Original Article

Combined treatment of sequential intensive immunosuppressive therapy and hematopoietic growth factors in patients with severe aplastic anemia: long-term outcome of hematopoietic recovery response

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Received August 17, 2018; Accepted September 10, 2018; Epub November 15, 2018; Published November 30, 2018

Abstract: This study aimed to assess hematopoietic recovery after combined treatment of sequential intensive immunosuppressive therapy (SIIST) and hematopoietic growth factors (HGF) in severe aplastic anemia (SAA) patients. Long-term follow-up was assessed in 79 SAA patients after treatment to assess healing and hematopoietic reconstruction. Meanwhile, the SAA and very severe aplastic anemia (VSAA) groups were compared for recovery status. The results showed that 29 (36.7%) and 17 (21.5%) cases showed complete and partial response, respectively. Among the patients with complete remission, >90% in SAA group recovered within 6 months after treatment and >80% in VSAA group recovered within 9 months after treatment. White blood cells returned to normal levels in 64 cases (81%): 35 cases from SAA group (100%) and 29 cases from VSAA group (65.9%). Red blood cells returned to normal levels in 33 cases (41.8%), including 23 (65.7%) cases in SAA group and 10 (22.7%) cases in VSAA group. The hemogram completely returned to normal level in 29 (36.7%) cases: 21 (61.0%) cases in SAA group and 8 (18.2%) cases in VSAA group. The SAA group showed overtly higher remission degree than VSAA group (P<0.05). For more than 80% cases, erythron returned to normal levels and began to form megakaryocytes within 6 to 9 months after treatment in SAA and VSAA groups, respectively. In SAA patients treated with SIIST, the fastest recovery was neutrophils (3 months), followed by erythroid cells, and megakaryocytes (6 to 9 months). Therefore, 6 and 9 months after treatment should be considered as observation points to assess the efficacy of first SIIS therapy in SAA and VSAA groups, respectively.

Keywords: Hematopoietic recovery, sequential intensive immunosuppressive therapy (SIIST), hematopoietic growth factors (HGF), severe aplastic anemia (SAA)

Introduction

Severe aplastic anemia (SAA) is a severe bone marrow hematopoietic failure associated with T-lymphocytes dysfunction [1, 2]. With the wide use of intensive immunosuppressive therapy (SIIST) drugs such as antilymphocyte globulin (ALG)/antithymocyte globulin (ATG) and cyclosporine A (CyA) in SAA, the early death rate of SAA has fallen to around 9.5%, with an efficiency of 70-80%. However, there are still 20~30% patients unresponsive to one course of SIIST [3-5]. Observation from several research centers have demonstrated that repeated SIIST is safe and effective. If a patient does not achieve remission after the initial SIIST, the remission rate for the second SIIST would reach at 40-77% [6-9], without significant difference in survival rate compared to the initial SIIST [7]. However, the initial SIIST outcome exhibited high heterogeneity in different patients with SAA; Therefore, there is no common rule for the judgment of the effectiveness of first therapy and whether a second therapy should be applied. However, existing studies have shown that the second SIIST was generally conducted 2 to 13 months after the first SIIST [7, 8]. In this work, retrospective analyses were conducted in 79 SAA patients after initial SIIST to assess hematopoietic recovery. The results provided a basic
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**Table 1.** Clinical characteristics of patients treated with SIIST

<table>
<thead>
<tr>
<th>NO. (%)</th>
<th>Median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All=79</td>
<td>SAA=35 (44.3)</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>45/34</td>
</tr>
<tr>
<td>Age (years)</td>
<td>22 (6-78)</td>
</tr>
<tr>
<td>ANC (×10⁹/l)</td>
<td>0.17 (0-0.48)</td>
</tr>
<tr>
<td>Ret (×10⁹/l)</td>
<td>6.48 (0.19-14.63)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Bone marrow aspiration</th>
<th>Neutrophils (iliac) (%)</th>
<th>Erythron (iliac) (%)</th>
<th>Infection (yes/no)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15.70 (0-50.5)</td>
<td>6.0 (0-30.5)</td>
<td>55/24</td>
</tr>
<tr>
<td></td>
<td>15.68 (3.5-26)</td>
<td>7.91 (0-30.5)</td>
<td>15/20</td>
</tr>
<tr>
<td></td>
<td>15.13 (0-50.5)</td>
<td>4.8 (0-24.5)</td>
<td>40/4</td>
</tr>
</tbody>
</table>

**Table 2.** Curative effect of patients treated with SIIST

<table>
<thead>
<tr>
<th>NO. (%)</th>
<th>All=79</th>
<th>SAA=35</th>
<th>VSAA=44</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR (n, %)</td>
<td>29, 36.7%</td>
<td>8, 18.2%</td>
<td>21, 61.0%</td>
<td>0.023</td>
</tr>
<tr>
<td>PR (n, %)</td>
<td>17, 21.5%</td>
<td>7, 15.9%</td>
<td>10, 61.0%</td>
<td>0.770</td>
</tr>
<tr>
<td>MR (n, %)</td>
<td>17, 21.5%</td>
<td>13, 28.3%</td>
<td>4, 11.4%</td>
<td>0.003</td>
</tr>
<tr>
<td>F (n, %)</td>
<td>16, 20.3%</td>
<td>16, 36.4%</td>
<td>0</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

CR: complete response; PR: partial response; MR: minimal response; F: failure. P was assessed between VSAA and SAA groups.

**Materials and methods**

**Patients**

The study population consisted of 79 patients (45 male, 34 female) with newly diagnosed (within 30 days) acquired SAA without concomitant or preceding neoplasia between July 2005 and December 2012. The characteristic details were listed in Table 1. All patients had anemia requiring red blood cell support, thrombocytopenia (platelet count less than 20×10⁹/L), neutropenia (PMN count ≤0.5×10⁹/L), reticulocyte count 20×10⁹/L and hypoaplastic marrow with less than 25% cellularity, or 25-50% with <30% residual haemopoietic cells as indicated by marrow histology. Very severe AA (VSAA) was defined as severe but with a neutrophil count ≤0.2×10⁹/L [10, 11]. Forty-four patients (55.7%) met the criteria for VSAA. Median age was 22 years (range 6-78 years). Cytogenetic analyses at diagnosis were available for all patients: all analyses were normal. Paroxysmal nocturnal haemoglobinuria (PNH) clones were always absent at diagnosis. Patients with an HLA iden-

**Therapeutic measures**

**SIIST therapy**

Rabbit ATG (5 mg/kg, Fresenius, Germany), or rabbit ATG (5 mg/kg, Polyclonals, France), or swine ALG (30 mg/kg, Wuhan Institute of biological products, China), was intravenously infused, from day 1 to day 5 according to the manufacture’s instruction; prednisone (1 mg/kg) was intravenously administered from day 1 to day 15 and the dosage was gradually tapered within 30 days; CsA (3-5 mg/kg, Novartis, Switzerland; Sino-American East China pharmaceutical company, China) was given orally from day 1 to 180-270 and the dosage was slowly tapered thereafter. The dosage was adjusted according to hepatic and renal function of the patients. The above therapies were applied to all the patients.

**Hematopoietic growth factors therapy**

Granulocyte colony stimulating factor (G-CSF, Hangzhou Jiuyuan, China; North China pharmaceutical company, China) and recombinant human erythropoietin (rhEpo, Kirin, Japan) were administered from day 1 to day 90. Recombinant human thrombopoietin (rhTpo, Shenyang Sansheng pharmaceutical company, China) was also applied in some patients according to their conditions. The G-CSF was applied with the dose of 5 μg/kg continuously. When the treatment response appeared, the frequency could be gradually reduced from 3 to 1 time per week to maintain white blood counts (WBC) between 4~10×10⁹/L. RhEpo was subcutaneously injected at 6000 U/d with the gradually reduced frequency from 3 to 1 time per week (3 times for the first month, 2 times for the second month, and 1 time for the third months, respectively). RhTpo was also subcutaneously injected at 15000 U/d and the frequency of RhTpo was same to that of RhEpo. The dose was properly adjusted according to the patient’s condition.
Basic treatment

Irradiated platelet concentrates were transfused to maintain the platelet count above 20×10^9/L when receiving ATG/ALG treatment, and to maintain the platelet count above 10×10^9/L at other time. Packed red blood cells transfusions were administered to maintain the hemoglobin above 7 g/dl. Patients were given testosterone undecanoate orally (80~120 mg/d) and the dosage was adjusted according to the liver function of the patients. They were also administered folate and VitB12 orally with the dosage of 30 mg/d and 500 µg/d, respectively.

Maintenance therapy

Among the responsive patients, if hemoglobin levels were below 120 g/L (for males) and 110 g/L (for females), the patients were required to receive original treatment (excluding ATG/ALG and HGF therapy) for more than two years. During the maintenance treatment period, the drug dosage was reduced every 3 to 6 months, and regular follow-up was conducted every 3 to 6 months. Treatment was not completed until WBC reached to normal levels and bone marrow hematopoietic progenitor cells recovered. CyA and testosterone undecanoate could not be withdrawn until the WBC recovered.

Evaluation criteria of therapeutic effect

The following evaluation criteria of therapeutic effect [12] were described as follows: complete response (CR), defined as trilineage haematological reconstitution (ANC ≥2.0×10^9/L, haemoglobin ≥11 g/dL and platelets ≥100×10^9/L); partial response (PR), defined as transfusion independence, WBC was a greatly improved (neutrophil count ≥0.5×10^9/L, platelet count ≥20×10^9/L and hemoglobin level ≥7.0 g/dl); minimal response (MR), defined as reduced frequency of blood cell transfusion, neutrophils counts and bone marrow parameters were slight improved; failure (F), blood transfusion was not reduced, and blood test was not improved.

The patients were followed until death day or September 30, 2013, and the median follow-up for surviving patients was 69.5 months (range 15~108). Patients were hospitalized until they were no longer at risk of serum sickness and were clinically stable. They returned to hospital for interval evaluations in 3 months, 6 months, 9 months, 1 year, and then yearly or as clinically indicated.

Statistical analysis

Data were expressed as the mean or median (min-max) and performed by SPSS Statistics V21.0 (USA). For parametric analyses, t-test was used for group comparison in normally distributed data and Rank sum test was adopted to analyze non-normally distributed data. Fisher’s exact test and chi-square test was used for differences between non-parametric analyses. P<0.05 was considered statistically significant.

Results

Curative effect

As was shown in Table 2, among the 79 patients, 29 patients (36.7%) had a complete response; 17 patients (21.5%) achieved partial response; 17 patients (21.5%) achieved minimal response; 16 patients (20.3%) failed (10 of them died, 5 of them quitted treatment and one patient underwent allogeneic bone marrow transplant (BMT) from HLA-matched donor on 21 months after treatment).

Among the 44 VSAA cases, 8 patients (18.2%) had complete response; 7 patients (15.9%) achieved partial response; 13 patients (28.3%)
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and 16 patients (36.4%) exhibited minimal response and failure, respectively. There were 8 deaths in 44 patients with VSAA (mostly due to infection).

Among the 35 cases of SAA group, 21 patients (61.0%) had complete response; 10 patients (28.6%) achieved partial response; 4 cases were minimal response, and no one was failed. These data indicated that the effective rate of VSAA patients was lower than that of SAA patients (88.6% vs 63.6 %, P=0.02).

Changes in blood product transfusion usage

Among the 46 patients who became transfusion-independent, the absolute reticulocyte count (Ret) and ANC in peripheral blood samples of SAA and VSAA groups within 1 year after SIIST treatment were shown in Figure 2.

The WBC of 64 cases (81%) returned to normal range, among which, 35 patients were SAA (100%) and 29 were VSAA (65.9%) (P=0.001). And the RBC of 33 cases (41.8%) returned to normal, among which 23 cases (65.7%) were SAA and 10 cases (22.7%) were VSAA (P=0.004). There were 29 cases whose hemogram parameters completely returned to normal level (36.7%), 21 cases (61.0%) from the SAA group and 8 cases (18.2%) from VSAA group (P=0.003). In all, the efficiency rates in the SAA group were higher than that of VSAA group at the third month after SIIST treatment.

Changes in bone marrow

Among the 46 patients who became transfusion-independent, the absolute reticulocyte count (Ret) and ANC in peripheral blood samples of SAA and VSAA groups within 1 year after SIIST treatment were shown in Figure 2.

Figure 2. Comparison of blood cell count values in the SAA and VSAA groups. A: The ANC levels of the patients at 0, 3, 6, 9, 12, and 15 months after treatment. B: The Ret levels of the patients at different times after therapy. Ret: reticulocyte count; ANC: absolute neutrophil count.

Figure 3. Comparison of erythron and neutrophils in bone marrow between SAA and VSAA groups. The ratios of erythron and neutrophils in bone marrow were detected at 0, 3, 6, 9, 12, and 15 months after treatment. A: Erythron ratios in bone marrow; B: Neutrophils ratios in bone marrow.

Among the 46 patients with complete or partial response after SIIST therapy, 15 cases were VSAA patients, and 31 cases were SAA patients. The ratio of patients who were free from blood product transfusion in the SAA and VSAA groups at different times after therapy was shown in Figure 1.

Six months after the treatment, 80.6% SAA patients became erythrocyte transfusion independent and 84.6% became platelet transfusion independent. Nine months after the treatment, 27.3% VSAA patients became transfusion independent. Among the patients who became transfusion independent in SAA group (31 cases) and VSAA group (15 cases), more than 90% of the patients in SAA group achieved remission within 6 months after treatment; and more than 80% of the patients in VSAA group achieved remission within 9 months after treatment.

Changes in peripheral blood

Among the 46 patients who became transfusion-independent, the absolute reticulocyte count (Ret) and ANC in peripheral blood samples of SAA and VSAA groups within 1 year after SIIST treatment were shown in Figure 2.

The WBC of 64 cases (81%) returned to normal range, among which, 35 patients were SAA (100%) and 29 were VSAA (65.9%) (P=0.001). And the RBC of 33 cases (41.8%) returned to normal, among which 23 cases (65.7%) were SAA and 10 cases (22.7%) were VSAA (P=0.004). There were 29 cases whose hemogram parameters completely returned to normal level (36.7%), 21 cases (61.0%) from the SAA group and 8 cases (18.2%) from VSAA group (P=0.003). In all, the efficiency rates in the SAA group were higher than that of VSAA group at the third month after SIIST treatment.

Changes in bone marrow

Among the 46 patients who were completely free from blood transfusion, ratios of neutrophils and erythropoietic in bone marrow were measured, and results were showed in Figure 3. Before treatment, the erythron ratio in bone marrow was higher in the SAA group (15.5±11.8) compared with the VSAA group (5.5±4.5) (P=0.030), while no significant difference was observed between two groups at other stages.
Table 3. Comparison of bone marrow recovery between the SAA and VSAA groups

<table>
<thead>
<tr>
<th>Time (month)</th>
<th>SAA (%)</th>
<th>VSAA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Neutrophils</td>
<td>Erythron</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>39</td>
</tr>
<tr>
<td>6</td>
<td>100</td>
<td>71</td>
</tr>
<tr>
<td>9</td>
<td>100</td>
<td>90</td>
</tr>
<tr>
<td>12</td>
<td>100</td>
<td>94</td>
</tr>
<tr>
<td>15</td>
<td>☆</td>
<td>★</td>
</tr>
</tbody>
</table>


As was shown in Table 3, neutrophils in both SAA and VSAA groups were recovered to a normal level within 6 months after treatment. For more than 80% patients both in the SAA and VSAA group, erythron levels were recovered to normal levels within 9 months after treatment. Besides, megakaryocytes of more than 80% patients both in the SAA and VSAA group began to start to appear within 9 months after treatment.

Discussion

It has been demonstrated that the efficacy of SIIST combined with hematopoietic for the treatment of SAA was about 70~80% [3, 4, 13]. This study provides data about the long-term outcomes after treatment of SAA with immunosuppression in a consistently treated population. The results showed that blood and bone marrow cells in 29 patients (36.7%) were completely restored to normal levels, indicating that complete response was achieved. A total of 17 patients (21.5%) became completely transfusion independent, indicating that partial response was obtained. Other 17 patients (21.5%) achieved minimal response, while 16 patients (20.3%) were failed.

Transfusion independence is a common standard in defining partial response. European group for blood and marrow transplantation (EBMT) in 1995 has stated that the overall survival rate of the patients who suffered from SAA for 18 months was 92%, the remission rate was 82% and the complete remission rate was 42% [3]. In our study, among the 79 patients, WBC returned to normal levels in 64 cases (81%), with 35 cases (100%) from the SAA group and 29 cases (65.9%) from the VSAA group. RBC returned to normal levels in 33 cases (41.8%), with 23 cases (65.5%) in the SAA group and 10 cases (22.7%) in VSAA group. Hemogram data completely returned to normal levels in 29 cases (36.7%), with 21 cases (61.0%) in the SAA group and 8 cases (18.2%) in the VSAA group, indicating a higher remission level in the SAA group compared with the VSAA group.

Compared to diseases with rapid response to immunosuppressive therapy, such as immune thrombocytopenic purpura (ITP) and autoimmune hemolytic anemia (AIHA), it took at least 4 weeks for most patients with SAA to improve peripheral blood cell number and reduce blood transfusion requirements [14]. In early studies, 3 months was considered as the observation point to determine remission in patients [15]. However, Speck et al. [16] demonstrated that among the patients who were recovered after ALG treatment, 50% of the patients achieved remission at 3 months after treatment and 90% at 6 months. According to a report from EBMT in 1995, the median time for transfusion independent is 115 days, and this ratio becomes 97% at 18 months after treatment [3]. As was reported in EBMT in 2000, the median time for transfusion independent was 96 days, with a total ratio for this parameter reaching 97% at 48 months after treatment [17]. Due to the fact that about 50% of patients achieved remission after 3 months of treatment, the time point has been considered as a demarcation point to determine the treatment effect, which would obviously underestimate the remission rate. However, the US study reported the remission rates were 60, 61 and 58% at 3, 6 and 12 months respectively among 122 patients, and suggested that 90% of patients who got remission within 6 and 9 months actually might have recovered within 3 months [18].
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To provide a basic understanding of the observation time at which the validity of the initial SIIST should be determined, retrospective analyses were conducted in 79 SAA patients in our study. Among the 46 SAA patients who were transfusion independent after SIIS therapy, 90% of the patients in the SAA group recovered at 6 months after treatment, and 80% of VSAA individuals recovered at 9 months after treatment. Neutrophils in both SAA and VSAA groups recovered to normal levels within 6 months after treatment. Erythron from more than 80% patients both in the SAA or VSAA group reached normal levels and megakaryocytes turned to normal form within 9 months. Therefore, it is appropriate to consider 6 and 9 months after treatment as the observation points for determining the validity of the first SIIS therapy for the SAA and VSAA groups, respectively.

Disclosure of conflict of interest

None.

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References

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