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## Decreased myeloid dendritic cells indicate a poor prognosis in patients with severe fever with thrombocytopenia syndrome



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### SUMMARY

**Objectives:** Severe fever with thrombocytopenia syndrome (SFTS) is a newly emerging infectious disease caused by a novel bunyavirus in which host immune system suppression is thought to be crucial in the development of disease. This study was designed to study the frequencies and activation status of dendritic cells (DCs) at different stages of SFTS and their association with disease severity.

**Methods:** All confirmed SFTS patients ( $N = 115$ ) were recruited from the Wuhan Union Hospital in 2015; routine laboratory parameters were collected. The frequencies, phenotypes, and subsets of circulating DCs, including myeloid and plasmacytoid dendritic cells (mDCs and pDCs), were analyzed by flow cytometry. Serum levels of interleukin (IL)-6, IL-10, and tumor necrosis factor alpha (TNF- $\alpha$ ) were detected by ELISA. The laboratory parameters and other clinical events related to mortality were assessed by multivariate logistic regression analysis and receiver operating characteristic (ROC) curves. **Results:** The frequency of circulating mDCs, especially from day 9 after disease onset, could serve as a valuable prognostic biomarker for the outcome in SFTS patients (area under the curve = 0.929,  $p < 0.0001$ ). In addition, persistent down-regulation of the co-stimulatory molecules CD80/CD86 on the mDCs was observed during the disease process. Moreover, levels of mDCs were inversely correlated with the production of IL-6, IL-10, and TNF- $\alpha$  and with viral load at admission.

**Conclusions:** The present results indicate that DCs might be functionally impaired in SFTS. A decreased level of circulating mDCs was closely correlated with the severity of SFTS.

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## 1. Introduction

Severe fever with thrombocytopenia syndrome (SFTS) is an emerging disease characterized by abrupt high fever, respiratory or gastrointestinal symptoms, leukocytopenia and thrombocytopenia, bleeding, and neurological symptoms, which may lead to multi-organ failure at the late stage of disease, or even death within 7–14 days after the onset of the disease.<sup>1</sup> SFTS virus (SFTSV) has been identified as the pathogen of this disease. Since 2009, SFTS has been reported in China, South Korea, and Japan, with a reported mortality rate varying between 2.5% and 30%.<sup>2–5</sup> It has become a substantial

risk to public health worldwide. In addition, humans may also be infected through direct contact with an infected patient's blood,<sup>6–8</sup> and no specific treatment for SFTS is available. Therefore, an understanding of the pathogenesis of SFTSV infection is important.

Both viral and host factors are crucial for the pathogenesis of SFTSV. Previous studies have shown that old age, high viral RNA levels in the blood at admission, and a cytokine storm (with abnormal expression of the cytokines interleukin 1 receptor antagonist (IL-1RA), interleukin (IL)-6, IL-10, tumor necrosis factor alpha (TNF- $\alpha$ ), granulocyte-colony stimulating factor (G-CSF), and interferon gamma-inducible protein 10 (IP-10)), play an important role in determining the severity and clinical outcome of this disease.<sup>9,10</sup> As reported previously, various immune cell populations, including T lymphocytes and natural killer cells, have been found to be abnormal in SFTSV-infected patients, especially during the acute phase and in severe cases.<sup>11</sup> Furthermore, elderly people are more susceptible to severe SFTSV infection, indicating that the

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impairment of host immunity is a contributing factor to the development and progression of the disease.<sup>12</sup> However, our understanding of the role of immune cells in SFTS patients is very limited.

Dendritic cells (DCs), in addition to their ability to control infections directly via innate immune functions, are key antigen-presenting cells that can orchestrate an effective immune response and are therefore responsible for the induction of adaptive immunity. DCs are thus recognized to be involved in various inflammatory diseases, including infections caused by Ebola virus (EBOV), influenza virus, and hepatitis C virus (HCV),<sup>13–15</sup> and are suggested to play an important role in disease processes. In addition, a recent study reported that human monocyte-derived DCs infected by dengue virus (DENV) showed a distinct activated phenotype, with increased production of proinflammatory cytokines and chemokines, except for type I interferons.<sup>16</sup> Furthermore, the infection of DCs by DENV may induce DC apoptosis and impair their ability to present antigens to T-cells, which contributes to the pathogenesis of dengue.<sup>17</sup> These studies suggest that functionally impaired DCs may mediate the suppression of host-specific T-cell immune responses and thus facilitate viral persistence and disease progression. However, the role of DCs in SFTSV infection has not yet been elucidated.

In this study, the frequencies and phenotypes of circulating DCs, including myeloid dendritic cells (mDCs) and plasmacytoid dendritic cells (pDCs), were examined dynamically to shed some light on the development of the disease and to facilitate a better understanding of the mechanisms underlying the progression of SFTS.

## 2. Materials and methods

### 2.1. Dynamic collection of clinical samples

The study protocol was approved by the Ethics Committee of Tongji Medical College of HuaZhong University of Science and Technology. Written informed consent was obtained from all patients prior to blood collection. The diagnosis was made according to the clinical guidelines on SFTS released by the Ministry of Health of the People's Republic of China in 2010. During the period April to November 2015, a series of blood samples was collected from SFTS patients every other day starting at the time of admission. In addition, 25 healthy volunteers were recruited for

the study. Patient details, including their clinical history, physical examination findings, and routine hematology laboratory results (white blood cell (WBC) counts, platelet (PLT) counts, elevated alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), and creatine kinase (CK)) were collected from the medical records to conduct a retrospective analysis. The basic characteristics and serum viral loads of these subjects at admission are listed in Table 1.

### 2.2. Isolation of peripheral blood mononuclear cells

Ethylenediaminetetraacetic acid (EDTA) anti-coagulated peripheral blood samples were collected from healthy subjects and SFTS patients. All samples were processed within the first 4 h after collection. Peripheral blood mononuclear cells (PBMCs) were separated by density gradient centrifugation with Ficoll-Paque Plus (DAKEW Biotech, China).

### 2.3. ELISA

The concentrations of TNF- $\alpha$ , IL-6, and IL-10 in the serum of SFTS patients were measured by ELISA using commercial kits (DAKEW Biotech, China) in accordance with the manufacturer's instructions. The detection limit for each cytokine was as follows: TNF- $\alpha$ , 8–1000 pg/ml; IL-10, 7.813–500 pg/ml; IL-6, 6.25–200 pg/ml.

### 2.4. SFTS viral load assay

The total RNA of every clinical patient's serum specimen was extracted using a viral RNA kit (DAAN Gene, Guangzhou, China) as per the manufacturer's instructions. The viral load of SFTSV RNA copies in the serum samples of SFTS patients was detected using a certified real-time PCR kit (SFDA Registration No. 340166, China) based on the detection of the SFTS viral S genomic segment with specific primers and probes. The detection sensitivity and specificity were demonstrated to be 98.6% and 99.1%, respectively.

### 2.5. Flow cytometry analysis

At least 200 000 events per tube were acquired by flow cytometry to determine the frequencies of mDCs and pDCs, as well as the expression of CD80/CD86. All antibodies were purchased from Biolegend (USA). CD80/CD86 levels were reported as the

**Table 1**  
Differences in clinical and laboratory characteristics on admission between patients with severe fever with thrombocytopenia syndrome (SFTS) who survived and those who died<sup>a</sup>

Characteristic	Healthy controls (n = 25)	All patients (n = 115)	p-Value	All patients		p-Value
				Survived (n = 94)	Died (n = 21)	
Age, years	58 (28–70)	60 (28–91)	0.4821 <sup>b</sup>	60 (28–83)	63.5 (48–91)	0.051 <sup>b</sup>
Male, sex, n (%)	13 (53.3%)	45 (39.1%)	0.161 <sup>c</sup>	34 (36.2%)	11 (52.4%)	0.169 <sup>c</sup>
Days of hospitalization	N/A	10 (2–35)	N/A	11 (3–35)	4 (2–14)	<0.0001 <sup>b</sup>
Plasma RNA, log <sub>10</sub>	N/A	4.37 (2.08–7.94)	N/A	4.10 (2.08–6.7)	5.81 (4.28–7.94)	<0.0001 <sup>d</sup>
WBC, 10 <sup>9</sup> /l	5.45 (3.92–9.45)	2.84 (0.27–12.13)	<0.0001 <sup>b</sup>	2.83 (0.27–9.75)	2.68 (1.11–12.13)	0.089 <sup>b</sup>
PLT, 10 <sup>9</sup> /l	174.5 (126–319)	41 (11–191)	<0.0001 <sup>b</sup>	45 (11–191)	35 (11–65)	0.0196 <sup>b</sup>
ALT, U/l	13.5 (5–39)	76 (17–445)	<0.0001 <sup>d</sup>	69 (17–384)	115.5 (35–445)	0.0003 <sup>d</sup>
AST, U/l	20.5 (12–38)	188 (12–2672)	<0.0001 <sup>d</sup>	150 (12–1318)	490 (74–2672)	<0.0001 <sup>d</sup>
GGT, U/l	22.5 (9–47)	53 (14–2012)	0.0416 <sup>d</sup>	47 (14–502)	99 (14–2012)	0.001 <sup>d</sup>
ALB, g/l	39.8 (35.9–49.2)	30.95 (19.7–46.6)	<0.0001 <sup>d</sup>	32.1 (19.7–46.6)	28.05 (22–37)	0.0019 <sup>d</sup>
CK, U/l	62 (13–119)	516 (31–13 113)	<0.0001 <sup>d</sup>	432.5 (31–6694)	1268 (250–13 113)	<0.0001 <sup>d</sup>
LDH, U/l	191 (18.2–223)	709 (137–11 108)	<0.0001 <sup>d</sup>	675 (138–2977)	1605 (392–11 108)	<0.0001 <sup>d</sup>

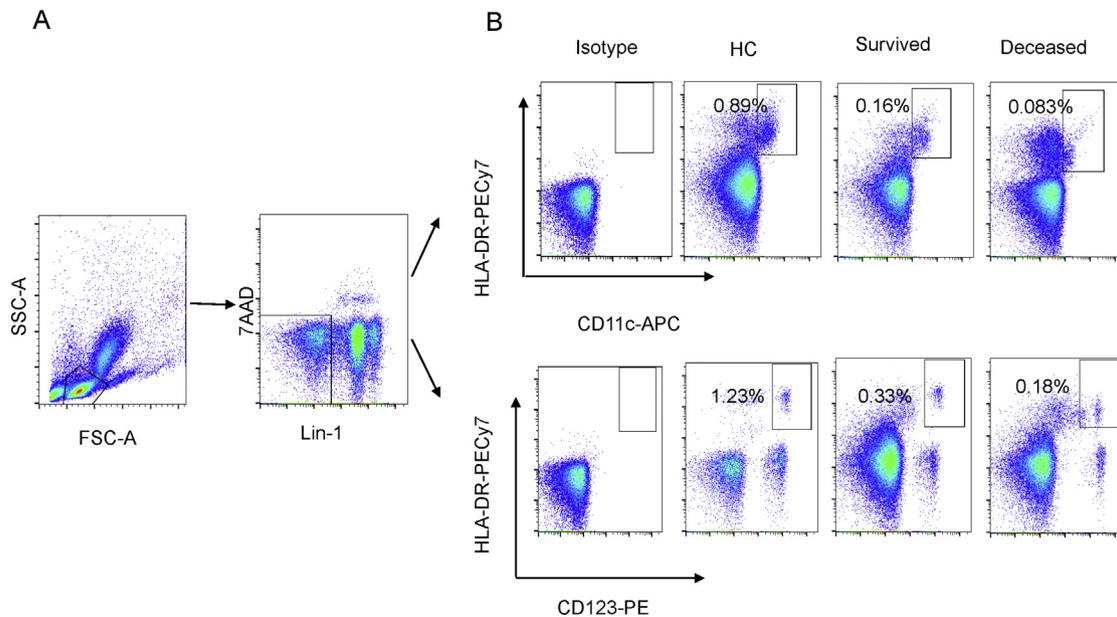
N/A, not applicable; WBC, white blood cell count; PLT, platelet count; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyltransferase; ALB, albumin; CK, creatine kinase; LDH, lactate dehydrogenase.

<sup>a</sup> Data are the median (range) unless specified otherwise.

<sup>b</sup> By means of the *t*-test.

<sup>c</sup> By means of the Pearson Chi-square test.

<sup>d</sup> By means of the Mann-Whitney *U*-test.



**Figure 1.** Gating strategy for the identification of myeloid dendritic cells (mDCs) and plasmacytoid dendritic cells (pDCs) from peripheral blood samples, and the frequencies as determined by flow cytometry. (A) The live Lin-1<sup>-</sup> cells were gated. Cells belonging to the Lin-1<sup>-</sup>HLA-DR<sup>+</sup>CD11c<sup>+</sup> subpopulation were identified as mDCs, whereas those belonging to the Lin-1<sup>-</sup>HLA-DR<sup>+</sup>CD123<sup>+</sup> subpopulation were denoted as pDCs. (B) Representative dot plots of mDCs and pDCs in peripheral blood mononuclear cells (PBMCs) from healthy controls, severe fever with thrombocytopenia syndrome (SFTS) patients who survived, and SFTS patients who died, in the acute phase.

mean fluorescence intensity (MFI) compared with the isotype controls. Logical gating was used to identify mDC and pDC populations.<sup>18</sup> mDCs were defined as Lin-1<sup>-</sup>HLA-DR<sup>+</sup>CD11c<sup>+</sup> cells, while pDCs were defined as Lin-1<sup>-</sup>HLA-DR<sup>+</sup>CD123<sup>+</sup> cells (Figure 1). The Lin-1 lineage cocktail used contained antibodies to CD3, CD14, CD16, CD19, CD20, and CD56. PBMCs were incubated with the appropriate antibodies for 30 min at 4 °C and then washed twice with phosphate buffered saline (PBS). Subsequently, the samples were resuspended in 200  $\mu$ l PBS before acquisition on a FACSCalibur Flow cytometer (BD Biosciences). All reagents were used in accordance with the manufacturer's instructions. Analyses were performed with CELLQuest software (BD Biosciences).

### 2.6. Statistical analysis

All results were analyzed using the statistical software package SPSS 17.0 for Windows (SPSS Inc., Chicago, IL, USA); a  $p$ -value of  $<0.05$  was considered to indicate a statistically significant difference. Continuous variables, including the median and range, were compared by Mann-Whitney  $U$ -test or unpaired Student  $t$ -test. Categorical variables, summarized as frequencies and proportions, were compared with Pearson's Chi-square test. The correlation between two variables was assessed by linear regression analysis. Univariate and multivariate logistic regression analysis was performed to identify variables that were associated with a fatal outcome in SFTS patients. Biologically plausible variables with a  $p$ -value of  $<0.10$  in the univariate analysis were entered into the multivariate logistic regression model by stepwise method. Receiver operating characteristic (ROC) curves and the area under the curve (AUC) were used to assess the diagnostic value of SFTSV and mDCs in predicting mortality in SFTS patients.

## 3. Results

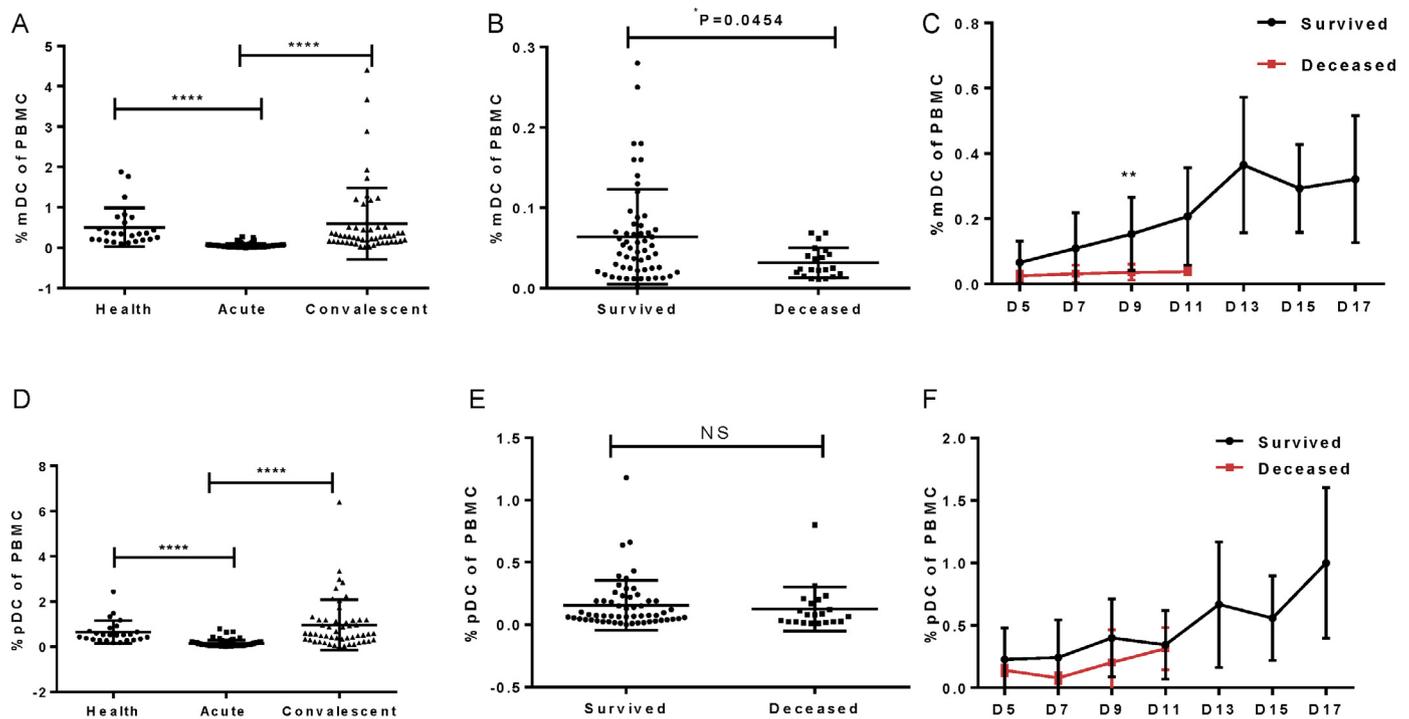
### 3.1. Characterization of deceased and surviving SFTS cases at admission

A total of 115 hospitalized patients (21 who died and 94 who survived) with laboratory-confirmed SFTS were enrolled in this

study. According to the demographic data, the median age of the patients who died was slightly higher than that of the patients who survived; however, the difference was not statistically significant. There was no sex bias in the deceased and surviving patients (11 male/10 female vs. 34 male/60 female, respectively;  $p = 0.169$ ). Among the 115 patients with confirmed SFTS, 21 (18.3%) died within 2–14 days of admission, and the viral load in the patients who died was substantially higher than that in the patients who survived. In addition, the SFTS patients who died showed significantly lower total leukocyte counts, albumin (ALB), and platelet counts, and higher serum ALT, AST, gamma-glutamyl-transferase (GGT), LDH, and CK levels on admission when compared to healthy individuals and patients who survived, which is consistent with previous reports.<sup>19</sup> In this study, the clinical symptoms began to resolve from day 13 after disease onset, and most clinical parameters gradually converted to normal physical ranges in survivors, which is consistent with previous studies.<sup>20</sup> The demographic and clinical information of the 115 SFTS patients enrolled in this study are summarized in Table 1.

### 3.2. The percentages of both mDCs and pDCs in PBMCs are decreased in SFTS patients

In this study, the frequencies of dendritic cells in SFTS peripheral blood were detected dynamically and compared with those in healthy controls. As shown in Fig. 2, the percentages of both mDCs (Figure 2A) and pDCs (Figure 2D) in PBMCs were markedly reduced in patients in the acute phase of SFTS compared with healthy controls ( $p < 0.0001$ ). However, in the convalescent phase, the circulating levels of mDCs and pDCs were back up to similar levels as those of healthy controls. Additionally, a significantly decreased percentage of mDCs in PBMCs during the acute phase was observed in patients who died when compared to those who survived ( $p = 0.0454$ , Figure 2B). However, the extent of the decrease in pDCs was similar between patients who died and those who survived (Figure 2E). Furthermore, the dynamic data showed that circulating mDC levels in surviving patients increased rapidly during the disease process. However, unlike pDCs, the levels of mDCs were persistently low in the patients who died,



**Figure 2.** Decreased percentages of myeloid dendritic cells (mDCs) and plasmacytoid dendritic cells (pDCs) in peripheral blood mononuclear cells (PBMCs) of severe fever with thrombocytopenia syndrome (SFTS) patients. (A) and (D): The mDC% and pDC% of PBMCs in healthy controls ( $n = 25$ ), SFTS patients in the acute phase ( $n = 75$ ), and SFTS patients in the convalescent phase ( $n = 54$ ). (B) and (E): The mDC% and pDC% of PBMCs at admission in patients who survived ( $n = 54$ ) and patients who died ( $n = 21$ ). (C) and (F): Dynamic changes in mDC% and pDC% in PBMCs of patients who survived and those who died, after disease onset. Data are shown as the mean with standard deviation. The statistical analysis was performed using the Mann–Whitney  $U$ -test. \*\* $p = 0.0015$ , day = 9; \*\*\*\* $p < 0.0001$ ; a  $p$ -value of  $< 0.05$  was considered significant.

especially from day 9 after the onset of fever ( $p < 0.001$ , Figure 2C and F).

### 3.3. Down-regulation of CD80/CD86 on mDCs of SFTS patients

The effect of SFTSV on the maturation and activation of DCs is unclear. The expression levels of the co-stimulatory molecules CD80/CD86 on mDCs and pDCs of patients with SFTS were quantified and compared to those of healthy controls. The results showed that the mDCs of acute SFTS patients expressed significantly lower levels of CD80 ( $p < 0.0001$ ) and CD86 ( $p < 0.0001$ ) when compared to healthy controls and convalescent patients (Figure 3A and D). However, on pDCs, a significant increase in CD80 expression was seen in acute SFTS patients compared with healthy controls (Figure 4C;  $p < 0.0001$ ). In contrast, no differences were found in the expression of CD86 on pDCs between SFTS patients and healthy controls (Figure 4A). No significant differences were observed in the expression of CD80/CD86 on mDCs and pDCs between patients who died and those who survived (Figure 3B and E; Figure 4B and D). Furthermore, the dynamic changes in CD80/CD86 on mDCs were investigated and it was found that there were no statistically significant differences between patients who died and those who survived during disease progression (Figure 3C and F).

### 3.4. The frequency of circulating mDCs is inversely correlated with cytokine levels and viral load in the acute phase of SFTSV infection

Specific cytokines were measured to identify those that may be involved in the pathogenesis of SFTSV.<sup>21</sup> The serum levels of TNF- $\alpha$ , IL-6, and IL-10 were significantly higher in acute SFTS patients, but declined to nearly normal levels in the convalescent phase when compared with healthy controls ( $p < 0.0001$ , Figure 5A). In addition, significantly higher levels of these three cytokines were found in patients who died compared to those who survived

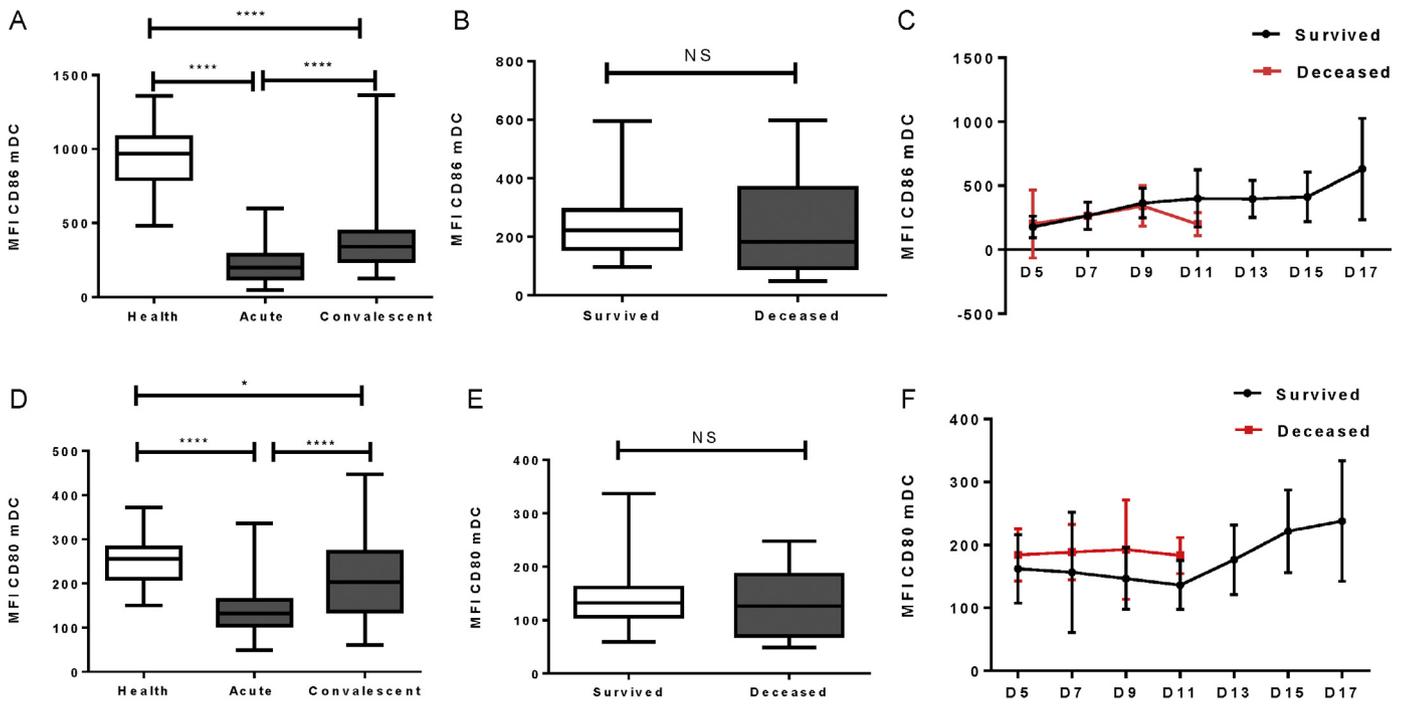
( $p < 0.0001$ , Figure 5B). A further analysis of the correlation of circulating mDC levels with viral load and the serum cytokine levels was performed. The data in Figure 5C–F show that the percentage of mDCs in PBMCs was inversely correlated with cytokine levels and viral load in the acute phase of SFTSV infection. In addition, in line with other reports,<sup>21</sup> positive correlations were found to exist between viral load and TNF- $\alpha$ , IL-6, and IL-10 (Figure 6). Furthermore, it was observed that mDCs were negatively correlated with serum ALT, AST, LDH, and CK levels, which were related to the severity of SFTS disease<sup>4</sup> (data not shown).

### 3.5. A high frequency of circulating mDCs is a protective factor in SFTS patients

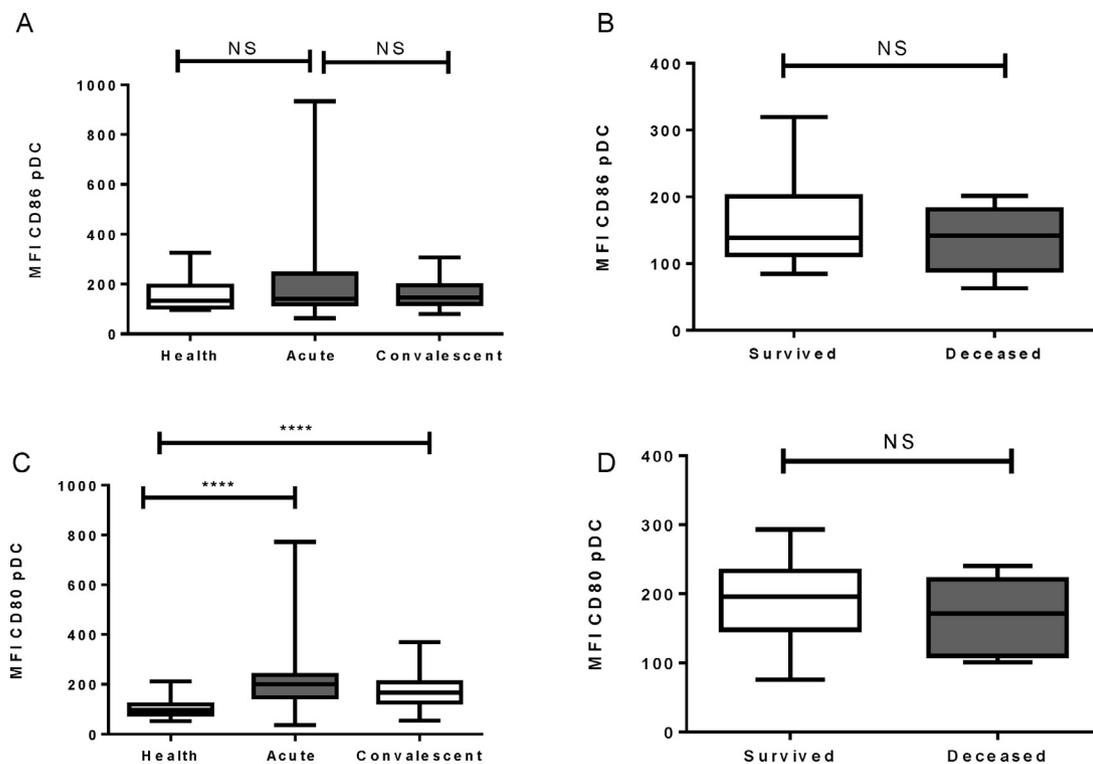
Data on the percentage of mDCs in PBMCs, serum viral load, age, and sex were included in the univariate analysis. In comparison to SFTS patients who recovered, a low percentage of mDCs in PBMCs from day 9 after the onset of disease, old age, and a high serum viral load were found to be significantly associated with a fatal outcome (Table 2). Multivariate regression analysis revealed that older age (odds ratio (OR) 1.104, 95% confidence interval (CI) 1.048–1.163;  $p < 0.0001$ ) and a high serum viral load on admission (OR 11.343, 95% CI 7.637–16.848;  $p < 0.0001$ ) were significant predictors of death (Table 2), which is consistent with previous studies.<sup>9,10</sup> In contrast, it was found that a high level of mDCs was a protective factor for SFTS patients, especially from day 9 after the onset of fever (OR 0.487, 95% CI 0.432–0.549;  $p < 0.0001$ ).

### 3.6. The prognostic values of serum viral load and mDC level according to ROC curves

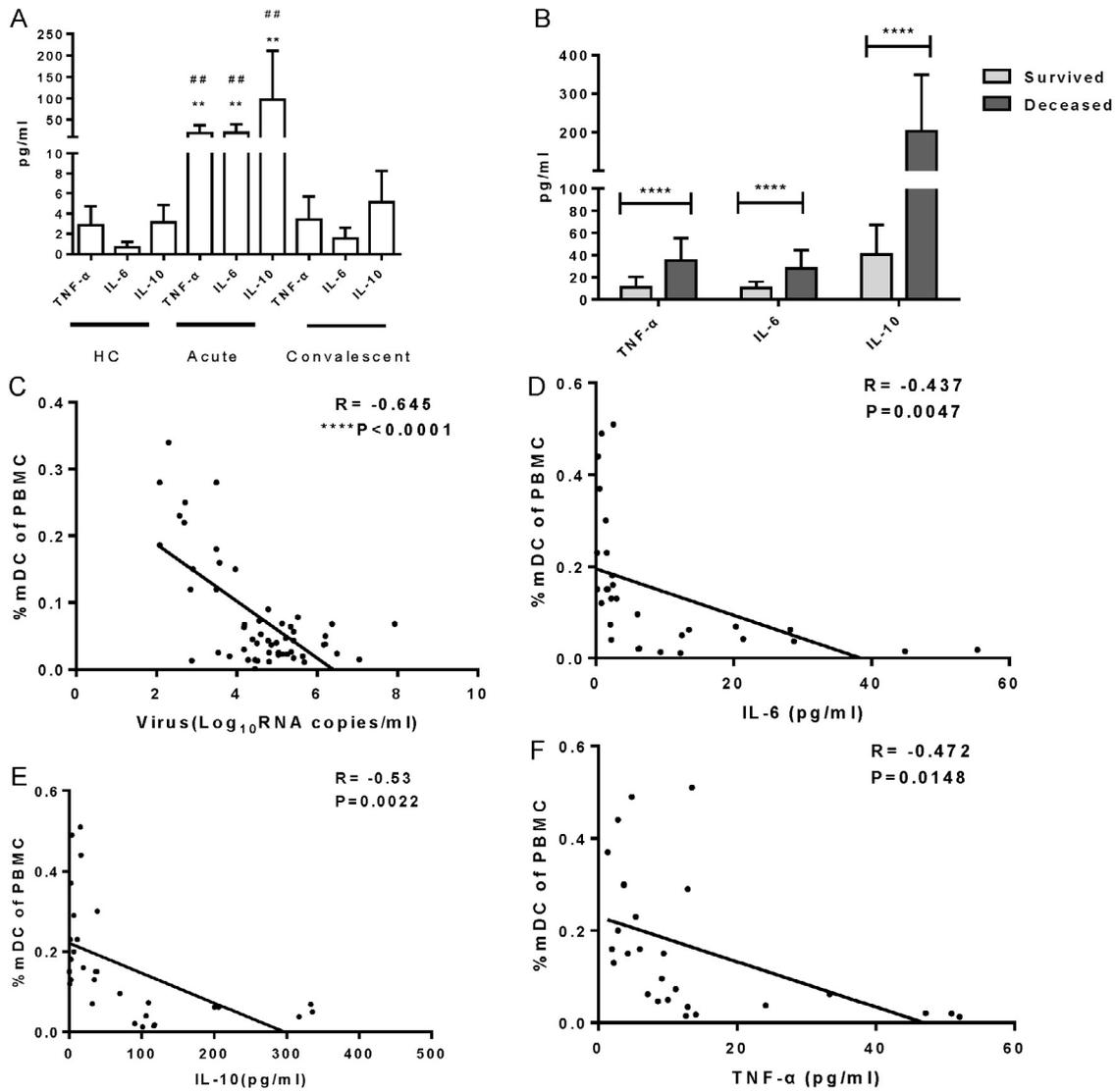
ROC curves and the AUCs were used to assess the capacity of serum viral load at admission and the percentage of mDCs in PBMCs from day 9 after disease onset to predict mortality of SFTS



**Figure 3.** Expression of the co-stimulatory molecules CD80 and CD86 on myeloid dendritic cells (mDCs) of severe fever with thrombocytopenia syndrome (SFTS) patients based on mean fluorescence intensity (MFI) analysis. (A) and (D): Analysis of CD80 and CD86 expression on mDCs of healthy controls ( $n = 25$ ), SFTS patients in the acute phase ( $n = 75$ ), and SFTS patients in the convalescent phase ( $n = 54$ ). (B) and (E): Expression of CD80 and CD86 in SFTS patients who survived ( $n = 54$ ) and those who died ( $n = 21$ ). (C) and (F): Dynamic changes in CD80 and CD86 on mDCs in patients who survived and those who died, after disease onset. Data are presented as the median and interquartile (25–75) range. The statistical analysis was performed using the Mann–Whitney  $U$ -test. \*\*\*\* $p < 0.0001$ ; \* $p < 0.05$ ; NS, no significance.



**Figure 4.** Expression of the co-stimulatory molecules CD80 and CD86 on plasmacytoid dendritic cells (pDCs) of severe fever with thrombocytopenia syndrome (SFTS) patients based on mean fluorescence intensity (MFI) analysis. (A) and (C): Analysis of CD80 and CD86 expression on pDCs of healthy controls ( $n = 25$ ), SFTS patients in the acute phase ( $n = 75$ ), and SFTS patients in the convalescent phase ( $n = 54$ ). (B) and (D): Expression of CD80 and CD86 in SFTS patients who survived ( $n = 54$ ) and those who died ( $n = 21$ ). Data are presented as the median and interquartile (25–75) range. The statistical analysis was performed using the Mann–Whitney  $U$ -test. \*\*\*\* $p < 0.0001$ , NS, no significance.



**Figure 5.** Correlation of the percentage of myeloid dendritic cells (mDCs) in peripheral blood mononuclear cells (PBMCs) with viral load and cytokines. (A) The serum cytokine levels of interleukin (IL)-6, IL-10, and tumor necrosis factor alpha (TNF- $\alpha$ ) were detected in healthy controls ( $n = 15$ ), severe fever with thrombocytopenia syndrome (SFTS) patients in the acute phase (20 who survived, 10 who died), and SFTS patients in the convalescent phase ( $n = 20$ ). (B) Serum cytokine levels of IL-6, IL-10, and TNF- $\alpha$  in 20 patients with SFTS who survived and 10 who died. (C) Correlation between the mDC% and viral load was analyzed based on the data obtained on the first day of admission. The correlation between the mDC% of PBMCs and the levels of IL-6 (D), IL-10 (E), and TNF- $\alpha$  (F) in the acute phase of SFTS was analyzed. (A)  $**p < 0.0001$  vs. normal control (NC) group;  $##p < 0.0001$  vs. convalescent group. (B)  $****p < 0.0001$ . The best-fit lines are shown on each graph. On the graphs, R and P indicate the correlation coefficient and the  $p$ -value of significance, respectively. Each dot shows an individual value. A  $p$ -value of  $<0.05$  was considered significant. Pearson's correlation tests were performed for correlation analysis.

patients (Figure 7A and B). The AUC of the serum viral load was 0.903 (Figure 7A; 95% CI 0.836–0.97;  $p < 0.0001$ ). The best diagnostic cut-off point was 5.1153 (copies/ml,  $\log_{10}$ ), with a sensitivity of 0.75 and specificity of 0.886, which is consistent with other studies. Additionally, the AUC of mDC% was 0.929 (Figure 7B; 95% CI 0.863–0.996;  $p < 0.0001$ ), and the best diagnostic cut-off point was 0.075%, with a sensitivity of 0.86 and specificity of 0.929.

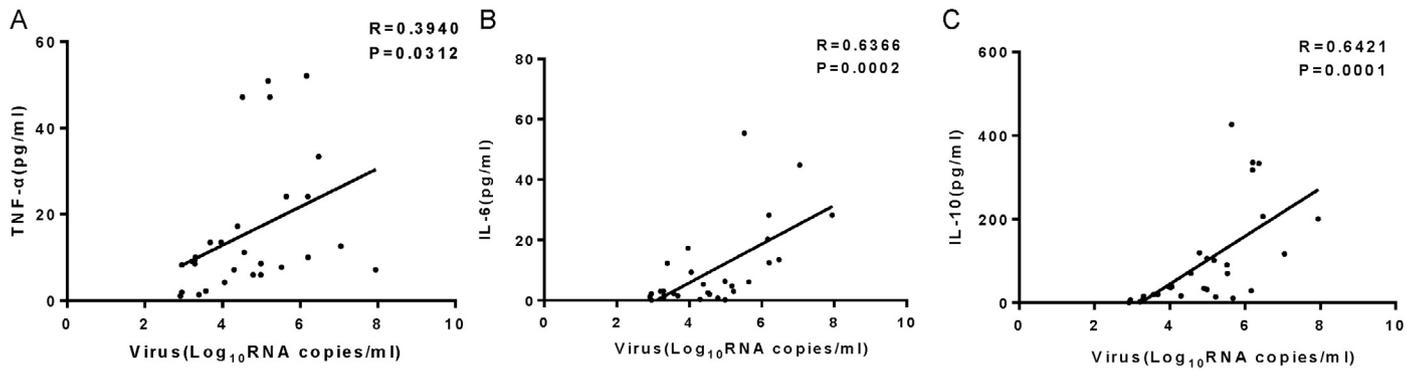
**4. Discussion**

As reported previously, immune-mediated responses play an important role in the pathogenesis and outcome of SFTSV infection.<sup>22</sup> In this study, it was observed that the percentages of both mDCs and pDCs in PBMCs were largely decreased in SFTS patients from the onset of illness, especially in those who died.

**Table 2**  
Predictors of mortality in patients with SFTSV disease by univariate and multivariate regression analysis

Characteristic	Univariate analysis			Multivariate analysis		
	OR	95% CI	$p$ -Value	OR	95% CI	$p$ -Value
Age, years	1.035	1.025–1.045	$<0.0001$	1.104	1.048–1.163	$<0.0001$
Male sex	1.941	0.748–5.04	0.173			
Plasma RNA, $\log_{10}$	5.322	4.613–6.14	$<0.0001$	11.343	7.637–16.848	$<0.0001$
mDC, day $<9$	0.812	0.534–1.234	0.329			
mDC, day $\geq 9$	0.513	0.477–0.551	$<0.0001$	0.487	0.432–0.549	$<0.0001$

SFTSV, severe fever with thrombocytopenia syndrome virus; CI, confidence interval; OR, odds ratio; mDC, myeloid dendritic cells.



**Figure 6.** Correlation of serum viral load and serum cytokine levels in patients in the acute phase of severe fever with thrombocytopenia syndrome (SFTS). The correlation between the viral load and the level of (A) tumor necrosis factor alpha (TNF- $\alpha$ ), (B) interleukin (IL)-6, and (C) IL-10 in the acute phase of SFTS was analyzed. The best-fit lines are shown on each graph. On the graphs, R and P indicate the correlation coefficient and the *p*-value of significance, respectively.

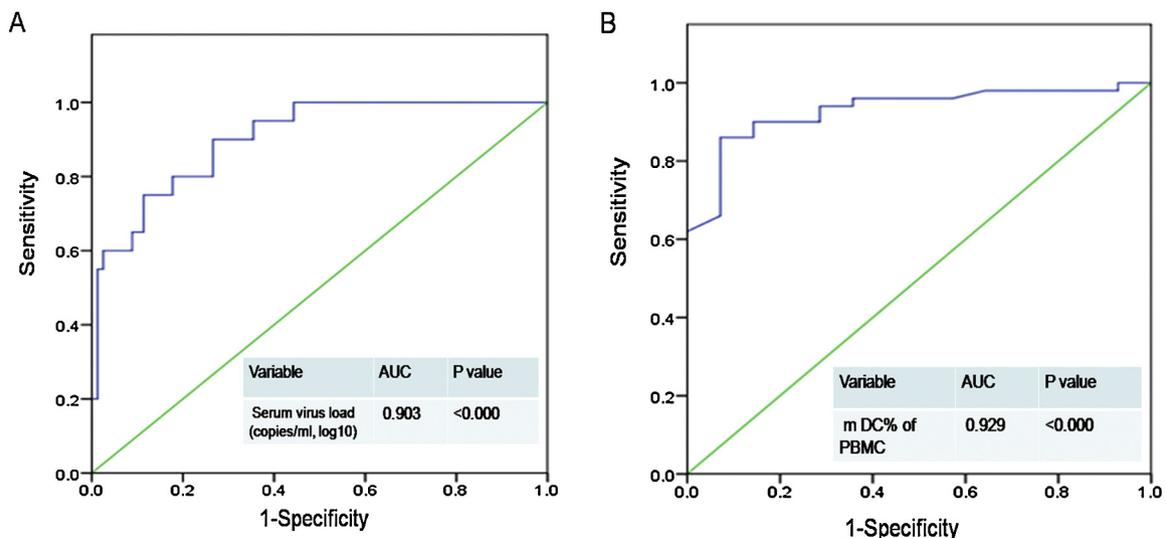
Interestingly, the patients who died, especially those with high serum viral loads, presented a persistently low level of circulating mDCs during the disease progression. Nevertheless, the level of pDCs in patients who died began to increase from day 7 after the onset of illness, which was parallel to the patients who survived. This phenomenon, which has also been reported previously for DENV infection,<sup>23</sup> demonstrated that a decreased level of circulating mDCs may be associated with a fatal outcome for SFTS patients. As expected, using multivariate logistic regression, the percentage of mDCs in PBMCs was found to be directly associated with the mortality rate, especially from day 9 after the onset of disease. Furthermore, its predictive properties were assessed and it was found that when the mDC% was lower than 0.075%, the SFTS patients might have a poor outcome (Figure 7B); this is an important and useful finding that may provide early guidance to clinicians.

In accordance with previous studies, old age, a high serum viral load, and cytokine release were identified as risk factors for death in patients with SFTS.<sup>9,10,24</sup> As previously reported, the clinical course of SFTS includes three stages: fever (1–7 days), multiple organ dysfunction (MOD) (7–14 days), and convalescence.<sup>25</sup> Although a high viral load is a critical predictive factor for a fatal outcome in SFTS patients during the early stage and this has been highlighted by most researchers, the SFTSV disappears quickly and almost all patients showed a decrease in viral load with the disease

process.<sup>26</sup> In contrast, circulating mDC levels as a protective factor for the prognosis represent a new promising biomarker to indicate the host's immune function in SFTS patients. Therefore, in the second week following disease onset, the mDC level is an alternative valuable factor to estimate the severity of SFTS. It is advised that this is monitored dynamically to guide the therapeutic approach and block the rapid development of illness.

Although the mechanism of the DC level decrease observed in this study remains to be elucidated, several explanations can be proposed for the low level of circulating DCs in SFTS patients: SFTSV directly infects cells and leads to the apoptosis or autophagocytosis of target cells, such as DCs or their precursors (monocytes);<sup>27–29</sup> DC migration to secondary lymphoid organs or the virus-driven impairment of bone marrow function leads to lower DC production; and IL-10 induces cell death by down-regulating anti-apoptotic proteins, such as B-cell lymphoma 2 (Bcl-2) and transforming growth factor- $\beta$  (TGF- $\beta$ ).<sup>29</sup> Any alterations in DC cell death would therefore have a significant effect on the antigen-specific immune responses. Nevertheless, further studies are needed to support and elucidate these hypotheses.

To the authors' knowledge, immature DCs continuously sample antigen, but represent poor inducers of immune responses.<sup>30</sup> Functional defects in DCs have been proposed to be responsible for the failure of antiviral immune responses in other diseases such as DENV, measles virus infection, and malaria.<sup>24,31–33</sup> In this study,



**Figure 7.** Receiver operating characteristic (ROC) curve of serum viral load and percentage of circulating myeloid dendritic cells (mDCs) in peripheral blood mononuclear cells (PBMCs) from day 9 after the onset of fever.

the maturation status of the circulating DC subsets in the SFTS patients was investigated and it was found that circulating mDCs of SFTS patients presented lower expression levels of CD80/CD86 than healthy controls. These primary findings indicate that infection with SFTSV may impair DC differentiation and attenuated DC function. This could have important consequences on the development of a specific immune response efficient to induce memory T-cells to control future infection. Therefore, it is speculated that the deficiency of DCs, especially mDCs, might contribute to the pathogenesis of SFTS disease. This speculation will only be better understood following a further study of monocyte-derived dendritic cells (MoDCs) from healthy controls infected with SFTSV *in vitro*.

In summary, despite the limitations of this study, it was demonstrated that the decreased frequencies of DCs, especially mDCs, in SFTS patients may influence the clinical outcome of the disease. Although the mechanisms for mDC deficiency need to be explored further, this study has facilitated a better understanding of the mechanisms underlying the progression of SFTS and provides a new promising prognostic biomarker. Further studies are required to assess the possibility of manipulating these cells to induce effective antiviral immunity and to elucidate how future DC-directed therapies may help induce protective memory responses and reduce SFTS pathogenesis.

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