Clinical outcomes of immunosuppressive therapy for severe aplastic anemia patients with absolute neutrophil count of zero

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Aplastic anemia; severe; zero; adult; anti-thymocyte globulin; cyclosporin A; immunosuppressive therapy; fulminant

ABSTRACT

Objectives: To evaluate the prognosis of adult severe aplastic anemia (SAA) patients with absolute neutrophil count (ANC) values of zero prior to immunosuppressive therapy (IST).

Methods: Patients with ANC values of zero prior to IST were separated from very SAA and analyzed in a prospective study. All patients received IST with rabbit anti-thymocyte globulin (ATG) and cyclosporine (CsA).

Results: A significantly lower response rate (RR) was identified in patients with ANC = 0 prior to IST when compared to patients with SAA after both 3 and 6 month periods or compared to those with vSAA at 3 months only. The efficacy of IST was inversely related to ANC = 0. The overall survival rate of the ‘zero’ group was significantly lower than that of the vSAA or SAA groups. Overall survival was closely associated with response to IST, and was inversely related to ANC = 0.

Discussion: In SAA patients, ANC is associated with prognosis, the elucidated overall survival improvement in patients without ANC = 0 occurred in conjunction with decreased infection-related mortality. Our study revealed that adult patients with ANC = 0 prior to IST responded poorly to IST, suggesting that having a very low number of neutrophils was a highly predictive factor for efficacy and survival of SAA patients treated with IST.

Conclusion: Adult SAA patients with ANC = 0 had a very poor prognosis and new therapeutic regimens may result in better outcome for these patients.

1. Introduction

Severe aplastic anemia (SAA) can be successfully treated with allogeneic hematopoietic stem cell transplantation (HSCT) from an HLA-matched sibling donor. Intensive immunosuppressive therapy (IST) consisting of anti-thymocyte globulin (ATG) and cyclosporine (CsA) is often used for patients with SAA who lack an HLA-matched sibling donor or who are not eligible for HSCT [1–5].

Early experiments showed absolute neutrophil count (ANC) was highly predictive of response and survival in SAA patients treated with IST. In a report by the European Group for Blood and Marrow Transplantation (EBMT), the actuarial 5-year survival rates were 46% in patients with an ANC of less than 0.2×10⁹/L and 61% in patients with an ANC between 0.2 and 0.5×10⁹/L, here the only significant pre-treatment variables were a low neutrophil count (p = 0.001) and increasing age (p = 0.05) [6]. A study analyzed 64 patients with aplastic anemia treated with antilymphocyte globulin between 1980 and 1985, and revealed 79% survival for non-severe aplastic anemia (NSAA), compared to 36% for SAA (p = 0.001). The neutrophil and platelet counts before treatment with ALG were highly predictive of survival [7]. A randomized trial from the EBMT SAA working party demonstrated that early mortality (<120 d) was correlated with the severity of disease (39%, 10% and 6% respectively in patients with neutrophil counts of < 0.2, 0.2–0.5, > 0.5×10⁹/L) [8]. Bacigalupo et al. further proposed that AA was considered very severe if the criteria for SAA were fulfilled and the neutrophil count was less than 0.2×10⁹/L. SAA was subclassified as very SAA (vSAA) (ANC of <0.2×10⁹/L) and SAA (ANC of 0.2–0.5×10⁹/L).

But recently, one study in children showed that younger age, higher baseline absolute reticulocyte count (ARC), and absolute lymphocyte count (ALC) were highly predictive of response to IST at 6 months (p = 0.018, p = 0.005 and 0.036, respectively) while ANC was not [9]. Another study even reported that patients with a white blood cell count (WBC) of less than 2.0×10⁹/L showed a higher response rate than those with a WBC of
hemoglobinuria (PNH) clone using flow cytometry and cytogenetics were performed on all patients. The presence of a paroxysmal nocturnal hemoglobinuria (PNH) clone was defined as the absence of glycosylphosphatidylinositol (GPI)-anchored surface proteins greater than 1% of neutrophils or monocytes in peripheral blood.

It may be controversial to suggest that the severity of the disease or neutrophil counts at diagnosis can predict the prognosis of SAA after IST.

A prospective Japanese study in children with SAA proposed further subclassification of SAA into fulminant aplastic anemia (FAA), if ANC = 0 for at least 2 weeks prior to and after IST. They found that the response rate (RR) of patients with the FAA was significantly lower than those with vSAA and SAA, but, overall survival (OS) was similar [11]. Does that mean ANC = 0 might be an effective prognostic factor to predict the efficacy of IST?

We retrospectively analyzed the efficacy of IST for adult patients with SAA who were enrolled in a prospective multi-center registration of the Chinese Eastern Collaboration Group of Anemia and evaluated whether the prognosis was impacted by ANC = 0 prior to IST.

2. Patients and methods

2.1. Patients

From January 2014 to March 2018, a total of 91 patients aged 18 years and over with vSAA or SAA in the Hospital A B and C were enrolled in this study. A diagnosis of SAA was given if at least two of the following peripheral blood count criteria were fulfilled: An ANC of <0.5×10^9/L, a reticulocyte count of <20×10^9/L, or platelet count of <20×10^9/L with a bone marrow cellularity of less than 25% [12].

A vSAA was diagnosis was applied if the criteria for severe disease. None remission (NR) was defined as a patient still fulfilling the severe disease criteria. The overall response rate (RR) was defined as CR or PR at 3 and 6 months after IST [1].

2.2. Treatment regimens

All patients were treated with a combination of intravenous rabbit ATG (Lymphoglobulin, Sanoﬁ, FR) at 3.5 mg/kg/day for 5 days and oral CsA at 6 mg/kg/day. The dose of CsA was adjusted to a trough between 150 and 200 ng/ml for at least 6 months. Granulocyte colony-stimulating factor was administered subcutaneously at 200 μg/m² only to patients with an ANC of <0.2×10^9/L.

2.3 Criteria for response to IST

The response was evaluated at 3 and 6 months after the initiation of therapy. A complete remission (CR) was defined as a hemoglobin level of more than 11.0 g/dL, a neutrophil count of more than 1.5×10^9/L, and a platelet count of more than 150×10^9/L. A partial remission (PR) was defined as a patient being transfusion independent and no longer meeting criteria for severe disease. None remission (NR) was defined as a patient still fulfilling the severe disease criteria. The overall response rate (RR) was defined as CR or PR at 3 and 6 months after IST [1].

2.4. Statistical analysis

Data was analyzed using SPSS 20.0. Differences among variables were evaluated by the χ² test (or Fisher’s exact test for cell frequencies less than 5) and a standard t-test was used for continuous variables. Correlation analysis of relevant factors and efficacy or death were analyzed by the log-rank test. Overall survival was estimated by using the Kaplan-Meier method. P values of less than 5% were considered statistically significant.

3. Results

3.1. Baseline characteristics

Patients were classified into the following three groups by neutrophil stratification: 20 with an ANC of ‘zero’, 26 with vSAA, and 45 with SAA. Eleven patients had PNH clones at the time of diﬀagnoses. The median interval between diagnosis and treatment was 9 (3–38), 15 (3–51), and 13 (3–97) days, respectively. The median follow-up at the time of analysis was 17.0 (1–51), 17.5 (4–41), and 27.5 (3–47) months, respectively. There were no significant diﬀferences elucidated between baseline characteristics when comparing groups (Table 1).

2.2. Treatment response

At 3 months after the initiation of therapy, 25 patients (55.6%) with SAA, 14 patients (53.8%) with vSAA, and 5 patients (25.0%) in the ‘zero’ group responded to the initial course of IST (Table 2). By 6 months, 33 patients (73.3%) with SAA, 15 patients (57.7%) with vSAA, and 9 patients (45.0%) with ‘zero’ group had become transfusion-independent. Six months later, 2 patients achieved CR in the ‘zero’ group, meanwhile, 3 patients achieved PR and 6 patients achieved CR in the SAA group. Overall, of the 91 evaluable patients who received an initial course of IST, 11 patients (12.1%)
Table 1. Patients’ characteristics.

|                          | ‘zero’ | vSAA | SAA | p value |
|--------------------------|--------|------|-----|---------|
| Patient number           | 20     | 26   | 45  |         |
| Sex (male/female)        | 5/15   | 14/12| 23/22| 0.120   |
| Median age, years (range)| 46.5(20–74) | 39(20–64) | 30.5(19–68) | 0.680   |
| Cause of aplastic anemia |         |      |     |         |
| Idiopathic               | 17     | 21   | 38  |         |
| Hepatitis                | 2      | 4    | 5   |         |
| Drug                     | 1      | 1    | 2   |         |
| Median days from diagnosis to treatment (range) | 93(3–38) | 15(3–51) | 13(3–97) | 0.583   |
| Median white blood cell count×10^9/L (range) | 0.94(0.01–2.10) | 1.69(0.61–2.42) | 2.21(0.81–5.17) | 0.678   |
| Median neutrophil count×10^9/L (range) | 0      | 0.13(0.05–0.19) | 0.47(0.3–1.9) | 0.433   |
| Median reticulocyte count×10^9/L (range) | 6.70(2.1–43.1) | 7.79(1.62–28.4) | 18(0.66–70.45) | 0.278   |
| Median platelet count×10^9/L (range) | 11.5(1–59) | 8(1–63) | 11.9(2–52) | 0.894   |
| Median observation time, months (range) | 17.0(1–51) | 17.5(4–41) | 27.5(3–47) | 0.130   |

vSAA: very severe aplastic anemia; SAA: severe aplastic anemia.

Table 2. Hematologic response in patients treated with immunosuppression.

| Cohort and Response | ‘zero’ | vSAA | SAA |
|---------------------|--------|------|-----|
| Rate at 3 Mo        |        |      |     |
| No. of patients     | 20     | 26   | 45  |
| Response – no. (%)  |        |      |     |
| CR + PR             | 1 + 4(25.0 [4.0–43.2]) | 1 + 13(53.8 [25.1–55.3]) | 1 + 24(55.6 [32.8–57.8]) |
| RR                  | 15(75.0 [34.9–69.5]) | 12(46.2 [21.1–50.9]) | 20(44.4 [26.8–51.3]) |
| Rate at 6 Mo        |        |      |     |
| No. of patients     | 20     | 26   | 45  |
| Response – no. (%)  |        |      |     |
| CR + PR             | 2 + 7(45.0 [19.3–51.2]) | 3 + 12(57.7 [28.0–58.4]) | 6 + 27(73.3 [45.4–70.2]) |
| RR                  | 11(55.0 [24.8–57.1]) | 11(42.3 [18.8–47.9]) | 12(26.7 [15.4–37.7]) |

RR: response rate, CR: complete remission, PR: partial remission, NR: none remission, vSAA: very severe aplastic anemia, SAA: severe aplastic anemia.

Figure 1 shows remission to IST, using the Kaplan-Meier method. The vSAA group showed a similar RR to the vSAA and SAA groups (57.7% vs 55.6%, p = 0.934). RR at 6 months was significantly lower in the ‘zero’ group than in either vSAA or SAA groups (25.0% vs 55.6%, p = 0.036). There was no significant difference between vSAA and SAA groups (53.8% vs 55.6%, p = 0.934). RR at 6 months was significantly lower in the ‘zero’ group than in the SAA groups (57.7% vs 73.3%, p = 0.031) while there was no significant difference between vSAA and ‘zero’ groups (57.7% vs 45.0%, p = 0.298), or between vSAA and SAA groups (57.7% vs 73.3%, p = 0.33).

Figure 1. Comparison of speed of getting RR in the ‘zero’, vSAA and SAA groups (Kaplan-Meyer method). The vSAA and SAA groups achieved remission faster than the ‘zero’ group, respectively (p = 0.001 and p = 0.001, respectively) (Kaplan-Meyer method).

3.3. Influencing factors of efficacy

Univariate analysis revealed that IST efficacy was related to the neutrophil count (p = 0.029), but was not related to age (p = 0.599), gender (p = 0.195), time from diagnosis to treatment (p = 0.195), lymphocyte count (p = 0.723), reticulocyte count (p = 0.285), platelet count (p = 0.963), or PNH clone presences (p = 0.256). Furthermore, the neutrophil count was the...
only factor shown to influence treatment efficacy when using multivariate analysis with a log-rank test \((p = 0.019, \text{Table 3})\).

### 3.4. Survival and outcomes

Overall survival in the ‘zero’, vSAA, and SAA groups were 65.0% (95%CI, 39.0–85.5%), 91.7% (95%CI, 75.2–99.9%), and 95.6% (95%CI, 89.0–99.9%) \((p = 0.001)\), respectively (Figure 2).

There were 7, 2, and 2 patients that died in the ‘zero’, vSAA, and SAA groups respectively. The deaths were due to the following causes: infection \((n = 6)\), respiratory failure \((n = 1)\), deadly hemoptysis \((n = 1)\), intracranial hemorrhage \((n = 1)\), and other reason \((n = 1)\). Among the 6 patients that died, there were 4, 1, and 1 from the ‘zero’, vSAA, and SAA groups respectively. Eight patients dropped out of IST: 5 patients had clonal evolution \((4 \text{ PNH and 1 MDS-EB-1})\), and 3 patients needed SCT.

When analyzing factors predictive of OS, including neutrophil count, IST efficacy, age, gender, time from diagnosis to treatment, lymphocyte count, reticulocyte count, platelet count and presence of PNH clones, only neutrophil count and IST efficacy were found to be predictive of improved survival when the multivariate analysis was applied \((p = 0.026, p = 0.010)\) (Table 4).

### 4. Discussion

Aplastic anemia (AA) is characterized by peripheral blood pancytopenia and classified according to the severity of cytopenia in clinic. In SAA patients a low ANC is associated with poor prognosis, therefore, patients with \(\text{ANC} < 0.2 \times 10^9/\text{L}\) are further defined as vSAA, indicating increased disease severity \([8]\). Some researches confirmed that patients might meet a higher incidence of fatal infection and a lower survival rate after ATG treatment with a low ANC \([6, 12]\).

The influences of neutrophils on prognosis are various and there are some important research results from several studies in children. A Japanese study showed that at 3 months after the initiation of therapy, 58% patients with SAA and 39% patients with vSAA responded to the initial course of IST. After 6 months, 66% patients with SAA and 72% patients with vSAA had evidence of a trilineage response and became transfusion-independent \([13]\). A German pediatric study even showed a significantly better CR and survival rate in response to IST in patients with vSAA compared to those with SAA \((\text{CR} 69\% \text{ vs } 44\%, \text{respectively, } p = 0.004)\) \([14]\).

The NIH group investigated the clinical outcomes of 420 patients with SAA over two decades, they showed that the overall 5-year patient survival level improved in conjunction with a decrease in infection-related mortality and frequency of invasive fungal infections (IFIs), as well as in the progression support treatment. When considering the reason for which the comparison of vSAA and SAA groups did not show significant differences in many studies, we postulate that more severe SAA could lead to fatal infection before any response to IST. However, importantly, this research did still show that absolute neutrophil count before IST was associated with prognosis \([15]\).

ANC fell to zero in some of the patients with AA in a Japanese study of SAA in children. The study proposed the concept of FAA and revealed lower RR when

**Table 3. Influence factors of IST efficacy for AA patients.**

| Category                        | Univariate | Multivariate |
|---------------------------------|------------|--------------|
| Age, years(>35; ≤35)            | 0.599      | 0.430        |
| Gender(Male; Female)            | 0.195      | 0.082        |
| Days from diagnosis to treatment| 0.195      | 0.065        |
| Neutrophil count×10^9/L         | 0.029      | 0.019        |
| Lymphocyte count×10^9/L         | 0.723      | 0.693        |
| Reticulocyte count×10^9/L       | 0.285      | 0.234        |
| Platelet count×10^9/L           | 0.963      | 0.914        |
| PNH clone                       | 0.256      | 0.819        |

PNH: paroxysmal nocturnal hemoglobinuria

**Table 4. Risk factors of death for AA patients after IST.**

| Category                        | Univariate | Multivariate |
|---------------------------------|------------|--------------|
| Age, years(>35; ≤35)            | 0.359      | 0.498        |
| Gender(Male; Female)            | 0.599      | 0.550        |
| Days from diagnosis to treatment| 0.599      | 0.881        |
| Neutrophil count×10^9/L         | 0.004      | 0.026        |
| Lymphocyte count×10^9/L         | 0.487      | 0.223        |
| Reticulocyte count×10^9/L       | 0.985      | 0.063        |
| Platelet count×10^9/L           | 0.421      | 0.149        |
| PNH clone                       | 0.345      | 0.799        |
| Efficacy                        | 0.001      | 0.010        |

PNH: paroxysmal nocturnal hemoglobinuria

**Figure 2.** The overall survival (OS) of patients in the ‘zero’ group was significantly lower than those in vSAA and SAA groups \((p = 0.001)\) (Kaplan-Meyer method).
comparing patients with vSAA and SAA [11], however, there was no difference in survival rate. Adult SAA patients often have different prognoses to children, since the efficacy of IST is age-related [1]. Therefore, it is necessary to analyze the results of IST in adult patients with SAA and an ANC of zero specifically.

In our study, patients with an ANC of zero took longer to achieve remission and had a lower response and survival rate compared to the patients with non-zero ANC. The presence of PNH clones was associated with a good response to IST and better prognosis [18–21]. However, there are also studies in children showing the response rate to IST was higher in patients without PNH clones [22]. These discrepancies were probably caused by the biological differences between adult and paediatric AA. Our analysis also did not find any correlation between PNH clones and the results of IST, as well as risk factors affecting survival. Other possible factors including gender, time from diagnosis to treatment, lymphocyte count, reticulocyte count, platelet count [3] were not shown to be prognosis predictors in our study.

In summary, this study revealed that adult patients with ANC values of zero poorly responded to IST, suggesting that very low neutrophils count, especially 0, was a highly predictive factor for efficacy and survival of SAA patients treated with IST.

Studies on patients with AA and very low neutrophils counts, especially those with zero neutrophils are still rare. Other treatments such as allogeneic hematopoietic stem cell transplantation, or IST combined with eltrombopag require improvements in efficacy, and could lead to better long-term survival for patients with an ANC of zero. Longer follow-up is also needed to determine the effect of eltrombopag, if any, on efficacy and OS in patients with a zero ANC.

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