

Original Article

Low-density neutrophils in severe fever with thrombocytopenia syndrome (SFTS) display decreased function to phagocytose SFTS virus and enhanced capacity to synthesize cytokines

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Abstract: Background: Severe fever with thrombocytopenia syndrome (SFTS) patients can quickly proceed to multi-organ dysfunction. Novel identified the low-density neutrophils (LDNs) roles on multiorgan dysfunction in SFTS are remained unclear. Methods: The levels of LDNs and high-density neutrophils (HDNs) from the blood of 20 SFTS patients were detected by Flow Cytometry. SFTS viral load in LDNs and serum were assayed by Real-time-PCR. Cytokines secreted by LDNs were detected by ELISA. Results: The percentages of LDNs were significantly elevated while percentages of HDNs decreased in SFTS patients compared to that of normal controls. The LDNs displayed the same phenotype as HDNs, but different levels of surface molecules. The further analysis showed that the heavy load of SFTSV in plasma correlated with level of LDNs, but not correlated with level of HDNs and SFTSV load was lower in LDNs than that in HDNs of SFTS patients. In addition, the LDNs secreted significantly increased levels of cytokines by SFTSV infection. Conclusions: The study demonstrates that LDNs are highly enriched in the SFTS patients and have a proinflammatory phenotype. LDNs display decreased phagocytosis of SFTSV and enhanced capacity to synthesize cytokines. LDNs may play more important roles than HDNs on multiorgan dysfunction of SFTS.

Keywords: SFTS, SFTSV, LDNs, neutrophil

Introduction

The severe fever with thrombocytopenia syndrome (SFTS), a novel emerging infectious disease, was reported in China, Korea and Japan [1-3]. The major clinical symptoms of the SFTS included fever, thrombocytopenia, gastrointestinal symptoms and leukocytopenia, the fatality rate for this severe disease was 30%. SFTS patients usually die due to multiple organ dysfunctions (MODS) [4, 5]. The causative agent of SFTS was identified to be a novel bunyavirus named SFTS virus (SFTSV), which is a phlebovirus in the Bunyaviridae family [6]. SFTSV is infected mainly by tick bites [7]. To date, the pathogenesis mechanism of fatal SFTSV infection remains poorly understood. Viral interaction with the innate immune system played a core role in determining the outcomes of the infection [8]. We have previously shown that

dysfunction of lymphocyte subsets caused immunological abnormality, which made SFTS disease deteriorate progressively [9, 10]. Previous studies have reported the overall levels of viral load, changes in key lab results, and certain immunological biomarkers in SFTS patients. The counts of white blood cells, neutrophils, lymphocytes and platelets were significantly lower in patients with SFTS [10-12].

Neutrophils are the most abundant leukocyte in human circulation and play a well-established role in host defense for which is the first immune cell to arrive at a site of pathogenic challenge [13]. The primary role of the neutrophil in the immune response is to combating pathogen invasion until adaptive immune responses can be initiated, and they do so mainly by ingesting microorganisms and dying cells [14]. However, recently, novel neutrophil functions

have emerged in addition to their classical anti-microbial role [13]. Neutrophils have been implicated in the antiviral immune response, as they constitute the first and predominant immune cell population that reaches the sites of viral infection. In general there is still little known about cell-mediated immunopathogenesis, especially neutrophils, in the pathogenesis of SFTSV. Controversy surrounds neutrophil function because neutrophils were shown to provide both beneficial and harmful effects for antiviral immune functions [15]. And disease processes that promote abnormal neutrophil activation can result in tissue damage and potentiation of aberrant immune responses. Nowadays, studies have found that neutrophil function differs based on specific density-dependent sub-population. Based on the difference in density, neutrophils that co-purify in the PBMC fraction as low-density neutrophils (LDNs) and neutrophils which sediment with erythrocytes as high-density neutrophils (HDNs) [16].

Low-density neutrophils (LDNs), that express the surface markers of mature neutrophils, yet their nuclear morphology resembles an immature cell. LDNs consist of both immature myeloid-derived suppressor cells (MDSCs) and mature cells that are derived from HDNs in a TGF- β dependent mechanism [17]. It appears that LDNs differ from their autologous high-density neutrophils (HDNs) in that they have a different nuclear morphology, show strikingly diminished phagocytic potential. LDNs mediate enhanced proinflammatory, cytotoxic responses and have immunosuppressive properties, characteristics that are in stark contrast to those of mature, high-density neutrophils (HDNs) [18].

Several studies have provided compelling evidence that low-density neutrophils (LDNs) are present in increased numbers in the peripheral blood mononuclear cell (PBMC) fraction of patients with systemic lupus erythematosus (SLE) and other systemic autoimmune diseases and in patients infected with human immunodeficiency virus (HIV) [19, 20]. Therefore, it is no surprise that these cells have a pivotal role in the virus infections. Until recently, there is no report on LDNs and HDNs studies with respects to SFTSV infection. In the present study, we analyzed their frequency correlated with markers of SFTS disease severity and their

possible roles in the acute phase of SFTS disease. Understand the inflammatory and immune response of SFTS by LDNs may provide new theoretical evidence and experimental basis concerning the inhibition of MODS occurrence in SFTS.

Materials and methods

Subjects

40 Subjects (20 SFTS patients and 20 volunteers.) were recruited from the Department of Infectious Diseases of Union Hospital, Tongji Medical College, Huazhong University of Science and Technology between May 2015 and June 2016. The procedures were in accordance with the ethical standards of the Helsinki Declaration. Subjects with the acute phase of SFTS patient were definitely diagnosed according to clinical symptom and laboratory examination. Healthy volunteers were normal controls who had no diseases via health examination and no treatment with drugs prior to and during the investigation phase.

Blood sampling and plasma preparation

Individual identification codes were given to each participant and blood samples were collected under identification codes. Peripheral blood was collected in EDTA tubes were brought to the laboratory within 12 h. The whole blood taken from every subject was centrifuged at $400 \times g$ for 5 min at $4^{\circ}C$ to be separated into plasma and cellular fractions. Then upper plasma was moved into a clean tube for further conduction.

Cell isolation

Peripheral blood was diluted 1:1 with phosphate buffered saline (PBS) and was centrifuged on a 3-ml cushion of Lymphocyte Separation Medium (Ficoll-Histopaque) in a 15-ml polystyrene conical centrifuge tube for 30 minutes at $400 \times g$ at room temperature without brake. The LDNs were carefully collected by aspiration from the plasma-lymphocyte separation medium interface, diluted 1:3 with PBS and centrifuged for 5 minutes at $300 \times g$ at room temperature. Red blood cells (RBCs) were eliminated by hypotonic lysis. The collected LDNs were washed twice in PBS. High-density neutrophils (HDNs) were collected from

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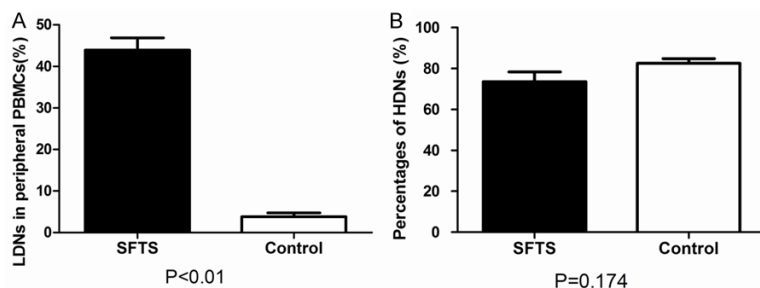


Figure 1. The percentages of LDNs and HDNs in SFTS patients and normal controls. A. The amount of LDNs in PBMCs of SFTS group was significantly elevated in comparison with that of control group. B. There was no significant difference between the percentages of HDNs in SFTS group and control group. Abbreviations: SFTS, severe fever with thrombocytopenia syndrome; LDNs, low-density neutrophils; HDNs, high-density neutrophils.

the granulocyte-erythrocyte pellet by 3 cycles of brief hypotonic lysis and were washed twice with PBS. The LDNs and HDNs were suspended in RPMI-1640 for cell counting and then analyzed immediately by flow cytometry.

Flow cytometry

To determine the markers on cytomembrane, LDNs or HDNs (10^6 cells each) cells were incubated for 20 min at 4°C . The following antibodies were used: fluorescein isothiocyanate (FITC)-conjugated anti-CD66b, peridinin chlorophyll-protein (PerCP)-labeled anti-CD45 and allophycocyanin (APC)-labeled anti-CD15 and phycoerythrin (PE)-conjugated anti-CD11b. Afterwards, cells were washed and resuspended in PBS. Analysis was performed on a FCM CaliburTM (BD, Bioscience) by CellQuest software (BD, Bioscience). Appropriate isotype control IgGs were used to eliminating nonspecific signal in the population. A total of 10,000 events per tube relative to the whole cells were acquired.

Cytokine quantification by ELISA

Plasma was collected as described above and all subjects were stored at -80°C until use. Concentrations of G-CSF and IL-17 in the plasma samples of SFTS patients and normal controls were quantified using enzyme-linked immunosorbent assays specific for human G-CSF and IL-17 (USCN, Wuhan, China). The assays were performed according to the recommendations of the manufacturer using standard curve for the cytokine. The results were reported as means of duplicate measurements. SFTS LDNs, HDNs and control neutrophils were

cultured in RPMI-1640/1% FBS for 48 h in the presence of SFTSV. Supernatants were then harvested and concentration of the cytokines IL-6, IL-8 and IL-17 were quantified using ELISA kits (USCN, Wuhan, China) as described by the manufacturer.

Real-time RT-PCR for SFTS viral RNA assay

RNA was extracted from LDNs and HDNs from SFTS by using the Blood RNA Kit (Omega) according to the manufacturer's instruction and then SFTSV RNA in LDNs and HDNs were detected by using a commercially available quantitative real-time PCR diagnostic kit, according to the manufacturer's instructions. Viral RNA was extracted from 200 μl serum samples by using the QIAamp Viral RNA Kit (Qiagen) according to the manufacturer's instruction. SFTSV RNA was detected by real-time reverse transcription polymerase chain reaction (RT-PCR) by using a commercially available quantitative real-time PCR diagnostic kit, which was quantified by quantitative RT-PCR with specific primers and probes targeted at the S segment [21], according to the manufacturer's instructions.

Statistical analysis

Unless indicated otherwise, results were summarized as means and standard deviations (mean \pm SD). Results were analyzed using the statistical software package SPSS 18.0 (SPSS, an IBM Company, Armonk, NY, USA). Comparison of various groups was performed using Student's t tests when the data were normally distributed; otherwise, the Mann-Whitney test was used. The correlation analysis of 2 variables among virus load, LDNs, HDNs, and cytokines were calculated using Pearson and Spearman test. $P < 0.05$ was considered statistically significant. The statistical graphs were performed by Graph Pad Prism 5.00 (GraphPad Software, San Diego, CA, USA).

Ethics statement

All participants signed informed consents before participation. The research protocol was approved by The Ethics Committee of Tongji

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Table 1. The MFI levels of LDNs and HDNs in SFTS patients and normal controls

Phenotype marker	SFTS		Control	
	LDNs	HDNs	LDNs	HDNs
CD11b	803.55±457.33	850.80±244.53	1500.65±897.79	967.25±193.52
CD15	44.30±29.24	72.10±50.23	74.10±44.67	45.55±7.32
CD45	165.47±55.97	220.59±71.99	327.57±189.42	275.42±67.56
CD66b	914.41±480.91	1070.72±490.49	640.58±331.50	377.60±86.23

The MFI of cell surface expression of CD11b, CD15, CD45 and CD66b were determined by flow cytometry. Results were summarized as means and standard deviations (mean ± SD). Abbreviations: MFI, mean fluorescence intensities; SFTS, severe fever with thrombocytopenia syndrome; LDNs, low-density neutrophils; HDNs, high-density neutrophils.

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Result

Clinical demographics of study subjects

Twenty patients hospitalized with laboratory-confirmed SFTSV infection were enrolled in this study. The median interval between the onset of illness and extraction of peripheral blood at admission was 3 days (range, 1-5 days). The median age of the patients was 59.50±8.85 years (range, 40-71 years), with a female to male ratio of 12:8. The median age of normal control was 54.70±7.82 years old (range, 40-66 years), with a female to male ratio of 10:10. There was no significant difference in average age and sex ratio between SFTS patients and controls.

LDNs and HDNs levels in SFTS

LDNs are the cells that co-purify with PBMCs following density gradient centrifugation. This difference in density distinguishes this population from the remaining granulocytes that co-purify with the erythrocyte fraction following density gradient centrifugation and thus have been named high-density neutrophils (HDNs). Then we detected the percentages of LDNs and HDNs in SFTS patients and normal controls. The peripheral blood mononuclear cell fraction isolated from patients with SFTS contains a pool of LDNs.

The percentages of LDNs in the PBMCs of SFTS patients was (43.94±13.08)% (mean ± SD). The level of LDNs of normal controls was (3.81±4.11)%. The LDNs level in SFTS patients was significantly elevated compared to that of normal controls ($P<0.01$). The percentage of HDNs of SFTS patients was (73.54±21.66)%. And the percentage of HDNs of normal controls

was (82.50±10.10)%. But there was no significant difference between them ($P=0.174$) (**Figure 1**).

The phenotype of LDN and HDN in SFTS

To answer whether LDNs and HDNs differ phenotypically and to look their function in SFTS patients, we assessed the expression levels of a panel of phenotypic markers of neutrophils. They were isolated as described in materials and methods and the mean fluorescence intensities (MFI) of cell surface expression of CD45, CD11b, CD66b and CD15 were determined by flow cytometry. The MFI levels of CD45, CD15, CD11b, CD66b of LDNs and HDNs in SFTS patients and normal controls were shown in **Table 1**.

We observed that the MFI levels of CD15 ($P=0.017$), CD45 ($P<0.01$) and CD11b ($P=0.017$) in LDNs of SFTS patients were significantly lower than that of the normal controls. The MFI levels of CD66b in LDNs of SFTS patients were higher than that of the normal controls, but there is no statistically significant between them (**Figure 2**).

The MFI levels of CD11b and CD45 in HDNs were lower than that of the normal controls, but there is no statistically significant between them. The MFI levels of CD66b, CD15 of HDNs in SFTS was elevated than that of normal controls, and there is statistically significant in CD66b between them ($P<0.01$) (**Figure 3**). We could conclude that LDNs and HDNs share the same phenotype, but they are different in the level of phenotypic expression.

The frequency of LDNs and HDNs correlates with G-CSF and IL-17 in SFTS patients

The cytokine interleukin (IL)-17 (also named IL-17) is emerging as an important cytokine in

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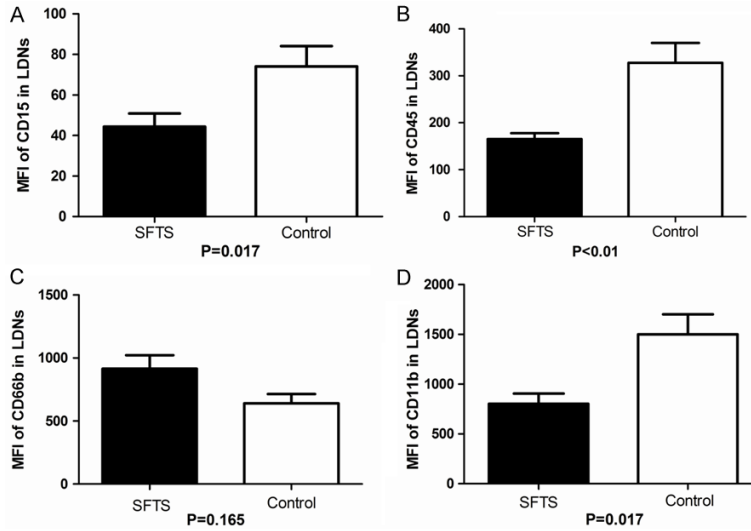


Figure 2. The expression of phenotypic markers in LDNs of SFTS patients and normal controls. The MFI values of CD11b, CD15, CD45 and CD66b were determined by flow cytometry. A, B, D. MFI levels of CD15, CD45 and CD11b in LDNs were lower than that in normal controls. C. MFI levels of CD66b in LDNs and normal controls has no significant difference. Abbreviations: LDNs, low-density neutrophils; MFI, mean fluorescence intensities.

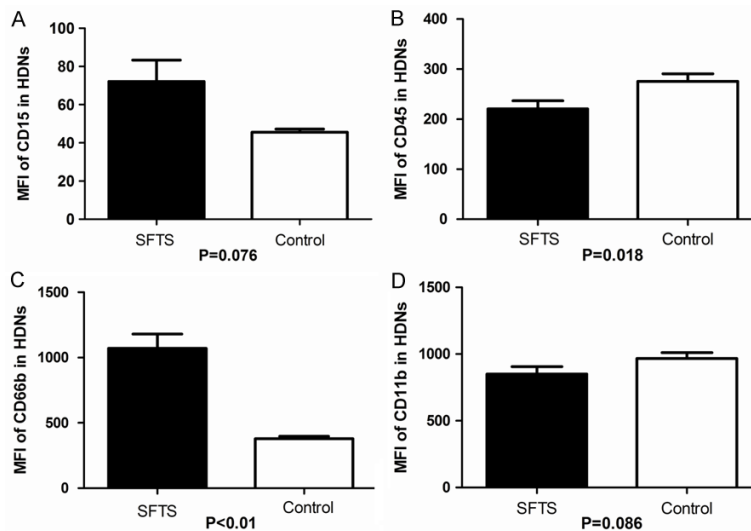


Figure 3. The expression of phenotypic markers in HDNs of SFTS patients and normal controls. The MFI values of CD11b, CD15, CD45 and CD66b were determined by flow cytometry. A, D. MFI levels of CD15 and CD11b in HDNs and normal controls has no significant difference. B. MFI levels of CD15 were decreased in HDNs of SFTS patients than of the normal controls. C. MFI levels of CD66b were increased in HDNs of SFTS patients than that of the normal controls. Abbreviations: HDNs, high-density neutrophils; MFI, mean fluorescence intensities.

the innate immune response against extracellular bacteria, which plays a role in stimulating structural cells to produce and release neutrophil-recruiting cytokines and growth factors at the site of infection, and thereby causes

accumulation of neutrophils [22]. We found that IL-17 levels in SFTS patients were significantly increased compared to that in control group (67.71 ± 76.18 versus 1.69 ± 3.97 , $P < 0.01$) (**Figure 4**). And we observed that there was a positive correlation between the level of IL-17 and the percentage of LDNs in SFTS patients ($r = 0.763$, $P < 0.01$) (**Figure 5B**). In addition, several studies had demonstrated that IL-17 was upstream of G-CSF. Indeed, there was a positive correlation between the level of IL-17 and G-CSF in SFTS patients ($r = 0.722$, $P < 0.01$) (**Figure 5C**). The level of IL-17 and the percentage of HDNs have no significant correlation ($P = 0.487$).

Granulocyte colony-stimulating factor (G-CSF) -that is known to mobilize stem cells out of the bone marrow and into the peripheral blood. G-CSF stimulates the production and maturation of neutrophils by promoting the proliferation and differentiation of myeloid progenitors [23, 24]. To analyze the mechanism of LDNs in the circulation of the acute phase of SFTS patients, we detected the concentrations of plasma G-CSF which was involved in neutrophils. We observed that the content of G-CSF in the acute phase of SFTS patients were obviously increased compared to that in control group (646.57 ± 387.05 versus 207.57 ± 51.38 , $P < 0.01$) (**Figure 4**). We also found that there was a significant correlation between the level of G-CSF and the percentage of LDNs in SFTS patients ($r = 0.938$, $P < 0.01$) (**Figure 5A**). But there was no significant correlation between the level of G-CSF and the percentage of HDNs in SFTS patients ($P = 0.127$).

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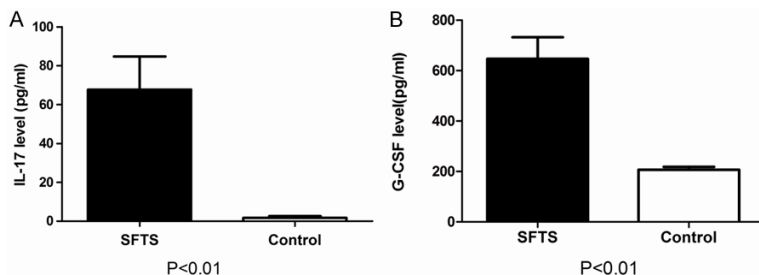


Figure 4. The concentration of G-CSF and IL-17 in SFTS patients and normal subjects. A. Level of IL-17 in SFTS was increased compared to that in control (67.71 ± 76.18 versus 1.69 ± 3.97 , $P < 0.01$). B. Level of G-CSF in SFTS was higher than that in control (646.57 ± 387.05 versus 207.57 ± 51.38 , $P < 0.01$). The Mann-Whitney test was used to compare variables. Abbreviations: G-CSF, Granulocyte colony-stimulating factor; IL, interleukin.

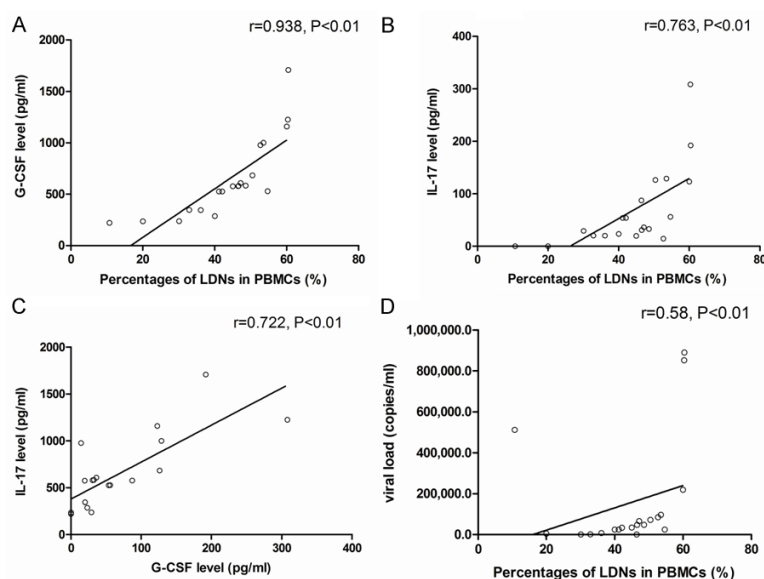


Figure 5. The relationship among IL-17, G-CSF, viral load and LDNs in SFTS patients. The best-fit lines are shown on each graph. On the graphs, r and P indicate the correlation coefficient and the P value of significance, respectively. A. A positive correlation was detected between G-CSF concentration and LDNs level ($P < 0.01$). B. There was a significant correlation between level of IL-17 and LDNs ($P < 0.01$). C. A positive correlation was detected between G-CSF concentration and the level of IL-17 ($P < 0.01$). D. There was positive correlation between level of LDNs and viral load amount in SFTS patients ($P < 0.01$). Pearson and Spearman test were used to show correlations. Abbreviations: G-CSF, Granulocyte colony-stimulating factor; IL, interleukin; LDNs, low-density neutrophils.

Association between load of SFTSV and the Frequency of LDNs and HDNs in SFTS

One of the most important markers of disease severity in SFTS is viral (bunyavirus) load. Therefore, we compared the SFTS patient viral load and the frequency of LDNs and HDNs. We found that there was no correlation between

SFTSV and percentage of HDNs. But we note that the SFTSV load correlates well with propagation of LDNs ($r=0.658$, $P=0.002$) (Figure 5D). These results suggested that in SFTS patients, the frequency of LDNs present in PBMCs correlated with SFTS disease severity.

LDNs in aged SFTS patient displayed decreased capacity to phagocytose SFTSV potential

Next, engulfment SFTSV capability of LDNs was evaluated. We explored the SFTSV loads in LDNs and HDNs from same SFTS patients (Figure 6). We observed that the load of SFTSV was lower in LDNs than that in HDNs of the SFTS patients, indicating that LDNs in SFTS patients display the different phagocytic SFTSV function and LDNs displayed decreased phagocytic SFTSV potential when compared to HDNs.

LDNs synthesize increased levels of proinflammatory cytokines in SFTS

As mentioned above, we found that SFTSV load correlates well with propagation of LDNs, but there was no correlation between SFTSV and percentage of HDNs ($P=0.696$). LDNs correlated with SFTS disease severity. Because IL-6, IL-8, G-CSF were elevated in SFTS patients and

produced at robust levels in fatal cases. In addition, IL-17 levels in SFTS patients were significantly increased compared to that in control group in this study. Therefore, the secretion of IL-17 was assessed in the supernatants of LDNs, HDNs from SFTS, and control neutrophils. Surprisingly, we found that LDNs from SFTS secreted significantly increased levels

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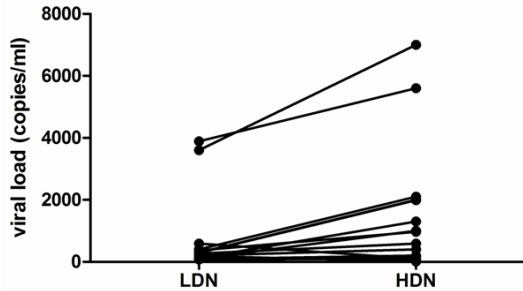


Figure 6. Comparison of capability of engulfment SFTSV of LDNs and HDNs. Each dot shows the SFTSV load in an individual. Each line connects a pair of SFTSV RNA samples collected from the LDNs and HDNs from the same patient. The load of SFTSV was lower in LDNs than that in HDNs of the SFTS patients in most cases. Abbreviations: SFTS, severe fever with thrombocytopenia syndrome; SFTSV, SFTS virus; LDNs, low-density neutrophils; HDNs, high-density neutrophils.

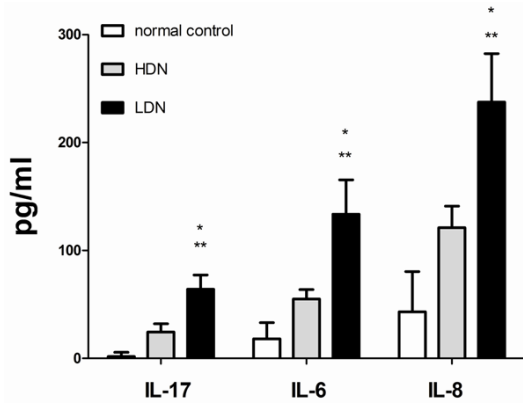


Figure 7. LDNs secrete increased levels of proinflammatory cytokines. Results represent cytokine concentration in cell supernatants in pg/ml. Measurements were done with supernatants harvested after 48 h in culture. * $P < 0.05$ when comparing LDNs to control neutrophils and ** $P < 0.05$ when comparing LDNs to HDNs. Results represent mean \pm SD of 20 controls and 20 SFTS patients. Abbreviations: IL, interleukin; LDNs, low-density neutrophils.

IL-17 proinflammatory cytokine than that of HDNs from SFTS. We further detected IL-6, IL-8 cytokines in the supernatants of LDNs, HDNs from SFTS, and control neutrophils. There was a trend for LDNs secreted significantly increased levels of IL-6, IL-8 cytokines by SFTSV infection, when compared to HDNs from SFTS or healthy control. There were statistically significant differences in IL-6, IL-8 and IL-17 cytokine secretion among the three groups of cells (**Figure 7**). These results indi-

cate that LDNs secrete increased levels of pro-inflammatory cytokines upon SFTSV infection.

Discussion

SFTS is one of the most severe emerging human infectious diseases in China. The natural history of SFTS encompasses three distinct stages: fever, MODS and convalescence [21]. Severe SFTS patient with MODS is a bottleneck to hinder the cure. So far, the mechanism of SFTS triggered severe inflammatory reaction in patients with multiple organ injury is not very clear. Here we detected the percentage of LDNs in the acute phase of SFTS patients. All SFTS patients so far examined display LDNs in their PBMC fractions. LDNs levels were significantly elevated in the PBMCs of subjects with SFTS patients than that in normal controls. The higher the level of LDNs, the higher number of SFTS viral copies. These findings are similar to the increased levels of LDNs that have been observed in patients with SLE and those with HIV [19, 25]. Those suggested that LDNs might play more important roles in inflammation and immune response induced by SFTSV. Morphological analyses showed that LDNs include increased numbers of cells with immature granulocyte morphology in cancer [17]. Therefore, expression levels of surface CD molecules by LDNs were further confirmed the identity of LDNs in the present study. Surface CD markers, which may appear with neutrophilic maturation, undergo several changes during neutrophilic maturation to accommodate the cell's function.

Here, we demonstrated that LDNs in patients with SFTS display decreased levels of CD45, CD11b, and CD15 expression and increases CD66b expression, compared with normal control. In SFTS, the most distinguishing phenotypic features of LDNs in the present study are near the 2-fold decrease in the level of CD45, CD11b, and CD15 compared to the normal control. In contrast, we confirmed a trend for decreased expression of CD45, CD11b, and increased expression of CD15 and CD66b in SFTS HDNs. We also found that LDNs and HDNs share the same phenotype in SFTS, but they are different in the level of phenotypic expression.

Integrins are critical for the migration and function of leukocytes in inflammation. CD11b,

belongs to the integrin $\beta 2$ family, participates in cell activation, chemotaxis, cytotoxicity and phagocytosis [26]. LDNs from SFTS expressed CD11b, but at lower levels than the other neutrophils subsets. Lower expression of CD11b in SFTS LDNs suggested that LDNs had less anti-virus function than the other neutrophils subsets. CD66b is a well-known cell marker of activation [27]. CD66b molecules seem to be capable of mediating a rapid and very effective cell adhesion and contribute to the supernormal adhesiveness to the endothelium [28]. SFTSV infection consequently resulted in CD66b cell-surface expression upregulation on human LDNs from patients with SFTS. LDNs can induce vascular damage [25]. The phenomenon that SFTS patients had vascular damage may not only be related to the functional role of LDNs in SFTS vascular damage, but also be related to CD66b expression upregulation on LDNs.

The exact role of LDNs in SFTS remains to be determined. One of the most important markers of disease severity in SFTS is viral (bunyavirus) load. Several studies proposed that SFTSV disable the host immune response by attacking and manipulating cells that initiate the antiviral response [10]. Our results showed that patients with the heavy load of SFTSV correlated with higher numbers of LDNs. By contrast, no appreciable associations have been found between load of SFTSV and numbers of HDNs.

In addition, we confirmed that the load of SFTSV was lower in LDNs than that in HDNs of SFTS patients, indicating a potential deficient ability for the clearance of SFTSV for infections in SFTS. Whether the abnormalities in SFTSV phagocytosis/clearance of LDNs was suggested to be induced by active viral replication and associated with disease severity remains to be determined.

Our previous studies have demonstrated that SFTS excessive immune responses can contribute to progressive organ damage [9, 10, 29]. We and other studies demonstrated that cytokine storm, a potentially fatal immune reaction occurring in the acute phase of SFTS [12, 30]. It has reported that LDNs secrete higher levels of this cytokine than high-density neutrophils [25]. In this study, the plasma level of IL-17 was significantly higher in SFTS patients, and

there was a correlation between the level of IL-17 and LDNs in SFTS patients. IL-17 is currently mainly regarded as a pro-inflammatory cytokine, one of its roles is stimulating structural cells to produce and release neutrophil-recruiting cytokines [22]. In our study, aside from IL-17, we also estimated the capacity of LDNs and HDNs from SFTS synthesize IL-6, IL-8 cytokines. Interestingly, LDNs had enhanced capacity to synthesize these cytokines when compared with HDNs from SFTS. It is possible that these cells may represent one of the sources of this cytokine in SFTS. Taken together, we can suggest that LDNs played a more important role than HDNs in the inflammation and immune response induced by SFTSV and may contribute to the amplification of inflammatory responses and tissue damage in SFTS.

Our results also showed that G-CSF was an aberrantly high production of several cytokines in SFTS. And G-CSF was further found to correlate with LDNs' amount, suggesting that the increased frequencies of LDNs in the peripheral blood of SFTS individuals might be caused by abnormal levels of G-CSF.

In summary, we, for the first time, identified a group of LDNs which were highly enriched in the acute phase of SFTS patients. LDNs have a proinflammatory phenotype. LDNs from SFTS patients displayed decreased phagocytosis of SFTSV and enhanced capacity to synthesize these cytokines when compared with HDNs from SFTS. LDNs are more important than HDNs in SFTS disease severity.

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Disclosure of conflict of interest

None.

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