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ORIGINAL ARTICLE

A Study of Immune Functionality of Newly Diagnosed Severe Aplastic Anemia Patients with Virus Infection

Jing Guan [*], Yingying Sun [*], Rong Fu, Huaquan Wang, Erbao Ruan, Xiaoming Wang, Wen Qu, Guojin Wang, Hong Liu, Yuhong Wu, Jia Song, Limin Xing, Lijuan Li, Hui Liu, Chunyan Liu, Zonghong Shao

[*] *These authors contributed equally to this work and should be considered co-first authors*
Department of Hematology, Tianjin Medical University General Hospital, Tianjin, China

SUMMARY

Background: **Previous research showed that virus infection is correlated with the occurrence, development, and prognosis of AA. This study was designed to explore the influence of virus infection on the immune functionality and immunosuppressive therapy (IST) efficiency of newly diagnosed SAA patients.**
Methods: **Fifty-six newly diagnosed SAA patients combined with virus infection treated in the Hematology Department of Tianjin Medical University General Hospital from October 2004 to July 2014 were studied. Various immune parameters were tested and compared for SAA patients with and without virus infection.**
Results: **When compared with SAA patients without corresponding virus infection, SAA patients with CMV-IgM, PVB19-IgM, and EBV infection had increased $CD8^+$ T cell percentage, decreased $CD4^+/CD8^+$ T cell ratios, and increased $CD8^+HLA\text{-}DR^+/CD8^+$ percentage. The absolute value of $CD8^+$ T cell of CMV-IgM group had increased as well. The CMV-IgM and PVB19-IgM groups showed decreased $CD4^+$ T cell percentage, and decreased $CD4^+HLA\text{-}DR^+/CD8^+HLA\text{-}DR^+$ ratio. The PVB19-IgM group exhibited decreased $CD4^+HLA\text{-}DR^+/CD4^+$ percentage, increased Th1 percentage and increased pDC percentage. Patients with EB virus infection showed lower NK cell percentage. Three years after IST, the treatment is significantly less effective for the SAA patients combined with virus infection than those without.**
Conclusions: **CMV, PVB19, and EBV infection worsen the immune functionality abnormality of newly diagnosed SAA patients and reduce the IST efficiency.**
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Correspondence:
Zonghong Shao
Tianjin Medical University General Hospital
Anshan St 154
Heping Dist.
300052 Tianjin
China
Phone: +86 13802036467
Fax: +86 22-60817092
Email: sun12345yingying@163.com

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KEY WORDS

SAA, EBV infection, CMV infection, PVB19 infection, immune functionality, IST efficiency

INTRODUCTION

Acquired aplastic anemia (AA) is a form of bone marrow failure caused by T cell-mediated immune destruction of hematopoietic cells. Previous research showed that virus infection is correlated with the occurrence, development, and prognosis of AA. In this study, we had selected 56 newly diagnosed acquired severe aplastic anemia (SAA) patients who were also infected with Cytomegalo Virus (CMV), Parvo Virus B19 (PVB19)

or Epstein Barr Virus (EBV). We analyzed various immune parameters and compared them to those of acquired SAA patients without virus infection (a total of 98 cases). We also analyzed the change of hematopoietic parameters to evaluate the efficacy of IST.

The goal of this study was to explore the immune functionalities of virus-infected SAA patients, as well as the influence of virus infection on IST curative effect. These results can be leveraged as the theoretical base for establishing scientific and effective AA treatment plans and early judgment of prognosis of SAA patients.

MATERIALS AND METHODS

Cases

We have collected samples and clinical data of 154 SAA patients in this study. They were treated at the Hematology Department of Tianjin Medical University General Hospital from October 2004 to July 2014. A total of 56 newly diagnosed SAA patients combined with CMV, PVB19 or EBV infection were selected. Characteristics of the cohort of patients was shown in Table 1. The number in each study group was shown in Table 2 and Table 3. The follow-up duration of these patients ranged from 3 to 72 months, with the median being 14.6 ± 15.1 months. The diagnosis of SAA complied with the 2009 British Council for Standardization in hematology aplastic anemia treatment guidelines [1]. The judgment of the treatment efficacy was in reference to the Camitta standard [2]. The study was approved by the Ethics Committee of the Tianjin Medical University. Informed written consent was obtained from all patients or their guardians in accordance with the Declaration of Helsinki.

Immune and hematopoietic parameters

Immune parameters monitored in this study include the T cell subset ($CD4^+/CD8^+$ ratio), activated $CD8^+$ T cell ($CD8^+HLA\text{-}DR^+$) percentage, DC subset (myeloid dendritic cell/plasmacytoid dendritic cell ratio, i.e., mDC/pDC ratio), Th cell subset (Th1/Th2 ratio), and NK cell subset ($CD16^+CD56^+$). These parameters were measured at diagnosis and were compared to those of SAA patients without the virus infection. We also analyzed the changes of hematopoietic parameters obtained from the blood routine tests and bone marrow smears in order to evaluate the IST efficiency.

Flow cytometric analysis

Peripheral blood T cell subset, NK cells, activated $CD8^+$ and $CD4^+$T cells, bone marrow DC, and Th cells were measured by flow cytometry, using the following directly labeled antibodies:

- T cell subset: anti-CD3-PerCP, anti-CD4-FITC, anti-CD8-PE
- Activated $CD8^+$ T cells: anti-CD8-PE, anti-HLA-DR-PerCP
- Activated $CD4^+$ T cells: anti-CD4-FITC, anti-HLA-DR-PerCP
- DC subset: anti-CD123-PE, anti-CD11c-PE. mDC is $CD11c^+$; pDC is $CD123^+$
- Th cell subset: anti-IFN-γ-PE, anti-IL-4-PE, anti-CD4-FITC. Th1 cell is $CD4^+$ IFN-γ^+; Th2 cell is $CD4^+$ IL-4^+
- NK cells: anti-CD3-PerCP, anti-CD16-FITC, anti-CD56-APC

All monoclonal antibodies are the products of BD Pharmingen.

Statistical analysis

The statistical analysis of the patients' data was done using SPSS 17 statistical analysis software. The results were expressed in terms of mean ± standard deviations. The independent sample mean comparison was done using the *t*-test (for data with normal distribution) and non-parametric test (for data without normal distribution). The efficiency comparison was done with the chi-square test. A value of $p < 0.05$ is considered statistically significant.

RESULTS

Immune functionality

T cell subset in peripheral blood

As shown in Figure 1 and Table 1, compared to those without corresponding virus infection, SAA patients with CMV-IgM, PVB19-IgM or EB virus infection had increased $CD8^+$ T cell percentage and decreased $CD4^+/CD8^+$ T cell ratio. Furthermore, the absolute value of $CD8^+$ T cells in the CMV-IgM group increased as well. The CMV-IgM and PVB19-IgM groups showed a decreased percentage of $CD4^+$ T cells. SAA patients with any of the three virus infections had an increased $CD8^+$ T cell percentage and decreased $CD4^+/CD8^+$ T cell ratio ($p < 0.05$). It is suggested that the virus infection in SAA patients aggravates the dysregulation of the T cell subset, and cytotoxic T cells have an advantage in quantity.

Activated state of T cell in peripheral blood

The percentage of $CD8^+HLA\text{-}DR^+/CD8^+$ is used to represent the activated state of $CD8^+$ T cell and the percentage of $CD4^+HLA\text{-}DR^+/CD4^+$ is used to represent the activated state of $CD4^+$ T cell.

As shown in Figure 2 and Table 2, compared to SAA patients without corresponding virus infection, SAA patients with CMV-IgM, PVB19-IgM or PVB19-IgG had an increased $CD8^+HLA\text{-}DR^+/CD8^+$ percentage. The $CD4^+HLA\text{-}DR^+/CD8^+HLA\text{-}DR^+$ ratio for the CMV-IgM and PVB19-IgM groups had both decreased. The $CD4^+HLA\text{-}DR^+/CD4^+$ percentage for the PVB19-IgG group had decreased as well ($p < 0.05$). There was no statistical difference in the above indicators between patients with and without EBV infection ($p > 0.05$). SAA patients with any of the three virus infections had an increased $CD8^+HLA\text{-}DR^+/CD8^+$ percentage ($p > 0.05$).

Table 1. Characteristics of the cohort of patients.

Characteristics	All patients (n = 154)	Virus infection group (n = 56)	Non-virus infection group (n = 98)
Gender			
Male, no. (%)	95 (61.7)	31 (55.4)	64 (65.3)
Female, no. (%)	59 (38.3)	25 (44.6)	34 (34.7)
Median age (years, range)	24.5 (3 - 79)	26 (5 - 78)	24 (3 - 79)
Etiology of SAA			
Idiopathic, no. (%)	146 (94.8)	52 (92.9)	94 (95.9)
Hepatitis, no. (%)	8 (5.2)	4 (7.1)	4 (4.1)
Severity of disease, no. (%)			
SAA	98 (63.6)	36 (64.3)	62 (63.3)
VSAA	56 (36.4)	20 (35.7)	36 (36.7)
Cell counts (Mean ± SD)			
ANC (x 10^9/L)	0.55 ± 0.75	0.72 ± 1.00	0.44 ± 0.53
RET (x 10^9/L)	11.63 ± 16.02	11.37 ± 14.67	11.77 ± 16.81
PLT (x 10^9/L)	24.96 ± 26.14	24.46 ± 27.58	25.24 ± 25.43
PNH, no. (%)	45 (29.2)	21 (37.5)	24 (24.5)
Therapy, no. (%)			
ATG/ALG + CsA	97 (63.0)	38 (67.9)	59 (60.2)
Others	57 (37.0)	18 (32.1)	39 (39.8)
Outcome after IST(ATG/ALG + CsA) at 3rd year, no. (%)			
Response	35 (53.0)	12 (38.7)	23 (65.7)
No response	31 (47.0)	19 (61.3)	12 (34.3)

SAA - severe aplastic anemia, VSAA - very severe aplastic anemia, ANC - absolute neutrophil count, RET – reticulocyte, PLT - platelet, PNH - paroxysmal nocturnal hemoglobinuria, ATG - rabbit antithymocyte globulin, ALG - antilymphocyte globulin, CsA - Cyclosporin A.

This suggests that the T cell activation of SAA patients is enhanced during virus infection, which aggravates the damage of T cells to bone marrow hematopoietic cells.

Th cell subset in bone marrow

As shown in Figure 3, SAA patients with PVB19-IgM had a higher Th1 cell percentage than SAA patients without (5.83 ± 3.77, n = 7 vs. 3.09 ± 1.97, n = 83; $p < 0.05$). It suggests that virus infection induces Th0 to polarize towards Th1, causing the balance of Th1/Th2 to skew towards Th1.

Dendritic cell subset in bone marrow

As shown in Figure 4, when compared to SAA patients without PVB19-IgM, SAA patients with PVB19-IgM exhibit an increased pDC percentage ($p < 0.05$). It is suggested that virus infection affects not only the down-stream of the SAA-associated immune cascade, but also the initial stage, making its abnormality more obvious.

Nature Killer cell percentage in peripheral blood

As shown in Figure 5, when compared to SAA patients without EBV infection, SAA patients combined with the EBV infection exhibit decreased NK cell percentage ($p < 0.05$). This demonstrates that the EBV infection weakens the protection from the NK cells.

Curative effect

Of all the 154 SAA patients, 97 of them had received immunosuppressive therapy (IST) which included anti thymocyte immunoglobulin (ATG)/anti human lympho-cyte globulin (ALG), and cyclosporine AA. Among them, 66 were followed up for more than 3 years. The curative effect was analyzed. We found that three years after IST, remission rate of the virus infection group was 38.7% (12/31), and remission rate of the non-virus infection group was 65.7% (23/35). The treatment is significantly less effective for SAA patients with virus infections than those without ($\chi^2 = 4.813$, $p < 0.05$). It is apparent that the virus infections reduced the IST effi-ciency for the SAA patients.

Table 2. Comparison of T cell subgroups between SAA patients with and without corresponding virus infection.

	n	CD4%	CD8%	$CD8^+$ T cell (*10^9/L)	$CD4^+/CD8^+$
CMV-IgM (+)	5	16.98 ± 12.88 ▲	79.92 ± 6.00 ▲	0.47 ± 0.33 •	0.21 ± 016 ▲
CMV-IgM (-)	123	41.26 ± 13.54	40.95 ± 16.13	0.26 ± 0.22	1.21 ± 0.67
PVB19-IgM (+)	12	31.15 ± 11.15 •	56.38 ± 19.43 •	0.33 ± 0.17	0.66 ± 0.36 ▲
PVB19-IgM (-)	119	41.26 ± 13.54	41.03 ± 16.78	0.27 ± 0.23	1.22 ± 0.69
EB (+)	20	36.51 ± 13.68	49.76 ± 18.03 •	0.29 ± 0.24	0.88 ± 0.58 •
EB (-)	111	41.02 ± 14.31	41.11 ± 17.19	0.27 ± 0.22	1.22 ± 0.69
Viral infection group	53	37.49 ± 14.41	46.20 ± 18.45 •	0.28 ± 0.21	1.03 ± 0.69 •
Non-viral infection group	78	42.26 ± 13.91	39.87 ± 16.51	0.26 ± 0.23	1.27 ± 0.67

• - compared to non-infected patients, $p < 0.05$, ▲ - compared to non-infected patients, $p < 0.01$.

Table 3. Comparison of activated T cells between SAA patients with and without corresponding virus infection.

	n	$CD4^+HLA\text{-}DR^+/CD4^+$	$CD8^+HLA\text{-}DR^+/CD8^+$	$CD4^+HLA\text{-}DR^+/CD8^+HLA\text{-}DR^+$
CMV-IgM(+)	4	25.03 ± 11.50	35.18 ± 11.29 •	0.10 ± 0.37 *
CMV-IgM(-)	44	14.50 ± 18.24	17.44 ± 16.53	1.08 ± 0.90
PVB19-IgM(+)	5	19.95 ± 12.74	33.78 ± 9.22 •	0.21 ± 0.14 •
PVB19-IgM(-)	44	14.73 ± 18.07	18.13 ± 16.90	1.06 ± 0.91
PVB19-IgG(+)	13	10.92 ± 8.01 •	26.59 ± 18.59 •	0.63 ± 0.72
PVB19-IgG(-)	36	21.63 ± 19.07	16.58 ± 12.75	3.40 ± 6.41
Viral infection group	15	12.67 ± 9.84	28.18 ± 17.14 ▲	0.54 ± 0.65
Non-viral infection group	34	21.99 ± 19.73	14.44 ± 11.39	3.79 ± 6.70

• - compared to non-infected patients, $p < 0.05$, ▲ - compared to non-infected patients, $p < 0.01$, * - compared to non-infected patients, $p < 0.05$ (non-parametric tests).

Table 4. A brief description of the effect of CMV, PVB19, and EBV on hematopoiesis.

Virus	Mainly affected hematopoietic cells	Possible influencing mechanism
CMV	various hematopoietic cell types, especially the hematopoietic progenitor cells, which is the main latent part of CMV	Establish latent infection within its host via inhibiting cellular immunity, escaping from the degradation of phagocytes lysosomes and inducing apoptosis. Reactivate via interhost transmission, differentiation and the role of cytokines. Damage immune surveillance by inhibiting DCs [9,10]. Fas-mediated apoptosis [11].
PVB19	erythroid lineage	Suppress erythropoiesis because of the specific receptor (blood-group P antigen) on the cell membrane [12]. Cause immune-mediated bone marrow suppression [13].
EBV	B cells T cells NK cells	Interfere with the MHC molecular expression of the host, affecting antiviral immunity. Stimulate the production of Th2 cells, the secretion of IFN-α, and other cytokines by expressing BCRF1. Cause immune abnormalities such as NK cells and EBV-specific cytotoxic T cells (EBV-CTL) impairment.

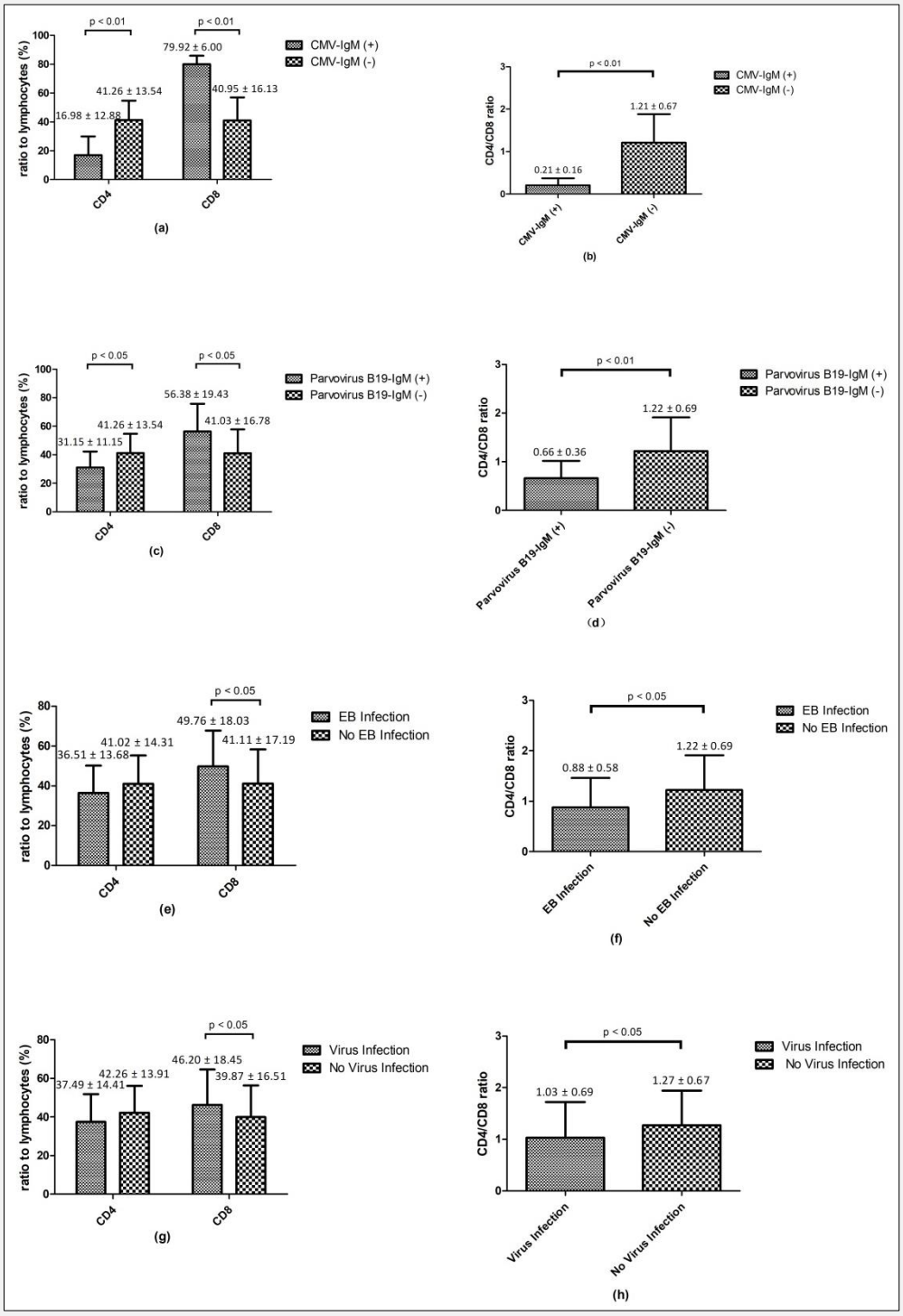

Figure 1. T cell subset in peripheral blood.

(a) Comparison of peripheral $CD4^+$ and $CD8^+$ T cell percentage in SAA patients with and without CMV-IgM. (b) Comparison of peripheral $CD4^+/CD8^+$ T cell ratio in SAA patients with and without CMV-IgM. (c) Comparison of peripheral $CD4^+$ and $CD8^+$ T cell percentage in SAA patients with and without PVB19-IgM. (d) Comparison of peripheral $CD4^+/CD8^+$ T cell ratio in SAA patients with and without PVB19-IgM. (e) Comparison of peripheral $CD4^+$ and $CD8^+$ T cell percentage in SAA patients with and without EBV infection. (f) Comparison of peripheral $CD4^+/CD8^+$ T cell ratio in SAA patients with and without EBV infection. (g) Comparison of peripheral $CD4^+$ and $CD8^+$ T cell percentage in SAA patients with and without any of the three viruses. (h) Comparison of peripheral $CD4^+/CD8^+$ T cell ratio in SAA patients with and without any of the three viruses.

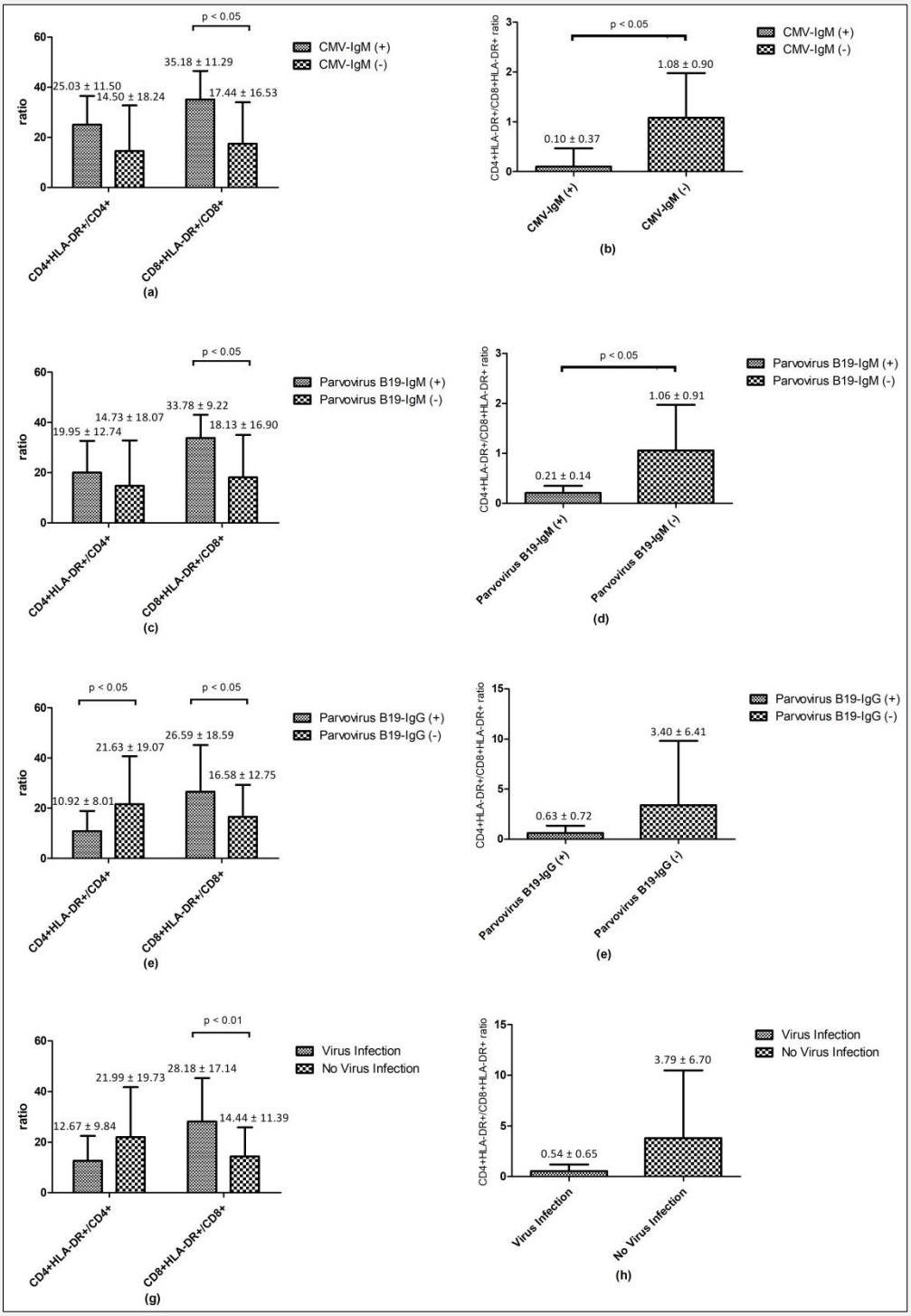

Figure 2. Activated state of T cells in peripheral blood.

(a) Comparison of peripheral T cell activation status in SAA patients with and without CMV-IgM. (b) Comparison of peripheral $CD4^+HLA\text{-}DR^+/CD8^+HLA\text{-}DR^+$ ratio in SAA patients with and without CMV-IgM. (c) Comparison of peripheral T cell activation status in SAA patients with and without PVB19-IgM. (d) Comparison of peripheral $CD4^+HLA\text{-}DR^+/CD8^+HLA\text{-}DR^+$ ratio in SAA patients with and without PVB19-IgM. (e) Comparison of peripheral T cell activation status in SAA patients with and without PVB19-IgG. (f) Comparison of peripheral $CD4^+HLA\text{-}DR^+/CD8^+HLA\text{-}DR^+$ ratio in SAA patients with and without PVB19-IgG. (g) Comparison of peripheral T cell activation status in SAA patients with and without any of the three viruses. (h) Comparison of peripheral $CD4^+HLA\text{-}DR^+/CD8^+HLA\text{-}DR^+$ ratio in SAA patients with and without any of the three viruses.

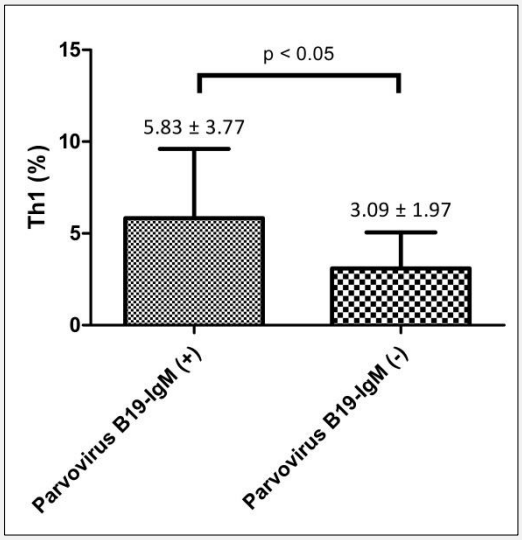

Figure 3. Comparison of bone marrow Th1 percentage in SAA patients with and without PVB19-IgM.

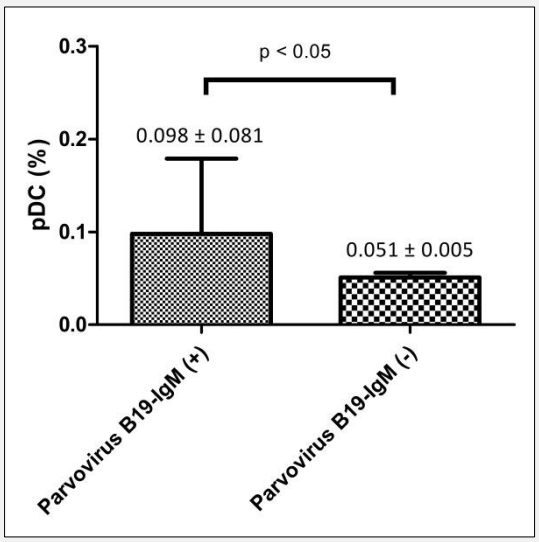

Figure 4. Comparison of bone marrow pDC percentage in SAA patients with and without EBV infection.

DISCUSSION

AA is a type of autoimmune disease mediated by T lymphocytes. It is understood that the immune abnormality plays an important role in the occurrence and development of AA, especially the functionality and quantity of abnormalities of T lymphocytes. Abnormal $CD8^+$ T cells (cytotoxic lymphocyte), $CD4^+$ T cells (T helper cells, including Th1 and Th2), regulatory T cells (Treg), Th17 cells, NK cells and NKT cells are all involved.

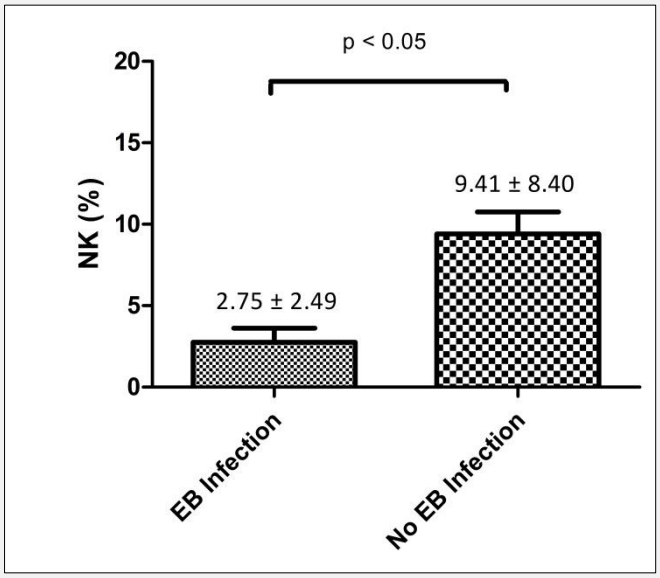

Figure 5. Comparison of peripheral NK cell percentage in SAA patients with and without EBV infection.

They secrete many cytokines inappropriately such as interferon-gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α), tumor growth factor-beta (TGF-β), etc. and work together to induce the apoptosis of hematopoietic stem/progenitor cells, leading to the onset of typical AA. Our previous studies have shown, it is possible that some antigenic substances trigger the increase of mDC activation [3], leading to the striking increase of the mDC/pDC ratio [4], secreting large amount of T-bet, inducing Th0 to polarize towards Th1, causing the balance of Th1/Th2 to skew towards Th1 [5]. The increased Th1 cells secrete many hematopoietic negative regulators such as INF-γ and others, mediate cell immunity, activate cytotoxic T cells ($CD8^+$ T cells), and cause the $CD4^+/CD8^+$ ratio to decrease [6]. The activated $CD8^+$ T cell percentage increases [7]. $CD8^+$ T cells are the main effector cells of AA pathogenesis. The above-mentioned immune functionality changes can be summarized as the "immune activation waterfall", which is the pathogenesis of AA.

The severity of SAA patients' condition at diagnosis varies significantly, and even if they receive the same initial treatment (e.g., immunosuppressive therapy, IST), the progression and outcome are significantly different. In addition to age, genetic background, and other individual differences, some complications also affect the prognosis of AA, such as viral infection. There has been many studies on the relationship between virus infection and bone marrow failure. Among them, are many studies on the hepatitis virus. Some scholars have pointed out that the prognosis of SAA patients with hepatitis virus infection is worse [8]. In this study, we focus on the impact of CMV, PVB19, and EBV on SAA patients. The influence of these three viruses on hematopoiesis is shown in Table 4.

In this study, when the 54 newly diagnosed SAA patients with virus infection started treatment, their humoral immune systems had started working and antibodies had already been produced in response to the corresponding viruses. At the same time, it is worth studying whether the virus infection can cause further cellular immune response, what effect the virus infection would have on cellular immune system, whether the virus infection can alter SAA's original immune pattern, and whether the virus infection can affect the curative effect of SAA.

$CD8^+$ T cells are the main effector cells in AA pathogenesis, and circulating activated T $CD8^+$ cells have been identified as a subgroup that inhibit hematopoiesis. The activated CTL kills the hematopoietic cells in three ways. It secretes hematopoietic negative regulators such as TNF-β, IFN-γ, etc., which bind to the corresponding receptors on the surface of target cells, and induce apoptosis of target cells. It releases perforins, which damage the hematopoietic cell membrane, killing hematopoietic cells directly or enters hematopoietic cells by releasing Granuase B, activating the apoptosis of target cells. It up-regulates the expression of FAS, which binds to FASL on the surface of hematopoietic cells, inducing apoptosis of target cells through the caspase cascade. As can be seen from the results, the aforementioned virus infection aggravates the original immune ab-

normalities of AA, mainly manifested as the increase in quantity and excessive activation of $CD8^+$ T cells, leading to more serious damage to hematopoiesis. Furthermore, $CD4^+$ T cell percentages in CMV and PVB19-infected SAA patients decrease, suggesting that T helper cells' function in stimulating non-specific immunity, humoral immunity, and regulating cellular immune function has been weakened. The reduced function of T helper cells causes the original infection to be difficult to control, and it is more likely to induce other new infections. The decrease of activated $CD4^+$ T cell percentage in PVB19-infected SAA patients further confirmed the decrease of T helper cells in both quantity and quality. Of the three viruses, CMV causes the most obvious change of $CD4^+$ and $CD8^+$ T cells. Not only the percentage, but also the absolute value of $CD8^+$ T cell increase, suggesting that CMV plays the strongest role in promoting immune abnormalities.

DCs are antigen-presenting cells and the most common division of DCs is myeloid and plasmacytoid, i.e., mDC and pDC. Stimulated by certain antigens which are still unknown, mDCs in SAA patients proliferate and activate, while pDCs play a critical role in antiviral responses. These pDC cells connect innate and adaptive immune responses by releasing high levels of type I interferon and processing virus/endogenous antigen into the form of MHC-peptide complexes onto cell membranes for T cells to recognize [14]. pDCs activated by a different kind of virus showed different regulatory effects on T cells. In general population, the number and function of DCs increase in response to virus infection. For SAA patients in our study, the ones who were infected by PVB19 had higher pDC percentages than those who were not infected, indicating that PVB19 infection causes new immune abnormalities, which may be related to the potent antiviral action of type I interferon.

AA is thought to be caused by increased Th1 and its downstream cells and cytokines - $CD3^+CD8^+$ T cells, TNF-α, IFN-γ, etc. DCs regulate the immune pattern of Th1 vs. Th2. During virus infection, the virus type, the stimulation of CD40 ligand, the virus strength and titer, and the microenvironment work together to determine the influence of activated DCs on $CD4^+$ T cells' differentiation direction. SAA patients combined with PVB19 infection at diagnosis exhibited higher Th1 percentage than those without, indicating that the infection aggravates polarization of T helper cells to Th1 in SAA. NK cells are closely related to T lymphocytes, B lymphocytes, and dendritic cells. They are immune cells that protect SAA patients and are involved in the pathogenesis of SAA in indirect ways with a decrease in number. NK cells combat viruses by directly recognizing and responding to the infected cells, they upregulate their cytotoxic potential and produce inflammatory cytokines [15]. We once studied the changes of the number and function of NK cells in peripheral blood of SAA patients. The number of NK cells of the untreated SAA patients was lower than the healthy control group, and it increased after IST, positively correlating with the change of hematopoiesis. In addition to the decrease in quantity, cytokines secreted by the $CD56^{bright}$ cells also decreased, resulting in the insufficient inhibition of T cells and mDCs, followed by the failure of hematopoiesis. We also noticed that NK cells did not kill the hematopoietic cells directly by the perforin/Granuase B pathway. Presumably the increase of perforin was in compensation for the decrease in the number of NK cells. We speculated that the decrease of the number of NK cells upregulated mDC and CTL, leading to the incidence of AA. At this time, NK cells expressed NKp46 strongly, which further activated NK cells to secrete a large amount of perforin, with a view to killing excessive mDC and CTL to terminate the progression of SAA. NK cells may be indirectly involved in SAA by regulating other immune cells. In this study, compared to SAA patients without EBV infection, SAA patients combined with EBV infection exhibited decreased NK cell percentage, inferring that EBV infection aggravated the decrease of NK cell percentage, making the immune abnormalities in SAA worse.

IST is the most commonly used and effective treatment for SAA. It is reported that nearly 80% of AA patients have a hematological reaction to IST. Large prospective studies conducted in Europe, USA, and Japan showed that 60% to 70% of AA patients had hematological relief and long-term survival after IST. The standard initial immunosuppressive therapy regimen includes ATG/ALG combined with cyclosporine A, and allows two-thirds of patients to eliminate blood transfusion, but 30 - 40% of patients still relapse or have life-long reliance on cyclosporine A. Therefore, although IST has significantly improved the prognosis of AA patients, there are still some patients who do not respond, relapse or have clonal evolution. One of the factors that affects the effectiveness of IST is virus infection.

We analyzed the cure rate at 0, 0.5, 1, 2, 3 year(s) IST and found that the cure rate of SAA patients with virus infection at 3 years after IST was only 38.7%, much lower than that of SAA patients without virus infection (65.7%). Virus infection not only aggravates the patient's immune abnormalities, but also affects the curative effect of IST. It is an adverse factor in the treatment process.

CONCLUSION

The above studies suggest that if SAA patients are infected with virus at diagnosis, the infection will aggravate the original immune abnormalities of these patients, worsening their conditions and reducing the IST efficiency. Further study is needed to understand whether the virus is the etiology of AA or only aggravates the immune abnormalities. However, the results of this study could help us establish scientific and effective AA treatment plans and early judgment of prognosis of SAA patients. SAA patients infected with virus should

be treated carefully, and we should screen the virus at the beginning of treatment and try to clear it.
However, our study had limitations. In general, the nucleic acid test is the gold standard for diagnosing viral infections. Due to the limitations of experimental techniques, we only performed serological detection for PVB19 and CMV, not the nucleic acid test. Despite these defects, our serological tests were also highly specific and the negative effects of viral infection on SAA were also demonstrated. Future studies will be required to explore the effects of specific viral loads on the pathogenesis and outcome of SAA patients.

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Declaration of Interest:
The authors declare no competing financial interests. There are no conflicts for each named author. There was no involvement of a pharmaceutical or other company. There were no sponsors in the article preparation.

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