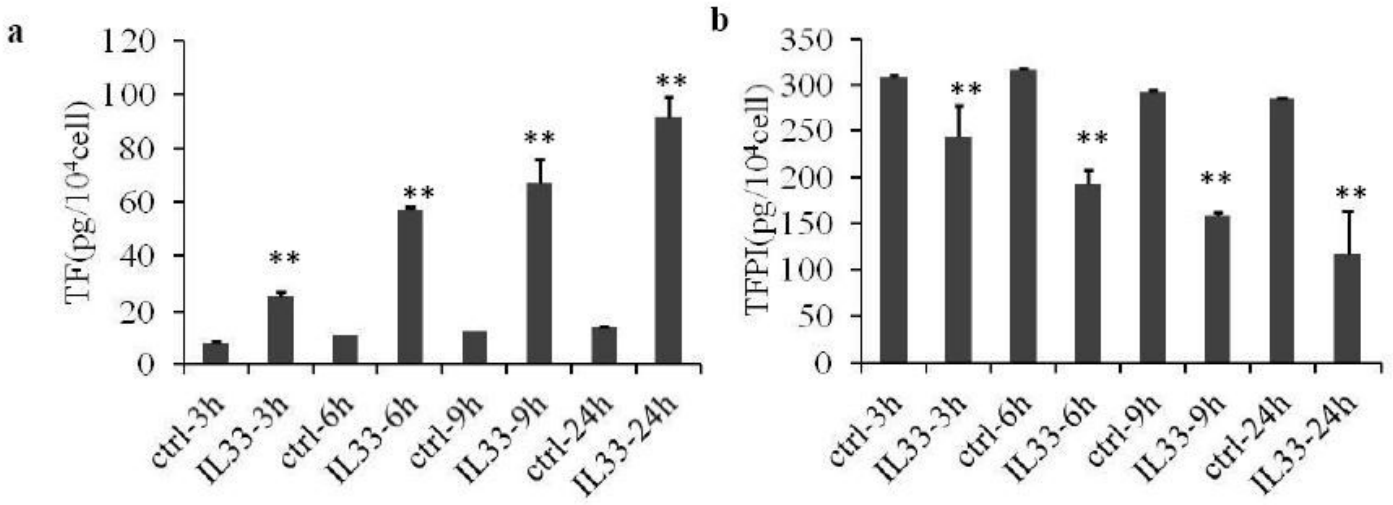


Figure 5: HCAECs were incubated for 3h,6h,9h,24h in the absence (Co) or presence of rh IL-33 (100 ng/ ml). a) rh IL-33 induces TF expression and the release of TF-positive MPs in HCAECs. b) rh IL-33 induces TFPI expression and the release of TFPI-negative MPs in HCAECs. ** means $P<0.01$

Figure 5

Figure 5

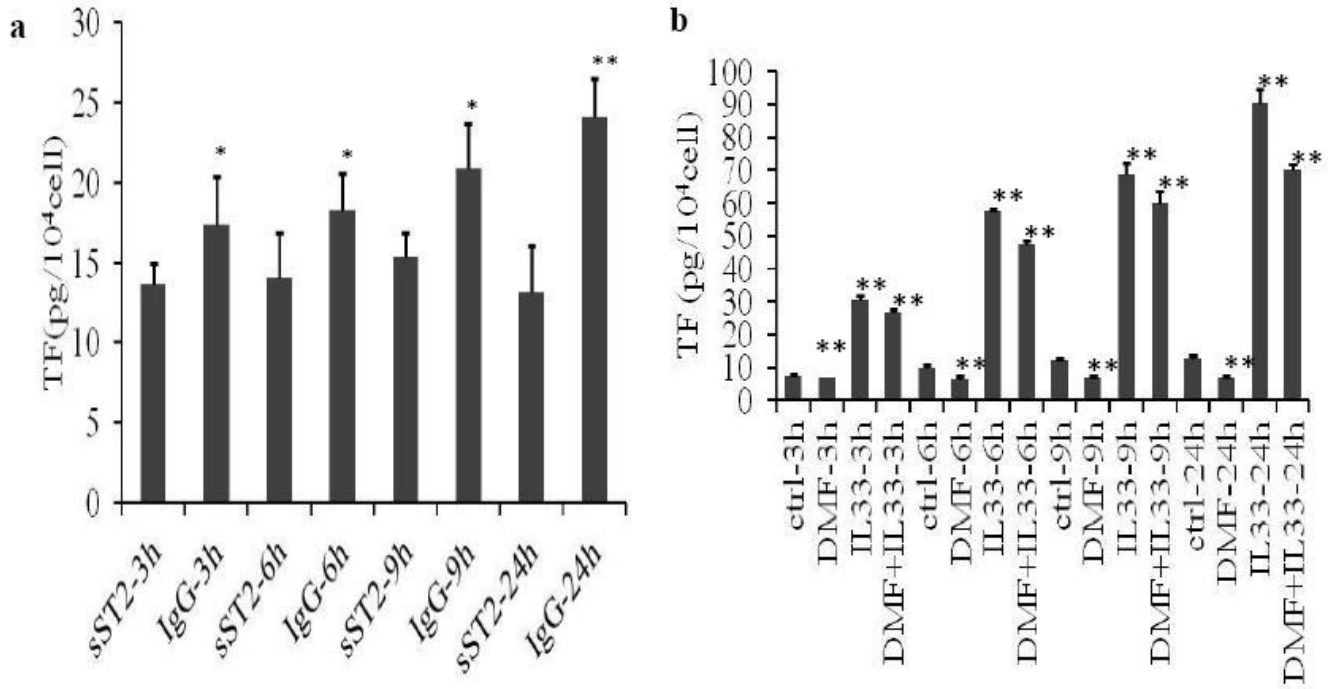


Figure 6: HCAECs were incubated for 3h,6h,9h,24h in the absence (Co), presence of DMF (100 ng/ ml), 5ug/ml sST2 or rh IL-33 (100 ng/ ml). a) Isolated MPs were incubated in the absence (Co) or presence of sST2 (5ug/ ml). b) Isolated MPs were incubated in the absence (Co) or presence of DMF (100 ng/ ml) and rh IL-33 (100 ng/ ml). * means $P<0.05$, ** means $P<0.01$

Figure 6

Figure 6