Clinical characteristics and treatment of chronic graft versus host disease in pediatric patients

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Background: **Chronic graft versus host disease (cGVHD) is an important complication and a cause of mortality in patients undergoing allogeneic hematopoietic stem cell transplantation (allo-HSCT). In recent years, new immunosuppressants such as tacrolimus (FK506) and mycophenolate mofetil (MMF) have been available and shown positive curative effects in cGVHD patients. This study was undertaken to analyze the clinical characteristics and risk factors of cGVHD after allogeneic hematopoietic stem cell transplantation and to assess the therapeutic effectiveness of methylprednisolone (MP) and MMF in combination with FK506 or cyclosporine A (CSA) in the immunosuppressive treatment of pediatric cGVHD.**

Methods: **In 40 patients who received allo-HSCT and engrafted, 25 were treated with umbilical cord blood transplantation (UCBT) and the remaining 15 with peripheral stem cell transplantation (PBSCT). GVHD prophylaxis employed CSA, MP and MMF. Treatment regimen consisted of MP, MMF and FK506 in one group, and MMF and CSA in the other.**

Results: **Eleven of the 40 engrafted patients (27.5%) developed cGVHD. The cGVHD morbidity was 20% among UCBT patients (5 of 25) and 40% among PBSCT patients (6 of 15). Eight patients presented the continuation of acute GVHD. One patient fully recovered after combined use of CSA and MMF, and 10 patients were given 1 "triple therapy" including MP, MMF and FK506, with an overall response rate of 100%. Three patients died of cytomegalovirus (CMV)-induced interstitial pneumonia, septicemia, and fungi pneumonia, respectively, giving a mortality rate of 27.3%. Seven patients showed an even-free survival (EFS) of more than 3 years, another patient has so far been surviving 29 months at writing. Hepatotoxicity, nephrotoxicity, hypertension, articular capsulitis and cardiac arrhythmia were side-effects most frequently observed. Infection was identified as the main complication and the major cause of death.**

Conclusions: **The incidence of cGVHD is higher after PBSCT than after UCBT, and the presence of acute GVHD is an important risk factor for subsequent cGVHD. A combination of MP, MMF and FK506 or CSA is safe and effective in the treatment of cGVHD in pediatric patients.**

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Key words: **chronic graft versus host disease; methylprednisolone; mycophenolate mofetil; tacrolimus**

Introduction

Chronic graft versus host disease (cGVHD) is an important complication, a cause of mortality and a threat to the life quality of patients undergoing allogeneic hematopoietic stem cell transplantation (allo-HSCT). Traditional multi-drug combination therapy is effective in only about 50% of the patients,[1] and the options for prophylaxis and treatment are generally not satisfactory. In recent years, new immunosuppressants, such as tacrolimus (FK506) and mycophenolate mofetil (MMF), have shown positive curative effects in cGVHD patients.[2,3] Single-drug immunosuppressive therapy is not very effective in individuals with severe or therapy-resistent cGVHD. In our institution, we have been working successfully with a "triple therapy" including methylprednisolone (MP), MMF and FK506 or cyclosporine A (CSA) since 1999.[4,5] The objective of this study is to assess the results and follow-up data of 11 patients with cGVHD.

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Methods

Patients

Between January 1998 and January 2005, a total of 58 patients underwent allo-HSCT at our institution. Forty of them engrafted, after peripheral stem cell transplantation (PBSCT) (15 patients) and umbilical cord blood transplantation (UCBT) (25). Twenty-four patients experienced acute GVHD (aGVHD) and 11 cGVHD. In the cGVHD patients, 6 were boys and 5 girls with a median age of 9 years (range, 2.5-12 years). They suffered from severe β thalassemia (7 patients), chronic idiopathic hemolytic anemia (1), chronic myelocytic leukemia (2), and acute lymphatic leukemia (1). Human leukocyte antigen (HLA) was completely matched (6/6) in 10 children and matched (5/6) in one child. Related donor-PBSCT was performed in 6 patients, related donor-UCBT in 1, and unrelated donor-UCBT in 4. The conditional regimen mainly consisted of busulfanum, cyclophosphamide and anti-human thymocyte globulin, with an additional treatment with fludarabin, melphalan, thiotepa, and total lymphnode irradiation.

Prevention and treatment of aGVHD

The aGVHD prevention regimen consisting of CSA, MP, methotrexate (MTX) and MMF was prescribed for the 11 children. In 10 children, aGVHD was treated with CSA + MP (3 patients), CSA + MP + MMF (3), and FK506 + MP + MMF (4), respectively. The remaining one (patient 6) without aGVHD received CSA + MP treatment continuously.

Treatment regimen for cGVHD

cGVHD was diagnosed as local or general cGVHD in accordance with the diagnostic criteria.[4] The clinical characteristics of our patients are listed in Table 1. Apart from patient 7, who received CSA + MMF treatment, the other 10 patients were given a "triple therapy" of MP + MMF + FK506. Treatment regimen and drug dosage were adjusted individually according to the severity of cGVHD, the course of the disease, organ function, and the blood concentration of the agents.

An acute episode of cGVHD in patient 5 required aggressive treatment with high dosages of globulin and MP. In general, CSA was initially administered by intravenous drop infusion of 3-3.5 $mg \cdot kg^{-1} \cdot d^{-1}$ and was continued per os at a three times higher dosage. Once the clinical symptoms were controlled successfully, the concentration of CSA plasma was maintained at 150-300 ng/L. CSA dosage was reduced or CSA was substituted by other immunosuppressive agents when symptoms of nephrotoxicity or other side-effects occurred. MP was given in patients with severe cGVHD with an initial intravenous dosage of 15-50 $mg \cdot kg^{-1} \cdot d^{-1}$ or 2-5 $mg \cdot kg^{-1} \cdot d^{-1}$. After the infusion took effect, the dosage was reduced by one-half every 1 to 3 days. After 7 to 10 days, 1-2 $mg \cdot kg^{-1} \cdot d^{-1}$ of MP or 1-1.5 $mg \cdot kg^{-1} \cdot d^{-1}$ prednisone was given orally. MMF was administered at a maximum dosage of 30 $mg \cdot kg^{-1} \cdot d^{-1}$ not exceeding 2 g/d (adult reference range 1.5-3

Table 1. Overview of 11 children with cGVHD

Patient	cGVHD		Treatment regimen	Duration of therapy	Side-effects	Time and localization of infection	Clinical outcome
	Time of occurrence	Type and localization					
1	Protracted aGVHD	Generalized; skin, liver, intestine	MP + MMF + FK506	3 y	Left hip, pharmacotoxic synovitis, abdominal pain	After 3 years septicemia	cGVHD controlled, died of septicemia
2	Protracted aGVHD	Local; skin	MP + MMF + FK506	2 y		After 120 days bronchitis	Recovered (5 y)
3	120 d	Generalized; skin, intestine	MP + MMF + FK506	3 y	Abnormal liver function, hyperazotaemia, hypertension	After 120 days bronchitis	Recovered (5 y)
4	300 d	Generalized; skin, intestine	MP + MMF + FK506	18 mon	Abnormal liver function, hyperazotaemia	After 125 days enteritis, after 300 days CMV interstitial pneumonia	Recovered (4.5 y)
5	Protracted aGVHD acute episode after 110 days	Generalized; skin, oral mucosa, liver	MP + MMF + FK506	2 y	Abnormal liver function, hyperazotaemia, arrhythmia	After 300 days pneumonia	Recovered (4.5 y)
6	210 d	Generalized; oral mucosa, liver	MP + MMF + FK506	9 mon		After 300 days bronchitis	Recovered (4 y)
7	Protracted aGVHD	Local; skin	CSA + MMF	9 mon		After 1 year bronchitis	Recovered (3.5 y)
8	Protracted aGVHD	Generalized; skin, liver intestine	MP + MMF + FK506	3 y		After 115 days CMV infection, after 720 days pneumonia	Recovered (4 y)
9	Protracted aGVHD	Local; skin	MP + MMF + FK506	4 mon	Abnormal liver function	After 150 days interstitial pneumonia	Recovered, died from interstitial pneumonia
10	Protracted aGVHD	Generalized; skin, intestine	MP + MMF + FK506	3 mon		After half a year transplantation fungi pneumonia	cGVHD controlled, died from fungi pneumonia
11	Protracted aGVHD	Local; skin	MP + MMF + FK506	2 y and on-going		After 150 days bronchitis	cGVHD controlled (2.5 y)

g/d). Once symptomatic relief was achieved, MMF was gradually reduced to a maintenance dosage of 0.5-1.0 g/d administered daily or every other day.

Individuals already on CSA therapy who were supposed to switch to FK506 had to stop CSA intake for 24 hours first. In severe patients, 0.03-0.05 $mg \cdot kg^{-1} \cdot d^{-1}$ FK506 was first given by intravenous drop infusion with a target blood concentration of 10-20 ng/L, followed by an oral administration of 0.01-0.08 $mg \cdot kg^{-1} \cdot d^{-1}$ after symptomatic relief. Less severe patients started with an oral dosage of 0.05 $mg \cdot kg^{-1} \cdot d^{-1}$ and a plasma concentration of 10-15 ng/L. Once the clinical symptoms were effectively controlled for more than 8 weeks, the FK506 dosage was gradually reduced once in four weeks to a maintenance dosage of 0.5-1.0 mg/d. After 6 months of successful "triple therapy", the dosage was further reduced, usually starting with MP, which was reduced once per month to 4-8 $mg \cdot kg^{-1} \cdot d^{-1}$. Subsequently, the dosage of FK506 and MMF was gradually decreased as well. The treatment took more than 1 year.

Blood pressure, urine volume, and renal function were monitored. When nephrotoxicity or other side-effects were noticed, the drug dosage was reduced or the patient was given other immunosuppressants.

Results

Incidence of cGVHD and its clinical characteristics

cGVHD occurred in 11 (27.5%) of the 40 engrafted patients. Six patients with cGVHD[6] were found in the 15 patients having related donor-PBSCT (40%), whereas 5 patients (three patients developed thalassemia after unrelated donor-UCBT) were encountered in 25 patients receiving UCBT (20%). This finding suggests a higher incidence of cGVHD in the patients having related donor-PBSCT. Local skin involvement was observed only in 3 patients, but 8 patients presented with a generalized cGVHD reaction involving the skin, intestinal tract and liver (2 patients), the skin and intestinal tract (3), and lichenification of the oral mucosa and liver (1). An acute episode of cGVHD occurred with the involvement of the liver and the skin all over the body and the lichenification of the oral mucosa in one patient.

Skin involvement appeared as hyperaemic maculopapular eruptions and areas of hypopigmentation. Skin biopsy in patient 5 showed degenerative changes of blood vessels in the basal layer, formation of eosinophilic bodies, and infiltration of lymphocytes, which were suggestive of cGVHD. The involvement of the intestinal tract was characterized by diarrhoea, watery stool, stool positive for white blood cells and ineffective antibiotic treatment. Patients with liver involvement showed impaired liver function (patient 6 GPT 1503 U/L, GOT 1630 U/L), hepatomegaly and icterus.

Ten of our 11 cGVHD patients had a history of aGVHD. In 8 of these patients, active treatment did not satisfactorily control the disease, which subsequently developed into a chronic state. Patients 3 and 4 in our series recovered from an aGVHD-related skin rash after 7-10 days of therapy, but showed generalized cGVHD after 120 and 300 days, respectively. One patient without a history of aGVHD presented with generalized cGVHD after 210 days with symptoms of lichenification of the oral mucosa, hepatomegaly, abnormal liver function, and icterus.

Clinical effects of "triple therapy" with MP + MMF + FK506

"Triple therapy" with MP + MMF + FK506 effectively controlled cGVHD in all patients receiving immunosuppressive therapy ranging from 4 months to 3 years (mean 21 months). Nine patients (81.8%) were cured, and 2 had the disease controlled. Event-free survival for three years was seen in 63.6% of our patients. Three patients died of cytomegalovirus interstitial pneumonia, fungi pneumonia, and septicemia at 4 months, 3 months and 3 years after therapy. The mortality rate in this study therefore was 27.3%.

Side-effects of cGVHD

The most commonly observed side-effects of our therapy were hepatotoxicity and nephrotoxicity. Four patients showed impaired liver and renal function during the course of the therapy and presented with icterus and elevated levels of liver enzymes (GPT 83-122 U/L, GOT 72-243 U/L), serum bilirubin and urea nitrogen (8.2-22.2 mmol/L). Reduced dose of FK506 (or CSA) and use of liver-and kidney-protective agents helped to normalize the levels of hepatic enzymes and urea nitrogen and eliminate icterus. The level of urea nitrogen was maintained >22 mmol/L in a patient for more than one month, but returned to normal after discontinuation of FK506, kidney-protective agents and symptomatic therapy.

FK506-related side-effect was the drug-induced synovitis of the hip joint observed in one patient presenting with arthralgia, fluid accumulation, and limitation of motion. The patient recovered after ultrasound-guided puncture, removal of the liquid, and immobilization for 2 weeks.

MP-induced side-effects were seen in 3 patients. One patient suffered from hypertension, one from repeated upper abdominal pain at a dose of 10 $mg \cdot kg^{-1}$

$\cdot d^{-1}$, and the other from cardiac arrhythmia at a maximum dose of 42 $mg \cdot kg^{-1} \cdot d^{-1}$. The symptoms disappeared after the reduction of MP dose to 10 $mg \cdot kg^{-1} \cdot d^{-1}$ as well as active treatment with cardio-protective agents.

Complicated infections during cGVHD treatment

All patients in our series suffered from respiratory tract infections or enteritis in the course of cGVHD treatment. The infections could be controlled with routine antibiotic treatment. Patient 1 died from enteritis and septicemia 3 years after transplantation while on MMF maintenance therapy. Patient 10 being treated with "triple therapy" died from fungi pneumonia 180 days after transplantation. CMV infection was discovered in another 3 cGVHD patients on days 75 to 105 after transplantation. In these patients, 2 were positive for CMV-Ig and 1 had a positive sputum CMV-PCR. Two of these patients (patients 4 and 9) developed interstitial pneumonia, and patient 9 died of CMV-interstitial pneumonia. No patient was infected with varicella-zoster virus or Epstein-barr virus. Up to date, 7 patients have shown an event-free survival of more than 3 years (Table 1).

Discussion

The key to cGVHD prevention is the avoidance of known risk factors for cGVHD, such as HLA mismatch, an older age of donor and/or recipient, a history of aGVHD (particularly 2nd or 3rd degree aGVHD), a short duration of preventive CSA treatment, and herpesvirus infection of donor and/or recipient around the date of transplantation.[7] Mohty et al[8] reported the incidence of cGVHD was significantly higher in patients with PBSCT (44%) than in those with bone marrow transplanation (17%). The incidence of cGVHD in this study was 27.5%, and compares well with 25% reported elsewhere.[9] We found the incidence of cGVHD in the related-donor PBSCT group was higher than in the UCBT group. In the 10 patients with a history of aGVHD (6 patients with 1st degree and 4 patients with 2nd degree aGVHD), 8 developed cGVHD from aGVHD. Allo-PBSCT and a history of aGVHD therefore appear to be high-risk factors.[10,11] Prevention of aGHVD as well as timely diagnosis and adequate therapy of cGVHD are important measures after transplantation.

CSA and glucocorticoids are the first choice for the treatment of cGVHD.[12,13] Single drug therapy with glucocoticoids or CSA can sufficiently treat mild cGVHD. "Triple therapy", however, is indicated for severe or therapy-resistant patients. It was reported that combined treatment with CSA and MP was effective in 50% of patients with progressive cGVHD with a survival rate of 50%-80.9%.[1] Gaziev et al[14] treated 45 patients with therapy-resistant cGVHD with a combination of CSA, MP and azathioprim. This regimen was tolerated well by the patients, and showed an effective rate of 77.5% and a survival rate of 80%. FK506 and MMF are new options for immunosuppressive treatment of cGVHD.[15,16] MMF can improve the pharmacological activity of FK506, and the combination of both agents shows satisfactory therapeutic effects in patients with refractory cGVHD.[17,18] The required dosage of MMF, FK506 (or CSA) and corticoids can be reduced by combining these drugs, allowing effective treatment with less toxicity and side-effects.[19,20] In this study, a combination of two drugs (CSA and MMF) was sufficient in one patient, whereas "triple therapy" was necessary for the other 10 patients to effectively control the disease. Curative effects were achieved in all our patients, 7 of whom by now showed an event-free survival of more than 3 years. Three patients died from severe infections, among them one patient had concurrent infection after 3 years. This study showed "triple therapy" with MP + MMF + FK506 can achieve a high response rate and is tolerated well by the patients.

The most frequently observed side-effects in this study were hepatotoxicity, nephrotoxicity, hypertension, synovitis and cardiac arrhythmia, which could be successfully treated by dose reduction and symptomatic therapy. Infection as the major cause of mortality in cGVHD patients deserves special attention. Intensification and conditional chemotherapy before HSCT affect the functioning of the immune system and the recovery mediated by the engrafted hematopoietic stem cells is slow.[21] The proliferation of T- and B-lymphocytes takes 10 to 12 months to recover in most individuals.[22] IgM secretion normalizes after 6 to 9 months, and IgG secretion after 18 months or longer. In addition, the mobility of granulocytes and monocytes is impaired and IgA secretion is reduced in cGVHD patients. Thus the treatment with immunosuppressants is another factor that adversely affects and slows down the recovery of the immune system. Patients with cGVHD are therefore susceptible to bacterial, fungal and viral infections, particularly infections with gram-positive cocci and varicella-zoster virus. Nine patients in this study suffered from respiratory tract infections of varying severity. Interstitial pneumonia after CMV infection and septicemia led to 2 deaths. Therefore, it is important to look for and monitor infections in such patients, to actively carry out anti-infective therapy, and to intravenously supplement IgG so as to reduce the cGVHD treatment-related mortality.

New immunosuppressive agents can greatly im-

prove the therapeutic effects in patients with cGVHD, which are particularly marked in those with moderate, severe and therapy-resistant cGVHD. This study used a two- or three-agent combination of MP, MMF and FK506 (or CSA), which was effective and tolerated well by the patients. We consider that this therapy may be of general interest for the treatment of pediatric patients with cGVHD.

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