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*Original Article*

# Upregulation of plasma SOCS-3 is associated with poor prognosis of acute myocardial infarction

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**Abstract:** Acute myocardial infarction (AMI) is a significant public health issue and risk stratification of the patients with AMI is crucial for timely interventions and the improvement of the clinical outcome. However, currently no standard prognostic biomarker is available for AMI. Therefore, the goal of this study was to determine the prognostic value of plasma SOCS-3 in AMI. Enzyme-linked immunosorbent assay (ELISA) was used to examine the expression level of plasma SOCS-3 in AMI patients and healthy controls. Then the potential clinical value of plasma SOCS-3 was evaluated. Our results showed plasma SOCS-3 was significantly increased in AMI and had a good diagnostic value of discriminating AMI patients from the healthy controls. In addition, plasma SOCS-3 was significantly associated with a number of clinicopathological parameters including hypertension, left ventricular ejection fraction (LVEF), eGFR, Troponin I, CK-MB, NT-proBNP, number of coronary artery stenosis and Killip classification. AMI patients with higher plasma SOCS-3 had a higher risk for suffering poorer major adverse cardiac events (MACE) and 5 year overall survival. Furthermore, plasma SOCS-3 was an independent risk factor for MACE. Taken together, plasma SOCS-3 was a valuable prognostic biomarker for patients with AMI.

**Keywords:** Acute myocardial infarction, SOCS-3, plasma, prognosis

## Introduction

Despite the improvement in the devices and medical treatments, acute myocardial infarction (AMI) is still a leading cause of morbidity and mortality around the world. The thrombosis resulting from atherosclerotic plaque rupture significantly reduces the blood supply and oxygen to heart wall, which is the main cause of AMI initiation and progression [1]. The use of biomarkers for early detection and prognostic classification of patients with AMI has been extremely valuable for improving the clinical outcome of deadly disease. However, available biomarkers are still not perfect due to the limited specificity and sensitivity [2, 3].

Suppressor of cytokine signaling (SOCS) proteins, a protein family of eight members (SOCS1-7 and CIS), have been demonstrated to regulate a number of cytokines including, but not limited to, interleukin-6, leukemia inhibitory factor, granulocyte colony stimulating factor, growth hormone and interferon-γ [4-7]. SOCS-3 plays a critical role in various biological processes such as infection, inflammation, embryonic development and insulin sensitivity [8-11]. Mice with a specific deletion of the Socs3 gene in hematopoietic cells developed neutrophilia and various types of inflammatory pathologies [12]. The expression level SOCS3 was increased in patients with rheumatoid arthritis. In addition, injection of SOCS3 adenovirus significantly reduced the severity of arthritis and joint swelling in the mouse model [13]. Glycoprotein 130 (gp130) could enhance the survival of cardiomyocytes and angiogenesis through activation of the Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway. SOCS-3 is a negative regulator of gp130-mediated STAT3 activation [14], suggesting upregulation of SOCS-3 might be unfavorable to the development of cardiovascular diseases.

Previous study reported that deletion of cardiac specific SOCS-3 was able to prevent the initiation of myocardial ischemia reperfusion injury, indicating that SOCS-3 might be closely correlated with the cardiac function [15]. However, the clinical significance of SOCS-3 in

AMI remains poor known. Therefore, the goal of the current study was to investigate the prognostic value of plasma SOCS-3 in the patients suffering from AMI.

## Materials and methods

### *Study population*

This study was performed in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Second Affiliated Hospital, Nanchang University. Written informed consent was obtained from the patients and their relatives. A total of 68 AMI patients who were admitted to the Department of Cardiology, Second affiliated hospital, Nanchang University and 20 healthy controls (normal electrocardiograms and no history of cardiovascular diseases) were included in this study. Patients with AMI were clinically diagnosed by elevation of cTnI (>0.1 ng/mL), acute ischemic-type chest pain, electrocardiography change, and coronary angiography.

### *Sample collection*

About 5 mL of venous blood collected in ethylenediaminetetraacetic acid (EDTA) coated tubes were obtained from AMI patients and healthy controls. All the samples were processed within 30 min to retrieve the plasma. Briefly, the blood samples were centrifuged at 4°C for 10 min at 1,000× g. Resulting supernatant was collected and stored at -80°C until RNA extraction.

### *ELISA*

The plasma SOCS3 concentration was determined by ELISA according to the manufacturer's instruction. The diluted plasma samples (100 μL; 1:80) were added to a microtiter plate that was pre-coated with a monoclonal antibody specific for human SOCS3 and incubated for 1 h at room temperature. Followed by 3 washes in PBS, a horseradish peroxidase-conjugated polyclonal antibody specific for SOCS3 was added and incubated for 1 h. Subsequently, substrate solution was added to the wells and incubated for 30 min before the reaction was stopped. The plates were read using a TECAN Sunrise plate reader at 450 nm. The standard curve was used to determine the abundance of SOCS3 in the unknown samples. The concentration read from the standard curve multiplied by the dilution factor was the final concentration of plasma SOCS3.

### *Statistical analysis*

Normally distributed variables were expressed as mean ± standard deviation and non-normally distributed variables as median. Mann-Whitney U test was used to compare the expression level of plasma SOCS3 between AMI patients and healthy controls. The median value of plasma SOCS3 was used to divide the AMI patients into high and low SOCS3 group. Student t test/Chi-square test was used to compare the differences about the continuous, normally distributed data/categorical data between high and low plasma SOCS3 expression group respectively. The diagnostic value of plasma SOCS3 was evaluated by the area under receiver operating characteristic (ROC) curve (AUC). Survival curves were calculated by the Kaplan-Meier method and the log-rank test. Multivariate Cox proportional hazards regression analysis was conducted to find out the independent prognostic factors for major adverse cardiac events (MACE). Two-sided *P* values <0.05 were considered to be statistically significant. All statistical analyses were performed using SPSS version 21.0 (SPSS Inc., Chicago, Illinois, USA) and Graphpad Prism software (GraphPad, La Jolla, CA, USA).

## Results

### *Expression level of plasma SOCS-3 was significantly upregulated in patients with AMI*

Our ELISA data demonstrated that the expression level of SOCS-3 was remarkably increased in the plasma samples derived from the patients with AMI compared to those from healthy controls (P<0.01) (**Figure 1A**). In addition, plasma SOCS-3 was able to discriminate AMI patients with elative high accuracy, with an optimal sensitivity and specificity of 70.0 and 85.5 respectively (AUC=0.856) (**Figure 1B**).

### *Association between plasma SOCS-3 and clinical features of patients with AMI*

Our results showed plasma SOCS-3 level was significantly associated a number of clinicopathological parameters including hypertension (P=0.002), left ventricular ejection fraction

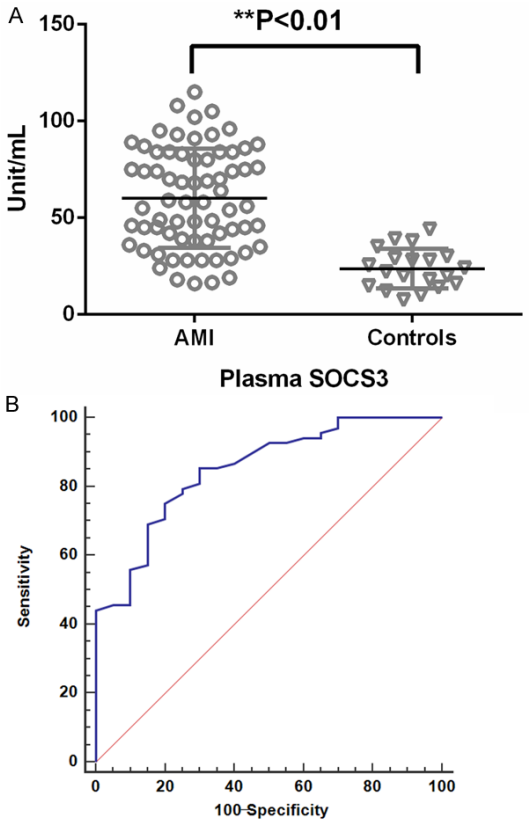


**Figure 1.** Expression level and diagnostic value of plasma SOCS3 in AMI.

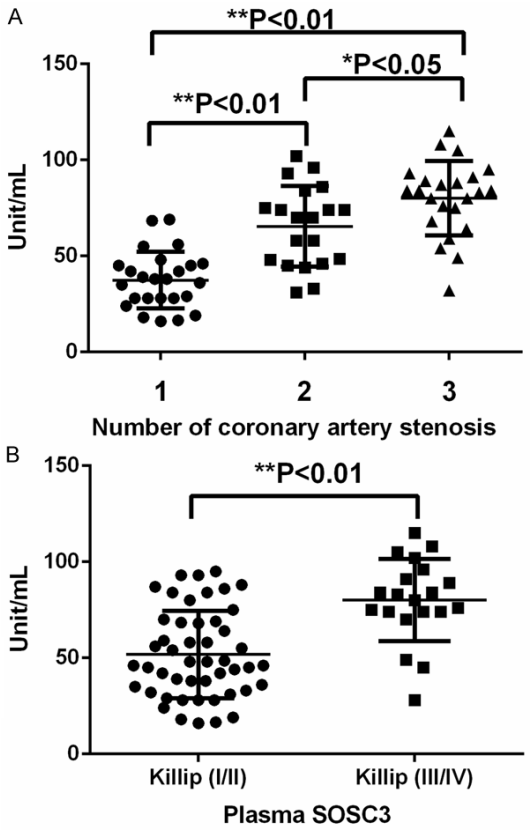


**Figure 2.** Association between plasma SOCS3 and Killip classification as well as number of coronary artery stenosis.

**Table 1.** Baseline characteristics of AMI patients based on the median expression of plasma SOSC3

| Variables | Low SOSC3 | High SOSC3 | P |
|---|---|---|---|
| Age | 64 | 65 | 0.716 |
| Male | 20 (56%) | 15 (47%) | 0.475 |
| Hypertension | 11 (31%) | 22 (69%) | 0.002 |
| Diabetes | 15 (42%) | 20 (63%) | 0.086 |
| Smoking | 18 (50%) | 15 (47%) | 0.797 |
| LVEF (%) | 58 | 54 | 0.004 |
| eGFR | 68 | 65 | 0.001 |
| Troponin I (ng/ml) | 3.2 | 4.1 | <0.001 |
| CK-MB (ng/ml) | 5.2 | 9.6 | <0.001 |
| NT-proBNP (pg/ml) | 541 | 875 | <0.001 |

(LVEF) (P=0.004), eGFR (P<0.001), Troponin I (P<0.001), CK-MB (P<0.001) and NT-proBNP (P<0.001) (**Table 1**). In addition, the AMI patients with increased number of coronary artery stenosis had higher concentration of plasma SOCS-3 (P<0.01) (**Figure 2A**). Furthermore, plasma SOCS-3 was significantly correlated with Killip classification. The expression level of plasma SOCS-3 was remarkably increased in patients in the higher Killip classification (P<0.01) (**Figure 2B**).

*Association between plasma SOCS-3 and MACE as well as mortality*

Kaplan-Meier survival analysis was conducted to compare the difference in MACE/5 year overall survival rate between patients in the high and low plasma SOCS-3 group. The AMI patients in the high plasma SOCS-3 group had a higher risk of suffering from MACE than the patients in the low plasma SOCS-3 group (P<0.01) (**Figure 3A**). Similarly, patients above the median levels of plasma SOCS-3 had a significantly shorter 5 year overall survival than the patients below the median levels of plasma SOCS-3 (P<0.01) (**Figure 3B**).

*Plasma SOCS-3 was an independent prognostic risk factor for MACE*

The multivariate analysis showed that troponin I (HR=1.82, 95% CI=1.31-2.84, P=0.011), LVEF

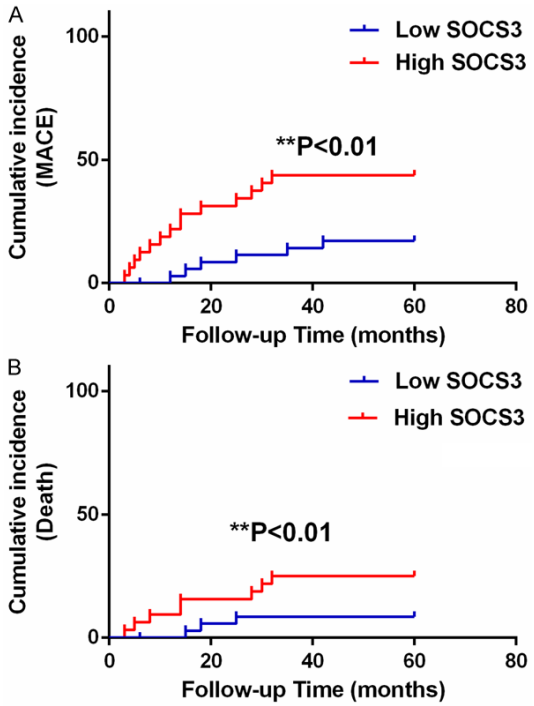


**Figure 3.** The association between plasma SOCS-3 and MACE as well as mortality.

**Table 2.** Multivariate analysis for MACE in patients with AMI

| Variable | HR (95% CI) | P |
|---|---|---|
| Troponin I (ng/ml) | 1.82 (1.31-2.84) | 0.011 |
| LVEF (%) | 1.38 ( 1.08-1.87) | 0.034 |
| Plasma SOSC3 | 1.54 (1.19-2.15) | 0.023 |

(HR=1.38, 95% CI=1.08-1.87, P=0.034) and plasma SOSC3 (HR=1.54, 95% CI=1.19-2.15, P=0.023) were all independent risk factors for MACE (**Table 2**). However, plasma SOSC3 was not an independent prognostic risk factor for mortality (Data not shown).

## Discussion

Subsequent to hospitalization for an AMI, patients still have risks of death and recurrent cardiovascular events. Thus identifying the biomarkers for patient risk stratification is important for disease monitoring. One of the major advantages of biofluid markers is that the real-time detection, which is very useful in the clinical setting. In this study, our results showed that plasma SOCS3 was remarkably increased in the patients with AMI and distinguished AMI patients from healthy controls with high accuracy. In addition, plasma SOCS3 upregulation was significantly associated with many clinical features, higher Killip classification and severity of coronary artery stenosis. The AMI patients in the high plasma SOSC3 group had a higher incidence of major adverse cardiac events and mortality compared with those in the low plasma SOSC3 group. Furthermore, plasma SOCS3 was demonstrated to be a significant independent predictor of MACE. These data indicate that increased expression of plasma SOCS3 is a poor prognosis predictor of AMI.

Consistent with our results, conditional deletion of SOCS3 in mice not only activated cardioprotective signaling molecules including STAT3, AKT, and ERK1/2, but also inhibited myocardial apoptosis and injury during the myocardial ischemia reperfusion injury process, suggesting that SOCS3 might play a central role in the development of myocardial injury [15]. Similarly, Ma et al investigated the effects of ischemic preconditioning (IP) on heart function and the expression of various molecules. The results showed that the expression level of SOCS3 was remarkably reduced in the mice with IP induction compared to those without IP induction, indicating that downregulation of SOCS3 is a possible molecular mechanism accounting for the cardiac protection of IP [16]. Liang et al reported that SOCS3 was significantly upregulated with increased feeding duration especially in those mice fed with high fat diet. In addition, a positive correlation was found between the total serum cholesterol levels and SOCS3 mRNA level in the peripheral blood mononuclear cells, indicating SOCS3 might crucial for the formation and development of atherosclerosis [17]. This result further corroborated that our findings that plasma SOCS3 level was positively associated with the severity of coronary artery stenosis. Furthermore, Li et al showed that SOCS3 deficiency could protect the mice from angiotensin II-induced endothelial dysfunction, indicating that SOCS3 has significant influence on the vascular system [18].

One limitation of the current study was the small sample size, further large cohort studies are required to validate the prognostic value of SOCS3 in AMI. Upregulation of SOCS3 is not only associated with atherosclerosis development, but also can exacerbate the progression of this AMI. Therefore, exploring the specific

inhibitors of SOCS3 might be an effective strategy for the prevention and treatment of AMI.

In conclusion, we demonstrated that the expression level of plasma SOCS3 was remarkably increased in patients with AMI and it level was negatively correlated with poor prognosis of AMI. Therefore, SOSC3 might be a valuable prognostic biomarker and potential therapeutic target in AMI.

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## Disclosure of conflict of interest

None.

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