Review Article

Aplastic Anemia and Therapeutic Options

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Abstract

Aplastic anemia refers to a failure of the bone marrow. The major characteristic of Aplastic Anemia (AA) is marrow hypoplasia and the exhaustion of hematopoietic precursors which then result in pancytopenia. Patients with AA exhibit symptoms that are related to a reduction in bone marrow production of hematopoietic cells. Signs and symptoms associated with Aplastic anemia include swelling of feet, gingival bleeding, petechial rashes. Palpitations, pallor, frequent headaches, dyspnea, and oropharyngeal ulcers. The disease is fatal and thus requires immediate interventions measures. The deficit in hematopoietic cellular often leads to hemorrhage and severe anemia. The most suitable and recommended method of treatment for patients suffering from AA is allogeneic bone marrow transplantation from a matched HLA sibling. The procedure has been carried out on various patients and has been found to be very effective in the treatment and control of the disease. However, the allogeneic bone marrow transplantation has only been effective in the treatment of young patients suffering from aplastic anemia. However, the procedure is ineffective when used for older patients. Additionally, the treatment method cannot be used for people who lack family donors. For such patients, autogenic mesenchymal bone marrow stem cell therapy is recommended.[1]

Keywords:

Aplastic Anemia; Allogeneic bone marrow transplantation; Autogenic mesenchymal bone marrow stem cell therapy

Introduction

The method used can be illustrated by evaluating a previous case. Un-mobilized, mononuclear cells were derived from four patients who had acquired aplastic anemia by aphaeresis.

The derived mononuclear cells were then cultured for three hours with the monoclonal antibody against the monomorphic regions.

The regions had the beta chan of MHC class II antigens. The process was used to derive autologous retro differentiared hematopoietic stem cells. The autologous cells (RHSC) infused into the patients without using any post or pre-conditioning regimen.

The efficacy of the RHSC was later assessed and the results analyzed. After the investigation, two out of the four patients were found to be transfusion independent the infusion of the RHSC.

The mode of engrafting used in the procedure was found to be similar to that used when obtaining transfusion of stem cells from close family members like twins, without any pre-conditioned regimen.[2]

The results were also analyzed for G-branding and karyotyping. The procedure was safe and secure since the karyotyping and G-branding remained the same before and after the procedure for the four patients. The results proved that the procedure's repopulation potential is long term. The above illustrated autologous approach creates more potential for the treatment and control of aplastic anemia. The autologous procedure will thus be a more suitable therapy for the treatment of aplastic anemia since it covers more patients including those suffering from hematological and non-hematological issues. The procedure hence offers treatment to aged patients and patients lacking a family donor.[3]

Methodology

Allogeneic BM transplantation for the Treatment of Aplastic anemia

The procedure is carried out by the transplantation from an HLA-identical donor. The transplantation allows the patient to

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survive for a longer period. Patients who do not have a sibling donor rely on an unrelated donor who is a well-match. After the transplanting, the application of peripheral blood stem cells is discouraged, and BM stem cell sources should be used. This is because the periphery blood stem cells are associated with an increased incidence of chronic GVHD which often leads to an increased risk of treatment-related mortality. Although the current age limit for patients is forty years although this limit is bound to increase due to the current research and study on the issue.[4]

Autogenic mesenchymal bone marrow stem cell therapy is whereby the BM Transplantation (BMT) is carried out from an HLA identical donor, related or unrelated, and is a common choice of treatment for young patients suffering from aplastic anemia. The procedure has a 95% rate of engraftment and over 90% survival rate. The treatment uses Cyclophosphamide (CY) with a conditioning regimen known as the Anti Thyroglobulin(ATG) and a GVHD prophylaxis (methotrexate) and close association of cyclosporine (CsA). This can be viewed as the standard treatment although there are other treatment modes.

The medical outcomes from the allogeneic HSCT have been enhanced for the last decade due to the research and improvement in various aspects of transplantation. Currently, most health facilities which treat patients suffering from the aplastic anemia prefer the Autogenic procedure for patients who have well-matched donors and are aged forty years and below.[5]

Conditioning Regimen with CY and ATG

Although the procedure successfully controls AA, the main challenge for this method is to carry out successful engrafting without any acute or chronic GVHD. Although there have been tremendous efforts to prevent graft failure, some patients still experience graft failure and have resulted in some deaths. Graft failure has occurred especially for patients who underwent the transplanting long time ago. To prevent graft failure, lymphoablative and immune-ablative components are usually used. ATG to CY dosage has been found to provide long-term outcomes as well as excellent engrafting. The dosage is administered at 200 mg of ATG per 1 kg CY. Previously, only CY was administered. However, research had shown that the combination of ATG and Cy improve the survival rate of patients and leads to a reduction in the cases of GVHD as compared to when only Cy-conditioning was used.

Research is still being carried out to establish a more suitable conditioning. For example, radiation-based regimens are being used. Although initial tests have shown that the regimens result in long-term morbidity and mortality, more research is currently underway to enhance the method.[2]

Use of BM as the primary source of stem cell

Since the use of peripheral blood stem cells (PBSCs) has been proven to cause chronic GVHD, nonmanipulated BM should be used. Research conducted on people of different ages has confirmed that BM grafting results to an increase in the survival rate. 3×108 mononuclear cells per 1 kilogram of stem cell dose should be used. A lower dose of stem cell increases the likelihood of the graft to fail.

Post-transplantation measures

Post-transplantation immunosuppression is vital to reduce the possibility of GVHD and suppress the host immune system. Measures should also be implemented to prevent graft rejection. The most recommended post-transplantation immune suppression are CSA and MTX. The research was conducted to analyze the effects of using CsA and MTX or CsA alone. The results showed that a survival rate of 94% was observed when both CsA and MTX were both used. A survival rate of 78% was obtained when only CsA was used. Thus, it is more beneficial to use a combination of CsA and MTX. However, CsA should be used with care, and chimerism and blood counts should be carried out frequently to avoid graft rejection.[5]

Issues related to Allogeneic

Transplantation for Patients older than forty years: Research on aplastic anemia yield different results. Some research suggests that older patients exhibit adverse effects of transplanting while other reports show that results are similar for younger and older patients. Therefore, there is a continuous debate on whether or not allogeneic should be used for patients regardless of their ages. Most medical professionals have however advised that allogeneic procedure should only be used for patients below the age of forty years. Older patients should be treated using other treatment procedures. The transplantation rate is recommended for the older people only when all other methods of treatment of aplastic anemia have failed, and there exists a well-matched donor. The most recommended treatment method for older people is Autogenic mesenchymal bone marrow stem cell therapy. Offering older patients treatment through the transportation may be fatal. The patient is exposed to an increased risk of GVHD. Therefore, older patients should be considered for other methods of treatment including IST. Mortality risks increase with age. Older patients have also recoded a lower performance score. In addition to that, older patients take a longer period from diagnosis to transplantation, unlike the younger patients. Therefore, other options should be considered for patients over the age of forty. Also, different conditioning regimens are considered for older patients. While CY and ATG are considered or younger patients, older patients require CY, Fludarabine, and either alemtuzumab or ATG. Currently, the regimens used for older people are being investigated.[4] Currently, there are no regimens agreed upon by all medical professionals that should be used for older patients who have undergone the transplantation procedures.

Chronic GVHD and other complications

Various researchers have concluded that BM grafts should be used instead of PNSCs. Some studies have shown that the

use of HSCT after it has conditioned with CY and ATG may yield better results where patients' survival rates increase, there is a limited amount of knowledge about the long-term effects since few patients have been observed for a long time after the procedure. The Hospital Saint Louis cohorts have shown that patients who were administered with HSCT experience some complications including tissue dysfunction, secondary cancer and delays in infections.[2]

Autogenic mesenchymal bone marrow stem cell therapy

Autogenic is also referred to as autologous bone marrow therapy. The procedure has been implemented to expand treatment for aplastic anemia to people who do not have a donor and older patients. In analogous treatment, mononuclear cells obtained from the patients are cultured with monoclonal antibody then re-introduced back into the bodies of the patients. Various researchers have shown the functional utility of reprogrammed cells, without any pre or post conditioning regimen. Although autogenic therapy is less effective than allogeneic procedures, autogenic therapy expands the patients' sample space. More patients including the aged have access to medical treatment. Aplastic anemia affects many people and can result in deaths if not properly handled. The main cause of the disease is immune-mediated pathophysiology. The hemopoietic cells are destroyed by various cytotoxic lymphocytes. Suppression is carried out with anti-thymocyte globulin (ATG). Cyclosporine restores the blood cell counts. However, after some time, the disease relapses due to renal failure and infections.

Stem cell transplantation (SCT) can be used to cure younger patients below the age of forty. The cells are derived from siblings of the patient. Although transplantation from related siblings has a higher survival rate that autogenic therapy, transplanting from siblings has various challenges. The negative impacts of allogeneic transplant result from the regimen used for immunosuppression. Cyclophosphamide is used for immunosuppression for long periods of time which then expose the patient to opportunistic diseases. Autogenic therapy also solves the challenge of graft failure. The cells that are introduced into the body of the patient were derived from the same patient hence graft failure cannot occur.[1]

The retro differentiation process is carried out by exposing leukocytes to culture conditions containing clone CR3/43 monoclonal antibody. This process does not involve any genetic modifier hence it is safer. The process results in the generation of numerous pluripotent stem cells. A faster recovery of the hematopoietic system is achieved by the combination of cyto reductive therapy. Engraftment is also enhanced by abrogation of GVHD.

Retro differentiation procedures

There is no specifically recognized procedure to carry out the process. However, research carried out at Tata Memorial Hospital can help establish a general outlook of the process. Before the patients undergo the procedure, their hemoglobin levels are maintained above 8g/dl. The platelets should be above 50* 109/ liter. The aphaeresis should be performed using the white blood cells separation kit and Cobe Spectra aphaeresis machine. CD34 cells were collected and analyzed before the retro differentiation process.

The buffy coat is then taken across the retro differentiation process. This is done under the hematopoietic culture condition. The procedure is the added 1000 µg of CR3/43 which is later diluted in Dulbecco's medium. The dilution is carried out aseptically in a white blood cell bag. The bag, together with its contents is then incubated at 37^{o} C and 5% Carbon dioxide for exactly three hours. The contents are incubated for the long period to make hematopoietic cells that have a loner repopulation potential.

After the incubation, the cells are tested for CD34+ contents. The cells are later washed with saline solution. Cobe cell processor should be used to wash the cells. Later, the cells are later infused into the patients through their jugular veins. After the process, patients are closely monitored and any changes in their bodies investigated. The above procedure was carried out on four patients. Two out of the four patients have survived for over seven years now.

HRCS was infused into the patients suffering from aplastic anemia. The post-infusion resulted in transfusion independence on all the four patients. However, independence was long-term for only two patients, patients 1 and 4. The long-term engraftment of the other patients can be attributed to the primitive RHSC which had CD34+CD3 immuno phenotype. Engraftment in patients 1, 2 and four can be attributed to mega doses of CD34+RHSC. Patient 3 was suffering from a hypoplastic anemia received large amounts of RHSC, but engraftment did not last long. The reasons why the patient failed to show long-term engraftment are still unknown. Investigation of the two patients who showed long-term engraftment has shown that they received high numbers of CD34 and CD45 cells.
Literature

Following a later research, patients numbers 2 and 3 failed to exhibit long-term engraftment due to increase in the number of CD61 cells that were not in the gates of the platelets. Graft rejection has been associated with platelet antigens and leukocytes interaction. Hb F switching can be viewed as a determinant of successful outcome. In other studies conducted on patients suffering from aplastic anemia, successful engraftment of HRSC has been proven to lead to Hb F switching.

The engrafting mechanism in the four patients can be determined by evaluating the transcriptional profiling of RHSC before and after the infusion process. A different study has shown that transcriptional profiling of RHSC derived from patients who are not suffering from aplastic anemia, showed upregulation of genes and primitive stem cell makers. The cell makers included embryonic and fetal erythropoiesis. The results of the experiment also showed that the hematopoietic engraftment that occurred in patients 1 and 4 was similar to those derived from twins without the

incorporation of any conditioning regimen. It was observed that the liver enzymes of the two patients were elevated before the autologous HRSC therapy. They were serologically negative for the virus that causes Hepatitis C. It is, however, unclear whether they tested negative for HCV by PCR.[5]

Since the two patients exhibited a long-term engraftment, it can thus be concluded that it is safe to infuse the RHSC in a hematological condition. Further research should be carried out to determine whether immunosuppression during the infusion of autologous HRSC in the patients who died would have yielded the required engraftment. Close examination showed that there was homing of the RHSC in the bone marrow. Before the infusion, none of the hematopoietic colonies was generated by the bone marrow of the patients under investigation. However, post-infusion samples showed that various hematopoietic cell colonies were being generated, as observed in their trephine section. The microenvironment of the patient numbers 2 and 3 may have contributed to the failure of the RHSC to repopulate well.

The elucidation of the mechanism used in retro differentiation provides credible information concerning normal and defective cell differentiation. The mechanism used in the retro differentiation process involves the loss of lineage makers in a population of a high number of committed cells, both at the protein and genetic levels. The rewinding of ontogeny to a stem cells state occur in a hierarchical manner just like in differentiation. For example, less mature cells will reach the stem stage before the mature cells of a specific lineage. Therefore, at the switch stage, less mature cells regain pluripotency before the mature cells. Thus, they can take part in re differentiation or trans differentiation. The reversed differentiation can either lead to trans differentiation or re differentiation of various specialized cells. This results in either different cells in trans differentiation or more rejuvenated tissues in the case of re differentiation.

The above illustration and experiment show that autologous RHSC can produce long-term engraftment results and increase the survival rates of patients suffering from aplastic anemia. The procedure is more suitable since it can be carried out without immunosuppression regimen or pre-conditioning. Thus, the patients are not exposed to diseases and health conditions associated with excessive immunosuppression. In addition to that, the procedure can be carried out on any person regardless of their age. Allogeneic procedures are only limited to younger people. However, Autogenic mesenchymal bone marrow stem cell therapy can be carried out on any patient suffering from aplastic anemia. [3]

Currently, there is an improved method of treating anemia especially in its mild stage. The method uses erythropoietin (EPO). Erythropoietin is a growth factor which enhances the production of red blood cells. The treatment is used to treat anemia that results from kidney failure or cancer treatment. The method is an alternative to allogeneic and Autogenic mesenchymal bone marrow stem cell therapy. The method of treatment involves the administration of myeloma drugs that control underlying cancer. Patients respond to the treatment have been noted to have an increase in the blood levels . The method of treatment also involves checking of other causes that would be leading to a low level of iron and other nutrients. The patients suffering from anemia have low levels of nutrients including folate and vitamin B12. Thus, erythropoietin serves to control the causative agent for anemia rather than treating the disease. Although some patients who take the medication for anemia have shown an improvement, transplantation and autologous bone marrow are recommended for patients who fail to improve.[2]

Conclusion

Aplastic anemia is a fatal disease which affects the ability of the body to create more red blood cells that are required to transport oxygen throughout the body. A patient suffering from aplastic anemia requires immediate intervention and treatment. The treatment of aplastic anemia is known as bone marrow transplant (BMT) or stem cell transplant (SCT). It is also called the hematopoietic stem cell transplant (HSCT). In the process, the unhealthy blood-making cells are replaced by healthy cells thus providing the cure. Although the treatment is not a guarantee, it increases the chances of survival by a large proportion depending on the age of the patient and the method used, among other variables. BMT is recommended for various patients although some patients are reluctant to undergo the procedure due to the risks involved and potential long-term effects. Also, some patients claim that it is not a perfect cure for aplastic anemia . There are two types of stem cell transplantation. The two types depending on whether the cells are derived from a donor or the patient. The first type is the autologous transplant where the patient's blood-forming cells are used. The autologous transplant is often recommended for patients who have bone marrow failure diseases. The second type is the allogeneic transplant which uses healthy cells from a well-matched donor, usually a sibling. To search for a donor, patients can search the registry of bone marrow which is managed by the National Marrow Donor Program. The success of stem cell transplant is dependent on the age of the patient, the stage or level of the disease and whether there is an available donor.

Due to the advancement in technology, stem cells should be derived from the patient's own body rather that from a donor. Although the procedure has been found to be less effective as compared to through a donor's cells, the side effects of allogeneic transplantation are major and increase the mortality rate of patients especially through immunosuppressing and graft failure. Therefore, the autologous therapy should be recommended and used. This is because it has fewer chances of side effects and can be carried out on patients without a donor and aged patients. The two methods, however, require more research and experiments . The major challenge of the allogeneic method is to find a matching donor and avoid graft failure. The major

challenge of the autologous method is lack of enough information and experiments to support its implementation.

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