Aplastic anaemia: a review

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ABSTRACT

Aplastic anaemia is featured by bone marrow hypocellularity and peripheral pancytopenia and is a potentially fatal disease. In recent years, insight in its pathogenesis has increased. It appears that activated autoreactive T lymphocytes induce apoptosis of haematopoietic stem cells resulting in a hypocellular bone marrow. Nowadays, it can be treated by stem cell transplantation or immunosuppressive therapy. This review focuses on the pathophysiology and treatment of aplastic anaemia.

INTRODUCTION

Aplastic anaemia is a serious medical disorder which, when untreated, has a median survival of less than ten months due to infections and haemorrhage. Fortunately, in the last decades its prognosis has improved dramatically and most patients now achieve durable responses. Aplastic anaemia is featured by hypoplasia of the bone marrow and peripheral pancytopenia (figure 1). The most commonly used criteria for the diagnosis aplastic anaemia are marrow cellularity of less than 25% of normal or less than 50% with haematopoietic cells representing less than 30% of the residual cells and at least two of the following peripheral blood counts: neutrophil count of less than 0.5 x 10⁹/l, platelet count of less than 20 x 10⁹/l, and/or anaemia with a reticulocyte count of less than 1%. Aplastic anaemia can be due to congenital (20%) or acquired causes (80%) (table 1). Congenital diseases leading to aplastic anaemia will not be further discussed here.

Figure 1
Bone marrow biopsy from a patient with aplastic anaemia (A) compared with a normal bone marrow (B)
Causes of acquired aplastic anaemia are very diverse, the most common being iatrogenic causes such as radiation and drugs directly cytotoxic to the bone marrow. Some haematological malignancies or infections can present as aplastic anaemia. In some cases, myelodysplastic syndrome (MDS) can occur with a hypocellular bone marrow and peripheral pancytopenia. In these cases, it is sometimes difficult to distinguish between MDS and aplastic anaemia. Distinction by cytogenetic analysis is not always possible because in about 4% of the patients with aplastic anaemia cytogenetic abnormalities are found, sometimes similar to those seen in patients suffering from MDS. Furthermore, not all patients with evident morphological MDS have cytogenetic alterations. Some institutions consider patients with findings fulfilling the diagnosis of aplastic anaemia and with cytogenetic abnormalities as MDS, independent of the marrow morphology.

The management of acquired aplastic anaemia due to such causes as aforementioned involves treatment of the underlying cause. This review, however, will focus on the remaining causes of acquired aplastic anaemia in which haematopoietic stem cell damage due to autoreactive T lymphocytes is the central event but in which the exact cause that elicits this reaction is largely unknown.

**Epidemiology and Aetiology**

Aplastic anaemia is a rare disease with a reported incidence of approximately 2 per 1 x 10^6 annually in Europe. In Asia, it occurs three times more often with an incidence of 5 to 7 per 1 x 10^6 annually. As will be described below, autoreactive T lymphocytes are thought to play an important role in the pathogenesis of aplastic anaemia. As in other autoimmune diseases, an association with certain HLA alleles has been described in patients with aplastic anaemia. These patients, an increased HLA-DR2 frequency of 58% is found, versus 28% in the normal population.

Because prognosis of aplastic anaemia is related to the number of neutrophils, aplastic anaemia is divided into nonsevere aplastic anaemia with neutrophils above 0.5 x 10^9/l, severe aplastic anaemia with neutrophils between 0.2 and 0.5 x 10^9/l, and very severe aplastic anaemia with neutrophils lower than 0.2 x 10^9/l.

The aetiology of acquired aplastic anaemia is very diverse. Aplastic anaemia usually occurs without a suggestive prior history and is labelled idiopathic. However, in some cases a clear inciting event can be identified. Many drugs and chemicals have been described as causing aplastic anaemia by a rare idiosyncratic reaction. The most well known are drugs such as chloramphenicol, but widely used medications as furosemide and allopurinol have also been associated with aplastic anaemia.

Approximately 5 to 10% of aplastic anaemia occurs after an episode of hepatitis in which no known viral pathogen or a relation with drugs can be identified. Patients who receive a liver transplantation for hepatic failure caused by such a seronegative fulminant hepatitis are at a high risk of developing aplastic anaemia. It occurs in about 25% of these patients. It is plausible that a not yet identified infectious agent is involved. Also parvovirus B19 is associated with aplastic anaemia. Relationships with pregnancy and autoimmune disorders such as eosinophilic fasciitis have also been reported. However, whatever the underlying cause, it does not seem to affect the response to treatment.

**Pathogenesis**

There are several indications that aplastic anaemia is due to a stem cell dysfunction. When stem cells of patients are seeded onto irradiated normal stromal cells in vitro, a reduced number of colony-forming units (CFU) is seen. In contrast, normal CFU forming is observed after placement of normal CD34-positive cells into stromal cells of patients. Therefore stromal dysfunction appears unlikely.
Furthermore, CD34-positive cells are decreased in blood and bone marrow,\(^{13}\) while in the remaining stem cells an increased percentage of apoptotic cells is seen.\(^{46}\) Evidence is mounting that a T-cell-mediated reaction may be responsible for the stem cell destruction. In bone marrow of patients an increased number of activated CD8 lymphocytes are seen.\(^{27}\) Moreover, T cells of patients are capable of reducing CFU forming in vitro, while depletion of these T cells restores CFU forming.\(^{18}\) Furthermore, T cells of patients produce cytokines such as interferon-\(\gamma\) and tumour necrosis factor-\(\alpha\) and increased expression of these cytokines has been shown in the bone marrow of patients.\(^{19}\) These cytokines can suppress stem cell proliferation and induce apoptosis of stem cells.\(^{20}\) The cytokine-induced apoptosis can occur directly and indirectly by the Fas-Fas-ligand system. Interferon-\(\gamma\) and tumour necrosis factor-\(\alpha\) are capable of inducing Fas expression on stem cells, and increased Fas expression is found in bone marrow of patients.\(^{21}\) Fas ligand, which can be found on activated T lymphocytes, can subsequently induce apoptosis of these Fas-expressing stem cells. The important role of these cytokines is underscored by the observation that in a murine model of aplastic anaemia a germ-line mutation in the gene encoding for the RNA component of telomerase results in decreased telomerase activity and subsequently shorter telomers, which may ultimately lead to reduced survival of stem cells and to aplastic anaemia.

**TREATMENT**

Allogeneic stem cell transplantation (allo-SCT) using bone marrow as treatment for aplastic anaemia was first applied in the early seventies. Nowadays, peripheral stem cells are being increasingly used. When successful, a fast haematological response is seen after allo-SCT with neutrophils above 0.5 x 10\(^9\)/l and platelets above 20 x 10\(^9\)/l after a median of about 15 and 20 days, respectively. However, allo-SCT can be accompanied with severe toxicity. The main mortality and morbidity is due to graft failure, drug-related toxicity, infections and the occurrence of acute and/or chronic graft-versus-host disease (GVHD). Recently the European Group for Blood and Marrow Transplantation (EBMT) retrospectively analysed the data of 2002 patients with aplastic anaemia who had received an allo-SCT between 1976 and 1998.\(^{35}\) Most patients (85\%) received a transplant from an HLA-identical sibling, 15\% from a matched unrelated donor, and 2\% from an identical twin. Graft type clearly affected survival in these three groups with 66, 37, and 91\% respectively. Data on responses were not given.

Mortality was strongly associated with age. In patients treated with allo-SCT from an HLA-identical sibling between 1990 to 1998, survival rates for patients aged <16, between 16-40, and >40 years were 77, 68, and 54\%, respectively. Remarkable was the improvement in time with an increase in survival from 57\% up to 1990 to 76\% after 1990. This improvement is mainly caused by a decline in the occurrence of acute GVHD due to more effective prophylaxis, better matching, and improved supportive care.

In patients who are not candidates for SCT, immunosuppressive therapy (IST) is employed. Mathe et al. reported autologous bone marrow recovery in some patients who rejected their grafts. These authors speculated that the antithymocyte globulin (ATG) used in the conditioning regimen had induced an anti-immune process.\(^{46}\) Since then, several randomised studies have been carried out. Drugs studied in a randomised setting are ATG, cyclosporin A (CsA), androgens, corticosteroids, growth factors and cyclophosphamide (table 2). However, these trials are very difficult to compare due to differences in inclusion criteria, definitions for response, time of follow-up, and treatment regimens. Until now, the best results have been reported with the combination of ATG and CsA. Both horse and rabbit ATG are frequently used and both are licensed for treatment in the United States. Data comparing both types ATG and several doses are not available. In our centre, the combination treatment consists of horse ATG 15 mg/kg administrated daily for five days and CsA for at least three months. Initially, CsA is administered orally twice daily at 3 mg/kg. Dosing of CsA is adjusted to...
maintain trough levels of 100 to 300 ng/ml from day 1 to 28; from day 28 target trough levels are 50 to 150 ng/ml. CsA can be tapered off when there is a good response, and if necessary, restarted in case of relapse. During administration of ATG, corticosteroids are given for attenuating ATG-induced side effects. The corticosteroids themselves probably have a minor effect in aplastic anaemia.34 Good responses by this combination treatment, defined as neutrophils above 1.0 x 10^9/l and transfusion independence, are obtained in 60 to 80% of the patients. These responses, however, are rather slow and usually take three to six months to occur. Furthermore the response is often not complete (normalisation of blood counts) and about 30 to 40% show a relapse after a few years. Over time, five-year survival has improved because of better supportive care. In 1981, the five-year survival was 58% compared with 75% in 1991.39

Concern for development of serum sickness by repeated courses of ATG was examined by Tichelli et al.40 These authors showed that a repeated administration of horse ATG is safe and well tolerated. Serum sickness occurred earlier but not more often.40 When disease is refractory to repeated courses of IST, an allo-SCT can be considered when the patient was not a candidate for primary treatment with SCT.

L O N G - T E R M  E F F E C T S

Because both allo-SCT and IST have rather good effects in the short term, long-term effects are important. About 10% of the patients treated with IST for aplastic anaemia develop paroxysmal nocturnal haemoglobinuria (PNH) with haemolytic anaemia.41 This does not occur in patients treated with allo-SCT. PNH is due to a mutation in a gene encoding for a protein which is involved in the synthesis of a glycosylphosphatidylinositol anchor (GPI).
responsible for the attachment of several proteins to the cell membrane. PNH usually presents with haemolytic anaemia but in 20 to 50% of the cases of aplastic anaemia a decreased expression of GPI-anchored proteins is found suggesting a strong link between these two conditions.41 PNH as a long-term side effect is only found in patients who initially present with aplastic anaemia combined with a decreased GPI-anchored protein expression.41 An explanation for this increased incidence of PNH after IST could be that stem cell clones with decreased expression of GPI-anchored proteins have a growth advantage compared with normal stem cells in aplastic anaemia. Stem cells with GPI deficiency, which can also be found in normal people,43 appear to be less prone to apoptosis than normal cells.44 In a disorder like aplastic anaemia, in which apoptosis-induced stem cell damage is important, this phenomenon may subsequently lead to a growth advantage for these GPI-deficient cells and ultimately to PNH.

Socie et al.45 compared the incidence of malignant tumours after treatment for aplastic anaemia. It was found that the number of cancers was clearly increased compared with the general population, especially the occurrence of acute leukaemia (115 times in patients treated with IST, 29 times in patients who received a transplantation). The incidence of the development of solid tumours was only significantly increased in patients who were treated with allo-SCT (5.7 times). Comparing both treatments, especially the occurrence of acute leukaemia and MDS was clearly increased in patients who were treated with IST compared with those who received allo-SCT. Altogether, this results in a cumulative ten-year incidence of all cancers of 18.8% in patients treated with IST and 3.1% in those who received an allo-SCT.41 The strongly increased incidence of cancer in patients treated with IST is reflected in the survival curve which is still declining after a follow-up of five years, while the curve of patients treated with allo-SCT reaches a plateau after this time period.40 The reason for the strongly increased incidence of cancer is not exactly known. The incidence of solid cancer after transplantation is associated to the use of radiation in the conditioning regimen.45 The strong increase of MDS in IST can be due to the fact that the initial diagnosis was not aplastic anaemia but hypocellular MDS. Another explanation could be that the remaining stem cells, not affected by the immune system, have an increased cell cycle to compensate for the damaged stem cells. The observed shortening of telomeres in peripheral leucocytes of patients is in agreement with this hypothesis.47 The increased cell cycle results in an augmented number of mutations which may lead to either MDS or acute leukaemia. Other possible mechanisms described as being related to the increased AML/MDS incidence are multiple courses of ATG45 and the use of G-CSF.48

BEST PRIMARY TREATMENT

An important remaining question is what the first-line therapy of a patient presenting with aplastic anaemia should be. Several studies comparing the efficacy of allo-SCT and IST have been performed (table 3). Again, these studies are difficult to compare. Most studies did not find a difference between these treatments, except for one study performed in children which showed an advantage for allo-SCT.52 However, the number of patients studied in these trials was rather small.

Therefore, the European Blood and Marrow Transplantation Group (EBMT) retrospectively analysed data on 1765 patients with acquired aplastic anaemia.40 Comparing both treatments, it appears that especially in younger patients up to 40 years with a strongly decreased number of neutrophils (<0.3 x 10⁹/l), allo-SCT is the best treatment, while in patients older than 40 years IST gives the best results.40 The advantage of transplantation compared with IST in younger, severely affected patients is probably due to a shorter time of improvement of blood counts which is especially favourable in those with a severe low number of neutrophils. The advantage for IST in older patients is probably caused by the increased mortality of allo-SCT in this group. However, the difference in advantage for IST in older patients decreases in time, probably

Table 3

<table>
<thead>
<tr>
<th>Author</th>
<th>Age (Years)</th>
<th>IST N</th>
<th>Survival (%)</th>
<th>Allo-SCT N</th>
<th>Survival (%)</th>
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</thead>
<tbody>
<tr>
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<td>69</td>
<td>18</td>
<td>44</td>
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<tr>
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<td>133</td>
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<tr>
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<td>49</td>
<td>55</td>
<td>72</td>
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</tbody>
</table>

IST = immunosuppressive therapy, allo-SCT = allogeneic stem cell transplantation. * Significant difference (p<0.05).
due to relapses and the increased incidence of cancer. In patients aged up to 40 years and with a moderately affected neutrophil number there is no clear difference in five-year survival in favour of either of the treatment options. However, because of the increased long-term effects of IST compared with allo-SCT, the latter is to be preferred.

So, in our institute, this has accumulated in the protocol as depicted in table 4.

Table 4
First, second, and third-line therapy for aplastic anaemia in the Erasmus Medical Centre

<table>
<thead>
<tr>
<th>NEUTROPHILS &lt;0.2 x 10^9/l</th>
<th>NEUTROPHILS &gt;0.2 x 10^9/l</th>
</tr>
</thead>
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<tr>
<td>Age &lt;45 years</td>
<td>Age &lt;20 years</td>
</tr>
<tr>
<td>1. sib-SCT</td>
<td>1. sib-SCT</td>
</tr>
<tr>
<td>2. IST</td>
<td>2. IST</td>
</tr>
<tr>
<td>3. MUD-SCT</td>
<td>3. MUD-SCT</td>
</tr>
<tr>
<td>Age ≥45 years</td>
<td>Age ≥20 years</td>
</tr>
<tr>
<td>1. IST</td>
<td>1. IST</td>
</tr>
<tr>
<td>2. sib-SCT (&lt;55 years)</td>
<td>2. sib-SCT (&lt;55 years)</td>
</tr>
<tr>
<td>3. MUD-SCT (&lt;50 years)</td>
<td>3. MUD-SCT (&lt;50 years)</td>
</tr>
</tbody>
</table>

sib-SCT = allogeneic stem cell transplantation from HLA-identical sibling, IST = immunosuppressive therapy, MUD-SCT = allogeneic stem cell transplantation from matched unrelated donor.

CONCLUSION

During the last decades, insight in the pathogenesis of aplastic anaemia has increased. In parallel with this, the prognosis of this potentially fatal disorder has improved radically. It can be expected that responses to IST will probably continue to rise partly due to new drugs tested for their efficacy in aplastic anaemia, such as mycophenolate mofetil, rapamycin, and monoclonals against the interleukin-2 receptor. Also results of allo-SCT will probably improve by better supportive care, more effective GVHD prophylaxis and less toxic conditioning regimens. This improvement of treatments urges that recommendations for treating aplastic anaemia will continue to be defined.

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REFERENCES


