Immunosuppressive treatment for aplastic anemia: are we hitting the ceiling?

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Aplastic anemia is a heterogeneous disease, a form of marrow failure that presents with pancytopenia of abrupt or insidious onset and an empty marrow. There are marrow and peripheral blood criteria (bone marrow cellularity <30%; reticulocytes <20×10^9/L, platelets <20×10^9/L, and neutrophils <0.5×10^9/L) to define the severity of the condition.[1] The disease is termed severe when two out of three peripheral blood criteria are met, moderate if less than two are met, and very severe if the neutrophil count is below 0.2×10^9/L. Severity has long been established as a prognostic criterion as it has been difficult to keep patients with severe neutropenia alive. The reticulocyte criterion will have to be re-evaluated as results of modern automated reticulocyte counters overestimate reticulocyte counts at low levels and do not, therefore, correlate well with manual counting results obtained by brilliant cresyl blue staining. Obviously, the diagnostic criteria established by Bruce Camitta were defined at a time when automated counters were not available. In a study published in this issue of the journal the reticulocyte criterion was set at 60×10^9/L using an automated counting method.

The diagnostic work-up includes testing for paroxysmal nocturnal hemoglobinuria by flow cytometry and cytogenetics and exclusion of hereditary marrow failure syndromes. This work-up is becoming increasingly complex as new hereditary forms are being identified and because the search for a hereditary form should no longer be limited to the pediatric age group.[2] The interpretation of clonal cytogenetic anomalies such as trisomy 8 and monosomy 7 is equally difficult as patients with a classical presentation of severe aplastic anemia and clonal hematopoietic anomalies should not be diagnosed as having a myelodysplastic syndrome based on the cytogenetic anomaly alone.

Aplastic anemia is rare. The incidence is 1-2 new cases per million per year. It occurs in all age groups but is found more commonly among the young. The incidence is higher in south-east Asia and in poor countries; this may be due to viral infections and exposure to toxins.

A series of studies in the past established that the combination of anti-thymocyte globulin (ATG) of horse origin, obtained by sensitizing horses with human lymphocytes or thymocytes, and cyclosporine A (CSA) is the standard treatment for aplastic anemia in patients not eligible for marrow transplantation.[3] Results of transplantation and immunosuppression are roughly equivalent with graft-versus-host disease and graft failure being problems associated with transplantation and treatment failure, relapse and secondary clonal disorders such as myelodysplastic syndromes, paroxysmal nocturnal hemoglobinuria and leukemia being associated with immunosuppressive treatment.[4-6]

Immunosuppression to treat severe aplastic anemia was initially pioneered by Georges Mathé and Bruno Speck among others. It is of interest that the first patients so treated received haploidentical marrow along with ATG and it took a while to discover that the response was due to ATG and not to the marrow infusion.[7]

Studies comparing ATG + CSA to CSA alone in patients with moderate aplastic anemia, and ATG + CSA to ATG alone in patients with severe aplastic anemia have established that the combination is the most effective treatment.[8-12] While ATG is administered as an intravenous infusion over 5 days in hospitalized patients, patients receive CSA orally for 6 months or more. There is controversy about the adequate duration of treatment, as differing recommendations are being made[13] based on little knowledge from clinical trials.

There are CSA-sensitive patients who require prolonged treatment and who relapse especially with thrombocytopenia whenever CSA is being tapered, whereas there are others who do not. These relapsing patients often respond when treatment is resumed. This touches on the controversy of over- versus under-treatment, which the authors of the study published in this issue of the journal try to address by following two different CSA tapering schedules.[14]

Patients with a full relapse after an initial response have a high rate of response to a second course of ATG[15-17] and some patients who have not responded to a first course may respond to a second course. Relapse is re-treatable and does not necessarily confer a bad prognosis. Patients not responding to two courses of ATG will not respond to a third course and this should, therefore, be avoided.[18] Whether such patients have other forms of marrow failure that are not responsive to immunosuppressants or whether the right immunosuppressant has not been found remains to be determined.

ATG + CSA has been the standard treatment over the last 20 years with continued improvement of results, as shown in Figure 1, probably more due to improvement in supportive care and increasing knowledge about best use of the established therapeutic tools than due to changes of the immunosuppressive treatment strategy. A sizeable number of patients are rescued by stem cell transplantation as second-line treatment. Several attempts at improving results of immunosuppressive treatment by adding growth factors, or other immunosuppressive drugs such a mycophenolate, have not produced higher response rates.[19-21]

ATG preparations are not all equal. In the USA the horse product is still available (ATGAM®) while in Europe horse ATG (Lymphoglobulin®) has been withdrawn in favor of a rabbit ATG (Thymoglobulin®) which is dosed differently and which has not been well documented as being efficacious as first-line therapy for severe aplastic anemia.[17] A third ATG, also of rabbit origin, is available: this form is produced not by sensitizing with human thymocytes but a T-acute lymphocytic leukemia cell line *i.e.* Jurkat cells. This obviously does not yield the same antibody specificities and in at least one clinical trial this form

of ATG has been shown to be inferior to horse ATG.[22] This goes to say that ATG is a combination of anti-human antibodies with a broad range of specificities and it is currently unknown which of these specificities are essential for efficacy in the treatment of severe aplastic anemia.

Scheinberg *et al.* are to be commended for having run a prospective clinical trial in the field of aplastic anemia.[14] This is an increasingly difficult field in which to conduct trials because of the rarity of the disease, because the outcome after standard treatment with ATG + CSA (60-70% response rate and 70-80% long-term survival probability) is demanding to top and, last but not least, because of the increasing burden of clinical trial regulations, which turn running academic trials in rare diseases into a nightmare. In fact, it is difficult to prove superiority of any new treatment over the old standard, once a high success rate has been achieved.

The authors chose response rate as the main outcome of their study which is wise, given the fact that even non-responders may enjoy prolonged survival because of improvement in supportive care such as transfusions and treatment of infectious complications. In spite of these improvements in supportive care, the primary treatment goal remains to achieve functioning hematopoiesis which ultimately protects patients from being at risk of dying of infectious complications or bleeding.

The pathopyhsiology of aplastic anemia has not been fully elucidated and the best evidence for an autoimmune pathogenesis is found in the response to immunosuppressive treatment.[23] As a substantial proportion of patients are non-responders the question remains as to whether these are patients with inadequately treated autoimmune marrow failure, who may be rescued by an improvement of immunosuppressive or immunomodulating treatment, or whether these patients may have a different pathophysiology and will, therefore, never respond to immunosuppressants, whatever the intensity or specificity. Such patients may include individuals with undiagnosed hereditary marrow failure syndromes, hypoplastic myelodysplastic syndrome and possibly also true quantitative stem cell failure.

The choice of Scheinberg *et al.*[14] to compare ATG + CSA with or without added sirolimus is highly rational, given the evidence of synergism between calcineurin and mTOR inhibitors in transplant medicine. The study was designed to detect an improvement in the 3-month response rate from 60% to 85% using the combination treatment.

The study was closed prematurely after some 2 years of accrual and after recruiting 64% of the targeted population of two groups of 60 patients each because of a low likelihood (<1%) of demonstrating a significant difference in the main outcome measure.

Some ink has been wasted in the past on the practice of closing trials prematurely because of lack of evidence on the ability to reach the targeted end-point. It is a completely different ballgame if a study needs to be closed for safety reasons. Obviously we are never sure whether a small or a late beneficial effect has been missed by closing a study early; however, the decision is much easier when the treatment under study is providing results that are rather worse than the standard treatment. Response

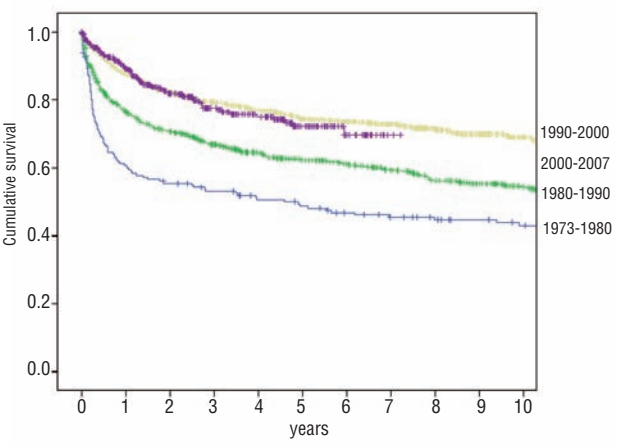

Figure 1. Survival up to 10 years for patients (n=2400) with severe aplastic anemia treated with ATG reported to the European Group for Blood and Marrow Transplantation (EBMT) database having received ATG ± CSA as a first line treatment. Patients were treated between 1973-2007. Five-year survival probabilities are: 49± 7% for patients treated between 1973-1980 (n=178), 62 ± 3% for those treated between 1980-1990 (n=850), 74 ± 3% for patients treated between 1990-2000 (n=928) and 72 ± 6% for those treated between 2000-2007 (n=444).

rates in the study by Scheinberg *et al.*[14] were 57% and 62% at 3 and 6 months in the standard treatment arm and 37% and 51% in the sirolimus study arm. In this study continuing the trial presented no particular risk for patients in the study arm in respect of response rate, survival or general outcome, with the exception of more dyslipidemia in the sirolimus arm. Given the slightly lower response rate in the experimental arm over the standard treatment arm this decision is rational, the public at large would probably feel more comfortable in settling the issue of mTOR inhibitors in this disease, had the study run to the end. Advancement of medical practice is based to an important degree on refuted hypotheses and the alternative to treat patients outside of clinical trials will not lead to progress in the field. We think that it is most important to publish negative studies such as the one presented in this issue of the journal.

Response to immunosuppressive treatment in severe aplastic anemia is gradual and many patients do not fulfill response criteria until 6 months into the treatment with few patients responding thereafter. This is of importance when choosing the main study outcome, as response by 3 months versus response by 6 months may not yield exactly the same results. It is unclear why the sirolimus group in this study had a slower response with slightly more patients responding between 3-6 months than in the ATG + CSA group. Even though the number of late responders was not high the combined response rates were 44% at 3 months and 57% at 6 months which goes to show that probably response by 6 months should be the standard end-point against which all new treatment schemas should be tested.

The authors stopped immunosuppression abruptly at 6 months in the ATG + CSA + sirolimus arm and withdrew CSA gradually over 2 years in the ATG + CSA arm. There was no difference in the early relapse rate between study arms but the study was not adequately powered to demonstrate non-inferiority of immunosuppression

withdrawal at 6 months versus over 2 years. A recently published paper by the Italian pediatric group came to the conclusion that CSA tapering was best done over many years to decrease relapse risks.[13] Continuing the study might have been worthwhile if only to shed more light on the important question of the impact of early versus late withdrawal of immunosuppressants. This study again confirms that short-term survival is excellent in these patients (>90%), and this includes obviously some of the patients who do not respond and patients who relapse, as well as the option of rescue bone marrow transplantation in patients in whom a donor can be found. Survival probabilities may be different with more follow-up and given a median age of the study patients of 26 years long-term disease- and complication-free survival is the main issue.

As shown in Figure 1 a lot of progress was made between the 1970s and 1990s, with some stagnation thereafter; renewed efforts are necessary to improve treatment results further. The authors of the sirolimus study have clearly defined the agenda for improving response rates to non-transplant treatment in severe aplastic anemia.

Lastly, the group at the National Institutes of Health led by Neil Young deserves a round of applause for working consistently over the years on elucidating the pathophysiology of marrow failure as well as on clinical trials to improve the outcome of affected patients.

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