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# Increased CD8$^+$CD27$^+$perforin$^+$ T cells and decreased CD8$^+$CD70$^+$ T cells may be immune biomarkers for aplastic anemia severity

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ABSTRACT

*Objectives:* Aplastic anemia (AA) is T cell immune-mediated autoimmune disease. Aberrant T cell activation involves an imbalance in T cell homeostasis in AA. However, whether the T cell activation molecule CD27 and its ligand CD70 participate in the immune pathogenesis of AA remains ill defined.
*Methods:* The frequencies of CD27/CD70 and perforin/granzyme B in different T cell subsets were detected in AA patients and healthy individuals by flow cytometry.
*Results:* We first time demonstrate a significantly elevated proportion of CD27$^+$ and significantly decreased CD70$^+$ T cells from AA. Changed frequency of CD27$^+$ and CD70$^+$ in different T cell subsets appeared to be associated with AA severity. In very severe aplastic anemia (VSAA) and severe aplastic anemia (SAA), increased CD8$^+$CD27$^+$ T cells present with a cytotoxic effector phenotype by elevating perforin proportion.
*Conclusions:* Elevated proportion of CD27 in T cells may contribute to distinct immune pathogenesis for different severities of AA. The CD8$^+$CD27$^+$perforin$^+$ T cells combined with CD8$^+$CD70$^+$ T cells may serve as an immune biomarker for AA severity estimation.

## 1. Introduction

Aplastic anemia (AA) is an autoimmune disease that is characterized by cytopenia and marrow hypoplasia. The stratification of AA severity depends on the degree of peripheral cytopenias and bone marrow cellularity. AA is further divided as very severe aplastic anemia (VSAA), severe aplastic anemia (SAA), and non-severe aplastic anemia (NSAA) [1,2].

Immunosuppressive therapy (IST) is recommended as first-line therapy for NSAA patients who require treatment, SAA or VSAA patients who lack a matched sibling donor, or aged between 35 and 50 years. Antithymocyte globulin (ATG) or cyclosporin A (CsA) are used as standard initial IST [3–5].

In general, patients with higher baseline absolute reticulocyte counts (ARC) and absolute lymphocyte counts (ALC) have a higher response rate [6,7]. The pathophysiology of AA is not fully understood. Dysregulated T cell immunity is implicated in hematopoietic stem cell injury [8].

Moreover, aberrant T cell activation was shown to be involved in T cell homeostasis imbalance in AA. T cell signaling activation molecules or regulatory factors is abnormally expressed in AA [9–12]. Our previous study also found that aberrant T cell activation might be distinct for different severities of AA. For instance, a more significantly increased CD3ζ mRNA expression was found in NSAA [10]. It may be possible that other T cell activation molecules also have diversity change tendencies in different AA severities.

Defective T cell activation is associated with severe immune deficiencies, whereas aberrant T cell activation contributes to the

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pathogenesis of diverse autoimmune and inflammatory diseases [13–15]. T cell activation requires signals provided by costimulators in addition to antigen-induced signals. The best characterized costimulator is CD28. The CD28 costimulatory signals work to promote T cell proliferation and differentiation by cooperating with antigen recognition [16].

In addition, novel T cell surface molecules also contribute to T cell activation. For example, CD27, a member of tumor necrosis factor receptor (TNFR) superfamily, also delivers costimulatory signals. CD27 is expressed on T cells, NK cells, and hematopoietic stem cells. This protein is also expressed on active and antigen-experienced B cells. The only ligand characterized for CD27 is CD70, a TNF superfamily member that is expressed on activated T cells, B cells, dendritic cells, and NK cells. The CD27-CD70 costimulatory pathway supports the proliferation and survival of activated T cells and positively regulates T cell effector functions and memory responses [17,18].

Little is known about whether the CD27-CD70 axis is involved in the immune pathogenesis of AA. In this study, we investigated the frequencies of $CD27^+$ and $CD70^+$ T cells in peripheral blood (PB) from AA. Moreover, we further analyzed the frequencies of $CD8^+CD27^+perforin^+$ and $CD8^+CD27^+granzyme\ B^+$ T cells in AA to gain a more detailed understanding of the role of CD27 in AA.

## 2. Materials and methods

### 2.1. Patients and healthy individuals

Peripheral blood samples were obtained from 58 AA patients (39 males and 19 females with a median age of 31 years), including 16 cases with VSAA, 28 cases with SAA, and 14 cases with NSAA and 60 healthy individuals as health controls whose race, sex and age were same as those of AA patients with a median age of 35 years (HIs; 31 males and 29 females, range, 22–67 years). PB samples from AA patients were collected at the time of diagnosis. We excluded AA patients and healthy controls with other haematological, autoimmune diseases or infections. The patients had not used corticosteroids or antibiotics during the last 3 months before study inclusion. The clinical characteristics of the patients are summarized in Table 1. All PB samples were obtained with consent. The study was approved by the Ethics Committee of School of Medicine of Jinan University. Written informed consents were obtained from patients in accordance with the Helsinki Declaration.

**Table 1**
Characteristics of the AA patients.

| Characteristics | VSAA ($N$ = 16) | SAA ($N$ = 28) | NSAA ($N$ = 14) |
|---|---|---|---|
| Age(year) | 36.8(14.1) | 33(14.7) | 32(15.0) |
| Sex | | | |
| Male | 11(68.7) | 19(67.9) | 9(64.3) |
| Female | 5(31.3) | 9(32.1) | 5(35.7) |
| Haematological parameters | | | |
| Haemoglobin(g/L) | 65.25(6.61) | 67.43(11.53) | 67.00(6.39) |
| Platelet count($\times 10^9$/L) | 19.94(13.47) | 30.86(18.19) | 28.71(19.29) |
| Absolute neutrophil count($\times 10^9$/L) | 0.45(0.83) | 0.94(1.04) | 1.63(1.08) |
| Absolute lymphocyte count($\times 10^9$/L) | 0.81(0.46) | 1.22(0.51) | 1.32(0.58) |
| Absolute reticulocyte count($\times 10^9$/L) | 6.54(7.79) | 20.68(23.36) | 31.96(19.89) |
| Treatment | | | |
| Hematopoietic stem cell transplantation | $N$ = 4 | $N$ = 6 | / |
| Immunosuppressive therapy (CsA) | $N$ = 9 | $N$ = 21 | $N$ = 12 |
| Immunosuppressive therapy (ATG + CsA) | $N$ = 3 | N = 1 | N = 1 |
| Transfusion | / | / | N = 1 |

The data are presented as means (standard deviation) for most variables; sex is presented as $n$ (%). N: number.

### 2.2. Antibodies and reagents

For flow cytometry, CD3-FITC (HIT3a), CD8-Percp-Cy5.5 (SK1), perforin-Alexa Fluor 647 (dG9), granzyme B-Pacific Blue (GB11), mouse (MPC-11) mAb IgG2b isotype control (Alexa Fluor 647 conjugate), and mouse (MOPC-21) mAb lgG1 isotype control (Pacific Blue conjugate) were obtained from Biolegend (San Diego, USA); CD4-APC-H7 (RPA-T4), CD27-PE-Cy7 (M-T271), mouse (clone MOPC-21) mAb IgG1 isotype control (PE-Cy7 conjugate), and CD70-PE (Ki-24) were obtained from BD Biosciences (San Jose, USA).

### 2.3. Flow cytometry analysis

In all cytometric analyses, a total of at least $1 \times 10^6$ cells were obtained by red blood cell lysis of blood from AA or HIs followed by analysis using a BD FACSVerse multicolor flow cytometer (BD Biosciences).

Cells were incubated with specific antibodies directed at surface markers including anti-CD3, anti-CD8, anti-CD4, anti-CD27, and anti-CD70. Then, the cells were washed and resuspended to prepare for detecting the CD27 or CD70 percentages in the different T-cell subsets.

To assess the perforin or granzyme B percentage in $CD8^+CD27^+$ T cells, cells were first labeled with anti-CD3, anti-CD8, and anti-CD27, fixed and permeabilized, and finally labeled with anti-granzyme B and anti-perforin antibodies.

Isotype controls were provided to enable compensation and confirmation of antibody specificity. Flow cytometry data were analyzed using FlowJo sofware (BD Bioscience).

### 2.4. Statistical analysis

All data are represented as medians, and statistically significant differences between AA and HIs were analyzed using the Mann-Whitney $U$ test for nonparametric values with SPSS software 13.0. $P$ values $< 0.05$ were considered statistically significant, and the following labels were used: $^*p < 0.05$, $^{**}p < 0.01$.

## 3. Results

### 3.1. Quantification of $CD3^+CD27^+$, $CD4^+CD27^+$ and $CD8^+CD27^+$ T cells in PB from AA patients

Flow cytometric analysis shown the percentage of $CD3^+CD27^+$ T cells in AA patients was 88.2%, which was significantly higher than that in HIs (median percentage: 78.9%). Moreover, this characteristic was also present for $CD4^+CD27^+$ and $CD8^+CD27^+$ T cells (Fig. 1A–D).

We further analyzed $CD27^+$ T cell alterations in different AA subtypes. Interestingly, a significantly elevated percentage of $CD3^+CD27^+$, $CD4^+CD27^+$ and $CD8^+CD27^+$ T cells was found in VSAA and SAA compared with HIs, while only a higher percentage of $CD4^+CD27^+$ T cells was found for NSAA. Moreover, the percentage of $CD4^+CD27^+$ and $CD8^+CD27^+$ T cells was higher in VSAA compared with SAA.

### 3.2. Quantification of $CD8^+CD27^+perforin^+$ and $CD8^+CD27^+granzyme\ B^+$ T cells in PB from AA patients

Because of $CD8^+CD27^+$ T cells characteristics in VSAA and SAA, we further analyze effector phenotype of cytotoxic $CD8^+$ T cells in AA. In this study, the median percentage of $CD8^+CD27^+perforin^+$ T cells in AA was 8.34%, which was significantly higher than that in healthy individuals (median percentage: 3.57%). The $CD8^+CD27^+perforin^+$ T

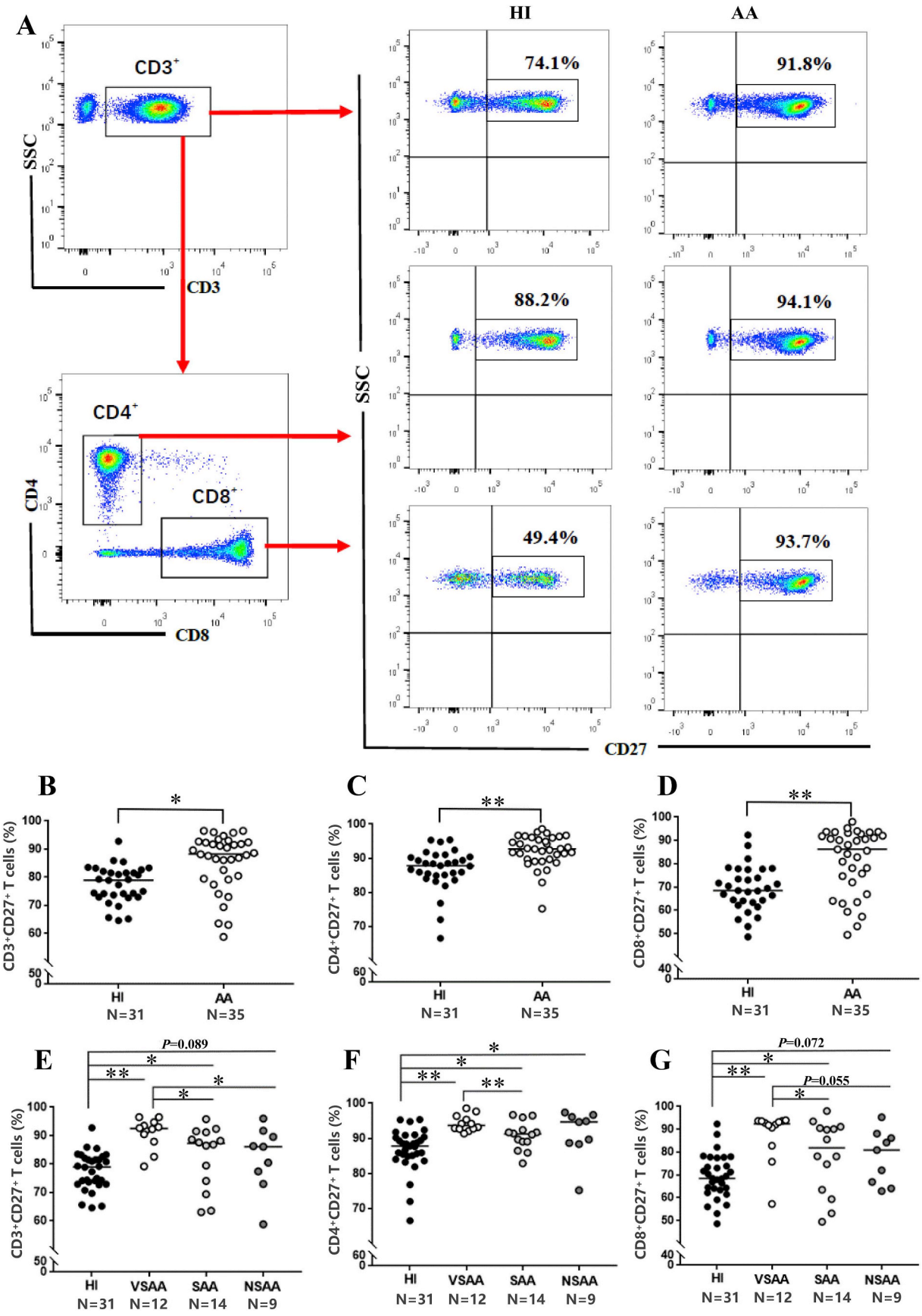


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**Fig. 1.** The frequency of CD27 in different T cell subsets a from healthy individuals and AA patients.
(A) Gating strategy for identifying the frequency of CD27 in different T cell subsets. (B–D) The median percentage of $CD3^+CD27^+$ (78.9%), $CD4^+CD27^+$ (92.7%) and $CD8^+CD27^+$ (86.2%) in AA patients was significantly higher than that in healthy individuals (88.2%, 87.8%, and 68.5%, respectively). (E) The median percentage of $CD3^+CD27^+$ T cells in VSAA, SAA, and NSAA was 92.5%, 87.3%, and 86.1%, respectively; (F) The median percentage of $CD4^+CD27^+$ T cells in VSAA, SAA, and NSAA was 93.7%, 91.3%, and 94.7%, respectively; (G) The median percentage of $CD8^+CD27^+$ T cells in VSAA, SAA, and NSAA was 92.4%, 81.9%, and 80.9%, respectively.

cells in VSAA (6.64%) or SAA (9.04%) were significantly higher than those in healthy individuals (Fig. 2A–C).

There were no significantly difference of the median percentage of $CD8^+CD27^+$granzyme $B^+$ T cells between healthy individuals (18.7%) and AA (16%) , while $CD8^+CD27^+$granzyme $B^+$ T cells in different severity of AA (VSAA, SAA and NSAA was 14.1%, 17.9% and 13.8%, respectively) also has no significantly difference compare with that in healthy individuals (Fig. 2D, E).

### 3.3. Quantification of $CD3^+CD70^+$, $CD4^+CD70^+$ and $CD8^+CD70^+$ T cells in PB from AA patients

Because of CD70 was also expressed in activated T cells, we analyzed the percentage of CD70 in different T cell subsets. In the present study, AA patients have a significantly decrease percentage of $CD3^+CD70^+$, $CD4^+CD70^+$ and $CD8^+CD70^+$ T cells in comparisons with healthy individuals (Fig. 3A-D).

In VSAA patients, not only $CD3^+CD70^+$ but also $CD4^+CD70^+$ and $CD8^+CD70^+$ T cells shown a significantly decrease feature in comparisons with HIs. Significantly decrease percentage of $CD3^+CD70^+$ and $CD8^+CD70^+$ T cells was found in SAA patients, whereas only significantly decrease percentage of $CD4^+CD70^+$ T cells was found in NSAA patients (Fig. 3E-G).

### 3.4. Correlations between CD27 or CD70 percentage in various T cell subsets and PB counts

In consideration of CD27 and CD70 percentage feature in various T cell subsets from different severity AA patients, we further analyze the correlation between CD27 and CD70 percentage in different T cell subsets and ANC or absolute lymphocyte count (ALC) or ARC. The analysis result shown that significant negative correlation between $CD3^+CD27^+$ or $CD8^+CD27^+$ percentage and ANC were found from all AA patients, while significant negative correlation between $CD8^+CD27^+$ percentage and ARC were found from all AA patients (Fig. 4A–C). Meanwhile, the significant positive correlation between $CD3^+CD70^+$ or $CD4^+CD70^+$ or $CD8^+CD70^+$ and ARC were also found from all AA patients (Fig. 4D–F).

The significant negative correlation between $CD3^+CD27^+$ or $CD8^+CD27^+$ percentage and ANC were found from SAA patients. Meanwhile, the significant positive correlation between $CD8^+CD70^+$ percentage and ARC were found from SAA patients (Fig. 4G–I).

Furthermore, the significant negative correlation between $CD3^+CD70^+$ or $CD4^+CD70^+$ percentage and ALC were found from VSAA patients (Fig. 4J, K).

## 4. Discussion

It is commonly recognized that AA is a T cell immune-mediated autoimmune disease. Previous studies have indicated that aberrant T cell activation signaling molecules play a role in AA immune pathogenesis [19].

In addition to antigens binding with T cell receptors on T cells, T cell activation requires costimulatory signals [20–22]. CD27 is an important costimulatory receptor. Costimulatory signals from CD27 play a key role in T cell activation, T cell clonal expansion, effector T cell differentiation, and T cell survival [23]. In mouse inflammatory bowel disease models, CD27-deficient $CD4^+$ T cells were significantly less pathogenic than wild-type cells, and anti-CD70 treatment prevented pathogenesis and ameliorated established disease [24]. It has been suggested that CD27 participates in the pathogenesis of autoimmune diseases, but whether it is also involved in AA pathogenesis is an interesting question. Therefore, in this study, we first analyzed changes in the percentage of $CD27^+$ T cells in PB from AA. Significantly elevated percentage of $CD3^+CD27^+$, $CD4^+CD27^+$ and $CD8^+CD27^+$ T cells were found in AA compared with HIs. These data provide the first evidence that CD27 may be one reason for aberrant T cell activation in AA.

The results of $CD27^+$ T cell alterations in different AA subtypes shown that significantly elevated percentage of $CD3^+CD27^+$, $CD4^+CD27^+$ and $CD8^+CD27^+$ T cells was only found in VSAA and SAA. The frequency of CD27 in the different T cell subsets was associated with disease severity or activity in rheumatoid arthritis (RA), inflammatory bowel disease, and chronic Trypanosoma cruzi infection [25–27]. We considered whether the increased numbers of $CD27^+$ T cells are related to disease status and if $CD27^+$ T cells could mediate aberrant T cell activation and contribute to AA pathogenesis or progression. Thus, we hypothesized that the alterations in $CD27^+$ T cells are an immune biomarker for AA severity.

In view of the above findings, we further analyzed the cytotoxic effector phenotype of the T cell subsets including $CD27^+$ T cells in AA. Antigen recognition by cytotoxic $CD8^+$ T cells triggers granule exocytosis. The secreted granules then release perforin and granzymes. Perforin forms pores that allow granzymes to enter target cells, initiating apoptosis. There are five human granzymes (A, B, H, K, M), and granzyme B is the most extensively studied in cytotoxic $CD8^+$ T cells [28]. There are a few studies regarding perforin and granzyme B alterations in AA, but the findings are inconsistent. For example, slightly reduced percentages of $CD8^+$perforin$^+$ T cells and comparable percentages of $CD8^+$granzyme $B^+$ T cells have been found in the PB of AA by flow cytometry [29], while significantly increased percentages of $CD8^+$perforin$^+$ and $CD8^+$granzyme $B^+$ T cells were found in PB from SAA [11]. Xu and colleagues reported a significantly increased perforin level in BM from AA by immunohistochemistry, but granzyme B was not increased [30].

We found a significantly elevated percentage of $CD8^+CD27^+$perforin$^+$ T cells in patients with AA or those with VSAA and SAA in comparison with HIs; however, there was no statistically significant difference in the $CD8^+CD27^+$ perforin$^+$ T cell percentage between the NSAA and HIs. For $CD8^+CD27^+$ granzyme $B^+$ T cells, there was no statistically significant difference between the AA subgroups and HIs.

These results suggest that $CD8^+CD27^+$ perforin$^+$ T cells may be involved in the abnormal T cell cytotoxicity found in AA, particularly for VSAA and SAA, while $CD8^+CD27^+$ granzyme $B^+$ T cells may play a limited role in abnormal T cell activation in AA. Second, these results may support the hypothesis that T cell immune pathogenesis in NSAA is different from that in VSAA and SAA. Thus, it may be possible that $CD8^+CD27^+$ perforin$^+$ T cells might serve as a potential clinical or laboratory immune parameter for AA severity judgment.

It should be point out that the characteristic of $CD27^+$ T cells in bone marrow from AA need to be analyzed in our ongoing studies to confirm effect of CD27 in different severity of AA.

Based on the finding of an alternative distribution of $CD8^+CD27^+$ T cells in AA, we were interested in characterizing the alterations of the CD27 ligand, CD70, in AA. In general, CD70 is expressed on various

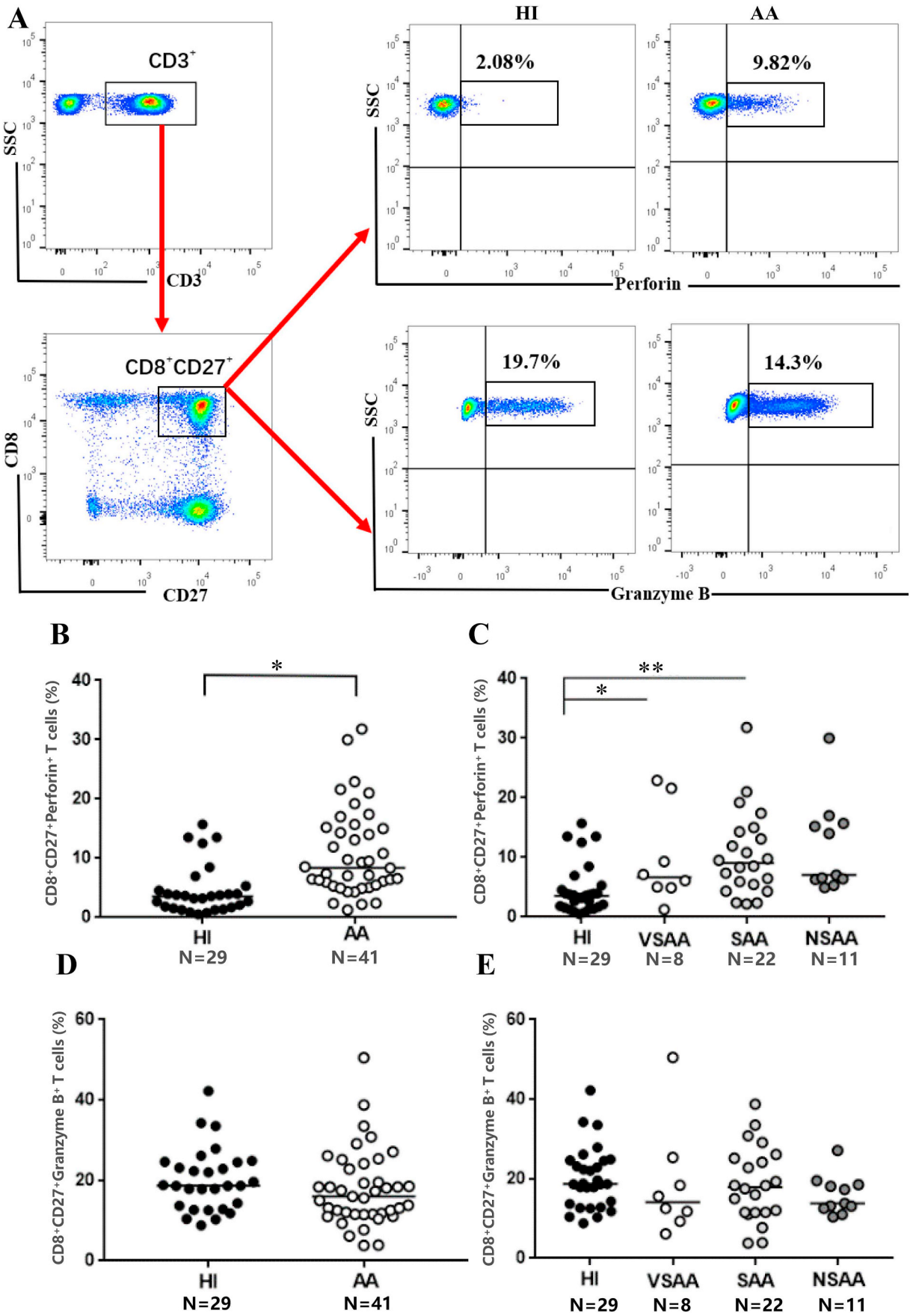


**Fig. 2.** The frequency of $CD8^+CD27^+perforin^+$ and $CD8^+CD27^+$granzyme $B^+$ T cells from healthy individuals and AA patients.
(A) Gating strategy for the identification $CD8^+CD27^+perforin^+$ and **$CD8^+CD27^+$granzyme $B^+$** T cells. (B) The percentage of $CD8^+CD27^+perforin^+$ T cells in PB between healthy individuals and AA patients. (C) The percentage of $CD8^+CD27^+perforin^+$ T cells in PB among healthy individuals, VSAA, SAA and NSAA patients. (D) The percentage of $CD8^+CD27^+$ Granzyme $B^+$ T cells in PB between healthy individuals and AA patients. (E) The percentage of $CD8^+CD27^+$granzyme $B^+$ T cells in PB among healthy individuals VSAA, SAA and NSAA patients.

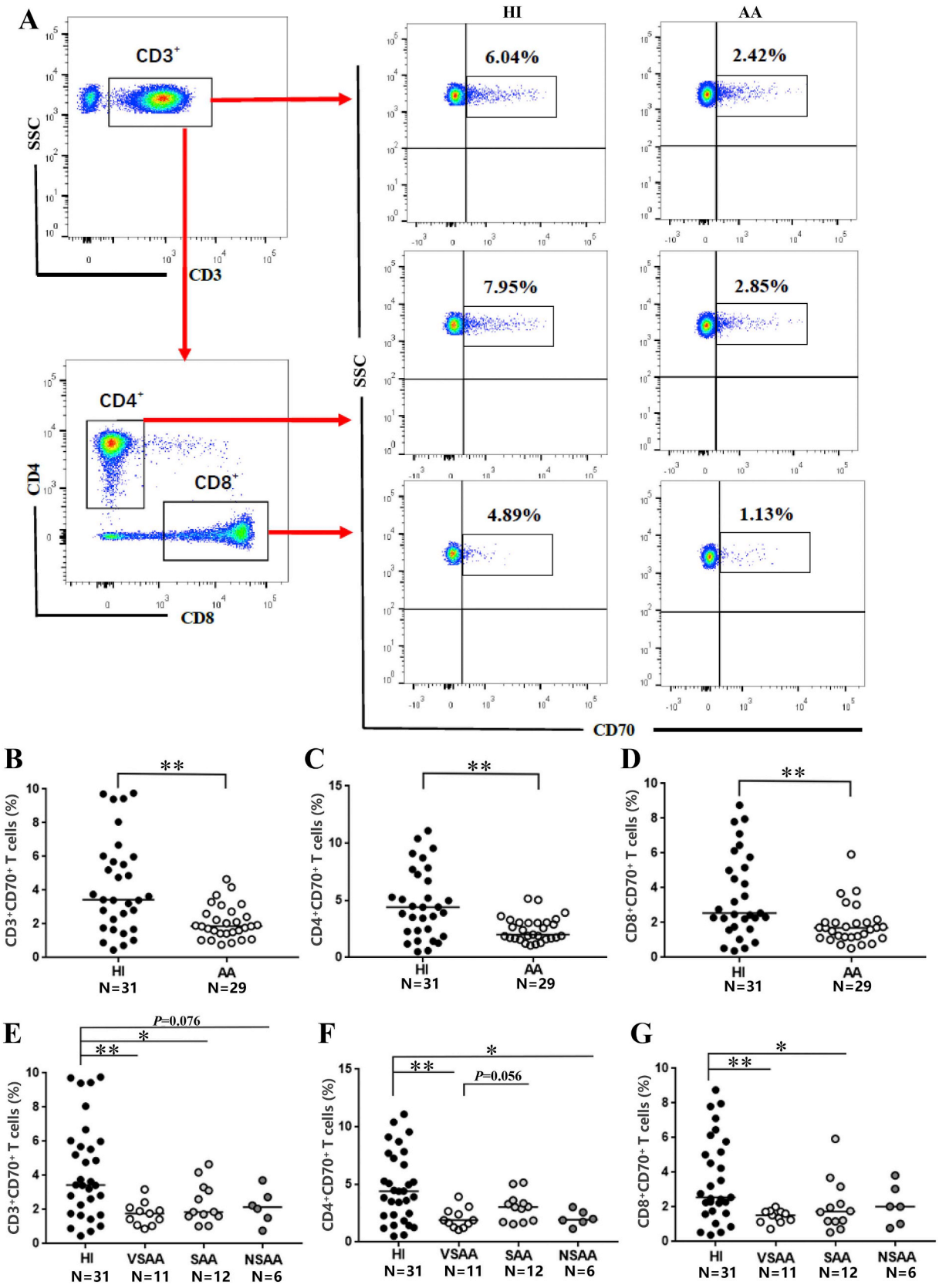


**Fig. 3.** The frequency of $CD3^+CD70^+$, $CD4^+CD70^+$, and $CD8^+CD70^+$ T cells in PB from healthy individuals and AA patients.
(A) Gating strategy for identifying the frequency of CD70 in different T cell subsets. (B–D) The median percentage of $CD3^+CD70^+$ (1.86%), $CD4^+CD70^+$ (2%), and $CD8^+CD70^+$ (1.7%) in AA patients was significantly lower than that in healthy individuals (3.44%, 4.4%, and 2.55%, respectively). (E) The median percentage of $CD3^+CD70^+$ T cells in VSAA, SAA, and NSAA was 1.77%, 1.86%, and 2.15%, respectively; (F) The median percentage of $CD4^+CD70^+$ T cells in VSAA, SAA, and NSAA was 1.9%, 3.05%, and 1.95%, respectively; (G) The median percentage of $CD8^+CD70^+$ T cells in VSAA, SAA, and NSAA was 1.52%, 1.73%, and 2.02%, respectively.

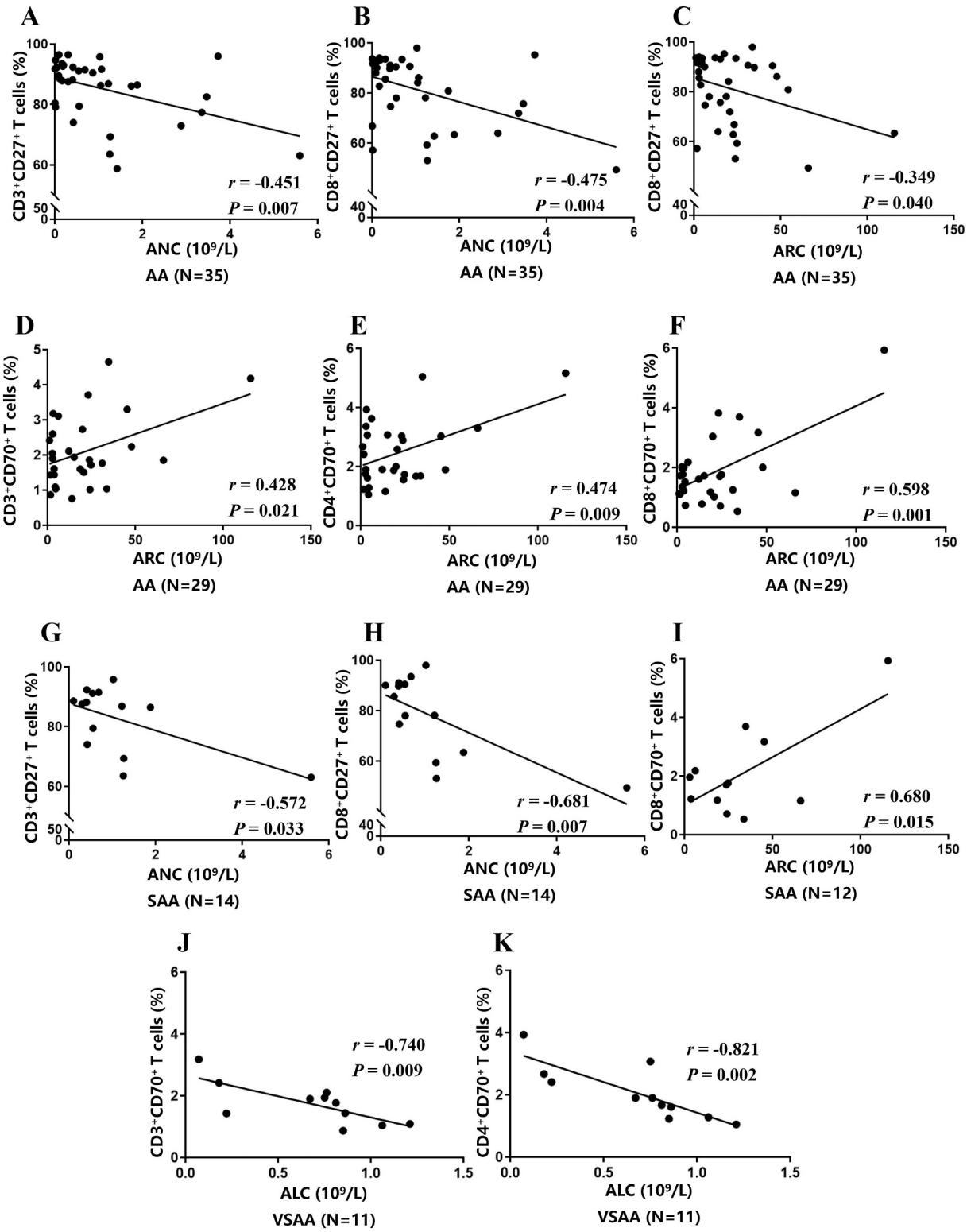


**Fig. 4.** Correlations between the CD27 or CD70 percentage in various T cell subsets and PB counts.
(A–B) A negative correlation between $CD3^+CD27^+$ or $CD8^+CD27^+$ T cells and ANC in AA is shown. (C) A negative correlation between $CD8^+CD27^+$ T cells and ARC in AA is shown. (D–F) A positive correlation between $CD3^+CD70^+$, $CD4^+CD70^+$, or $CD8^+CD70^+$ T cells and ARC in AA is shown. (G–I) In SAA, negative correlations between $CD3^+CD27^+$ T cells and ANC, $CD8^+CD27^+$ T cells and ANC, and a positive correlation between $CD8^+CD70^+$ T cells and ARC were found. (J–K) In VSAA, a negative correlation between $CD3^+CD70^+$ or $CD4^+CD70^+$ T cells and ALC was found.

types of antigen-presenting cells, while CD70 is also expressed on activated T cells [17]. However, the function of T cell-derived CD70 remains unclear. Elevated frequencies of $CD4^+CD70^+$ were found in RA and systemic lupus erythematosus (SLE) patients. The effects of elevated $CD4^+CD70^+$ in RA and SLE are different. In the former, increased $CD4^+CD70^+$ T cells increase the T cell autoreactivity response risk, while for the latter, increased $CD4^+CD70^+$ T cells lower the B cell differentiation threshold [31,32]. Recently, CD70 has been reported to

play an immune checkpoint role by inhibiting inflammatory T cell responses using adoptive-transfer models [33]. These findings indicate that the effects of CD70 on T cells are complicated and may depend on the different pathological states in different diseases.

To the best of our knowledge, we for the first time analyzed the distribution of $CD70^+$ T cells in AA. The changes in $CD70^+$ T cell subsets appear to be related to disease status. In VSAA patients, the percentages of $CD3^+CD70^+$, $CD4^+CD70^+$, and $CD8^+CD70^+$ T cells were significantly decreased, and in the SAA group, a lower percentage of $CD3^+CD70^+$ and $CD8^+CD70^+$ T cells was found, while in the NSAA group, a change was found only for $CD4^+CD70^+$ T cells. These results are consistent with the change in $CD27^+$ T cells in different severity of AA and further support the speculation that the characteristic proportions of $CD70^+$ T cells in different T cell subsets are related to the severity of AA and may serve as an auxiliary parameter for evaluating AA severity.

In this study, the finding of a decreased percentage of $CD8^+CD70^+$ T cells in SAA patients was contradictory to Wang's report in which a significantly higher proportion of $CD8^+CD70^+$ T cells were detected [34]. The reason for the inconsistent results between the two studies may be due to the selection of samples because the other group analyzed samples including cases with relapse AA who had undergone IST. IST may influence the distribution of T cell subsets. Further investigation is needed to compare the differences in the alterations of $CD70^+$ T cells and $CD27^+$ T cells in AA before and post IST.

It has been reported that patients with lower ARC and ALC have a lower response to IST compared with those with higher baseline values, and patients with lower ANC although have a lower response to IST that is not as strong as with ARC and ALC [5]. It has been suggested that higher baseline ARC and ALC may indicate better residual marrow function and the presence of sufficient stem cells to support blood cell production after IST.

In consideration of the characteristic percentages of CD27 and CD70 in various T cell subsets in AA with different severities, we further analyzed the correlation between the CD27 or CD70 percentages in different T cell subsets and ARC, ALC, or ANC. Interestingly, the proportionate number of CD27 or CD70 in various T cell subsets positively correlates with the baseline ANC or ARC that was found for AA or SAA patients. In SAA, the CD27 or CD70 percentage indexes and ARC or ANC together may serve as indicators of response following IST.

It is interesting to note that a negative correlation between the $CD3^+CD70^+$ or $CD4^+CD70^+$ percentage and ALC was found for VSAA patients. Also, it is known that most AA patients present with decreased ALC [35]. Whether these results imply that $CD4^+CD70^+$ T cells play a protective or regulatory role rather than a disease-inducing or disease-favoring role in VSAA remains an open question.

## 5. Conclusion

In conclusion, we for the first time demonstrate an elevated proportion of CD27 in T cells from patients with AA, which may contribute to distinct immune pathogenesis for different severities of AA. In VSAA and SAA, increased $CD8^+CD27^+$ T cells present with a cytotoxic effector phenotype by elevating the perforin proportion. These results preliminarily illustrate that CD27 participates in aberrant T cell activation in AA.

We first found a decreased proportion of CD70 in T cells from patients with AA. Although the exact role of CD70 in AA has not been characterized, the varies characteristics of the CD70 percentage in T cell subsets among different severities of AA suggest that $CD8^+CD70^+$ T cells combined with $CD8^+CD27^+perforin^+$ T cells may serve as potential immune biomarkers for estimating the severity of AA.

## Abbreviations

| | |
|---|---|
| AA | aplastic anemia |
| HIs | healthy individuals |
| VSAA | very severe aplastic anemia |
| SAA | severe aplastic anemia |
| NSAA | non-severe aplastic anemia |
| IST | immunosuppressive therapy |
| ATG | antithymocyte globulin |
| CsA | cyclosporin A |
| ARC | absolute reticulocyte counts |
| ALC | absolute lymphocyte counts |
| ANC | absolute neutrophil count |
| TNFR | tumor necrosis factor receptor |
| PB | peripheral blood |
| BM | bone marrow |
| RA | rheumatoid arthritis |
| SLE | systemic lupus erythematosus |

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## Conflict of interest

The authors declare that they have no competing interests.

## Sources of funding

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## Authors' contributions

BL and YQL contributed to the concept development, study design, coordinate the study and draft the manuscript. SWZ performed flow cytometry and data analysis, prepared figure and table. YPZ, WFL, MZ, YML and JL provided all samples and clinical data. GXH and YKX were responsible for the collection of clinical data. All authors read and approved the final manuscript.

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