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Haemolytic anaemia after lung transplantation: an immune-mediated phenomenon?

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Summary

Background: Haemolytic anaemia is believed to occur only rarely after solid organ transplantation. During the past eight years we have repeatedly observed cases with otherwise unexplained haemolysis in our lung transplant recipients.

Methods: We conducted a retrospective cohort analysis to determine the prevalence of haemolytic anaemia after lung transplantation and to elucidate possible pathogenetic mechanisms.

Results: Whereas in three cases haemolytic anaemia was possibly caused by cytomegalovirus disease or drug-related side effects, sixteen out of the remaining 81 lung transplant recipients at risk (20 percent) developed otherwise unexplained haemolysis. All except one haemolytic episode occurred during the first postoperative year and their degree was mild to moderate in 14 cases. A case control study of these patients with those who did not develop haemolysis revealed that this phenomenon occurred less often in patients receiving immunosuppressive therapy with mycophenolate mofetil and prophylaxis for cytomegalovirus disease with intravenous immune globulin.

Conclusions: We found a high prevalence of otherwise unexplained, mostly mild to moderate episodes of haemolytic anaemia after lung transplantation occurring predominantly during the first year after transplantation. The fact that this phenomenon was observed less often in patients receiving mycophenolate mofetil instead of azathioprine as well as in those receiving regular immune globulin prophylaxis with potentially immunomodulatory effects may indicate an immune-mediated pathogenesis.

Key words: lung transplantation; haemolytic anaemia; mycophenolate mofetil; immune globulin

Introduction

Haemolytic anaemia after solid organ transplantation is believed to be a rare event. It has been described after minor AB0-incompatible renal, liver and lung transplant procedures [1–4]. Single cases of graft-versus-host antibody production after liver transplantation [5] and donor anti-D antibody-induced haemolysis after heart-lung transplantation [6] have been reported. Microangiopathic haemolytic anaemia is a rare but well-known complication of cyclosporine and tacrolimus immunosuppressive therapy [7, 8]. Only exceptionally may haemolysis be due to an autoimmune process associated with post transplant lymphoproliferative disorders [9].

During the evolution of our lung transplant program we have repeatedly observed haemolytic episodes that could not be ascribed to any of the above mentioned pathogenetic mechanisms. Therefore, the present study was undertaken to describe the incidence of otherwise unexplained haemolytic anaemia after lung transplantation and to elucidate possible pathogenetic mechanisms.

Methods

Patient population

The medical records of 94 patients receiving a lung transplant between the start of our program in November 1992 and April 2000 were retrospectively reviewed. Ten patients who died within 30 days of transplantation due to multiple organ failure (5), hypoxic-ischaemic encephalopathy (2), hyperammonaemia (2) and cardiac failure (1) were excluded from the study. The remaining 84 patients, 46 females and 38 males with a median age of 39 (12 to 66) years, received 64 bilateral and 20 single lung transplants

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after a median waiting time of 97 (1 to 891) days. The indications for lung transplantation were end-stage lung diseases due to bronchiectasis including cystic fibrosis (30), pulmonary emphysema or obstructive airway disease (22), diffuse parenchymal lung disease (16), pulmonary hypertension (9) and lymphangioleimyomatosis (7).

Surgery and clinical management

Details on surgery and clinical management have been described in detail elsewhere [10]. The operative procedure was the same throughout the study period according to standardised techniques. All transplants were ABO-identical and none was HLA-matched. The induction immunosuppressive regimen consisted of cyclosporine, azathioprine and antithymocyte globulin. Only 7 of the last 13 patients received basiliximab for induction immunosuppression. Maintenance immunosuppressive drugs were cyclosporine, aiming at a monoclonal cyclosporine A trough level of 180–250 µg per litre, azathioprine 2 mg/kg/d and prednisone 0.5 mg/kg/d, tapered to about 10 mg per day within the first six postoperative months. No patient received tacrolimus. From August 1997 to March 1999 seventeen patients were randomised to receive either azathioprine (8) or mycophenolate mofetil 3 grams per day for three months and 2 gram per day thereafter (9) in an open, unblinded fashion. From April 1999 mycophenolate mofetil was routinely used instead of azathioprine. Perioperative antibiotic prophylaxis consisted of ceftriaxone or an anti-pseudomonas combination therapy tailored to the pre-transplant bacteriological results in cystic fibrosis patients. Postoperative antibiotic treatment was adapted according to the detected bacterial strains. Pneumocystis carinii prophylaxis was carried out with cotrimoxazol three double strength tablets per week. Bacterial, fungal or protozoal infections were treated according to standard criteria.

Patients seronegative for cytomegalovirus receiving an organ from a seropositive donor were given monthly intravenous immune globulin at a dose of 0.5 g/kg of body weight. Initially, patients seropositive for cytomegalovirus were given acyclovir 800 mg three time daily irrespective of the donor serology. From May 1994 to April 1995 this was given intravenously at a dose of 5 mg/kg of body weight twice daily for 2 to 3 weeks postoperatively. Thereafter, patients received prolonged ganciclovir prophylaxis on an out-patient basis. Initially, intravenous ganciclovir 5 mg/kg of body weight per day was used up to about postoperative day 90. From May 1995 all patients received oral ganciclovir 1 gram three times daily until the prednisone dose was reduced to less than 15 mg/d [11]. Cytomegalovirus infection was defined as identification of the virus by shell-vial or conventional cultures of blood, urine, throat swabs or bronchoalveolar lavage fluid (i.e. viral shedding), pp-65 antigenaemia or asymptomatic seroconversion. Cytomegalovirus disease was defined as histological evidence of pneumonia, colitis, or gastroenteritis, accompanied by a positive viral culture or detection of cytomegalovirus by polymerase chain reaction.

Post transplantation surveillance protocol

Daily routine clinical, laboratory, functional and radiological evaluations were performed during the first postoperative week and every 2–3 days during in-hospital stay. Thereafter, clinical assessment was done weekly during the second postoperative month and every second week during the third postoperative month. Laboratory assessments including red blood cell count were done twice weekly during in-hospital stay, weekly during the second postoperative month and every second week during the third postoperative month.

Routine surveillance bronchoalveolar lavage and transbronchial lung biopsy procedures were performed monthly in clinically and physiologically stable recipients during the first six postoperative months as reported previously [12] and in each patient with new symptoms, signs, roentgenographic infiltrates or declining lung function. The severity of acute rejection was graded according to the criteria of the International Society for Heart and Lung Transplantation [13]: grade A0 = no significant abnormality; grade A1 = minimal; grade A2 = mild; grade A3 = moderate; and grade A4 = severe. Acute rejection episodes were treated with prednisone pulses of 0.5 to 1 gram per day for three days. Antithymocyte globulin was given in three and OKT-3 in one patient with recurrent acute rejection episodes or bronchiolitis obliterans syndrome.

Definition of haemolytic anaemia

In the case of otherwise unexplained anaemia the reticulocyte fraction R3500 was determined by the coulter counter method. A value greater than 17‰ was considered to be abnormal. Haemolytic anaemia was diagnosed in the presence of reticulocytosis and elevated serum lactate dehydrogenase levels (normal <420 U/L) after the exclusion of other causes of elevated reticulocyte counts such as previous haemorrhage. Reasons for increased erythropoiesis other than haemolysis were recent blood loss (3 patients), recovery after mild transient aregenerative anaemia due to treatment with antithymocyte globulin (3) and administration of vitamin B_{12} (1) or folic acid (1). These patients were included in the non-haemolysis group. Automatic blood counts were cross-checked by the microscopic examination of peripheral blood smears for the absence of Howell-Jolly bodies, fragmentocytes and basophilic stippling. Direct and indirect Coombs testing was performed by standard methods. No irregular red cell antibodies could be detected in any of the patients. Severity of anaemia was graded as follows: mild = haemoglobin <12 g/dL, moderate = haemoglobin <10 g/dL, and severe = haemoglobin <8 g/dL.

Statistics

Results are expressed as median and ranges. Statistical comparisons between groups were performed with the Mann-Whitney U test or the Wilcoxon matched pairs test for continuous variables and the Fisher's exact test for discrete variables. A p value of 0.05 or less was considered to be significant.

Results

There were 19 cases of haemolytic anaemia among the 84 lung transplant recipients. Haemolysis could be explained by secondary causes in 3 patients, one case each of a haemolytic episode possibly due to cytomegalovirus disease and drug-related side effects of ribavirin and valacyclovir respectively. These patients were excluded from further analysis. Thus the overall incidence of otherwise unexplained haemolytic anaemia was 16 of 81 lung transplant recipients (20 percent). Haemo-

Table 1

Baseline characteristics of the two study populations.

	no haemolytic anaemia (n = 65)	haemolytic anaemia (n = 16)	p value
Age, yr (range)	35 (12–66)	46 (21–60)	0.13
Female sex (percentage)	33 (51)	11 (69)	0.16
Diagnosis			0.60
Cystic fibrosis or bronchiectasis	24	5	
Obstructive airway disease / emphysema	16	4	
Pulmonary hypertension	7	2	
Parenchymal lung disease	11	5	
Lymphangioleiomyomatosis	7	0	

lytic anaemia developed after a median postoperative time period of 200 (range 39 to 376) days. All except one case occurred within the first year after lung transplantation. The haemoglobin value decreased from 12.1 (10.2–14.3) to 9.5 (7.8–11.9) g/dL ($p < 0.001$). The nadir of the reticulocyte fraction R3500 was 38‰ (21–87). Lactic dehydrogenase increased to 736 (433–2038) U/L. Total bilirubin was only slightly elevated to 14 (4–28) µmol/L. Eleven anaemic episodes were graded as moderate, three as mild, and one as severe. One patient merely showed an increased reticulocyte fraction of 35‰ and a stable haemoglobin value around 12 g/dL. The anaemia persisted for 117 (22–328) days. In one female patient stable mild haemolytic anaemia with haemoglobin levels around 10 g/dL is still detectable more than 4 years after its onset on postoperative day 198. A Coombs test was available in 10 patients and was negative in all cases. No patient showed signs of microangiopathic haemolytic anaemia.

The patient group suffering from post transplant haemolysis was then compared with the remaining lung transplant recipients at risk i.e. those surviving at least 30 days after transplantation. Neither age, sex nor the distribution of the underlying diagnoses differed between the two groups (table 1). The comparison of possible pathogenetic factors between the two study populations is shown in table 2. Two patients, positive for rhesus blood group antigens after receiving an organ from a rhesus-negative donor, developed haemolytic anaemia compared to three in the non-haemolysis group (p = NS). There were no ABO blood group mismatches. The two groups were also comparable with respect to the number of pregnancies, the number of females with at least one pregnancy, the incidence of cytomegalovirus infection and disease, the frequency of acute rejection episodes per patient and the number of patients suffering from at least two acute rejection episodes. However, patients suffering from post transplant haemolytic anaemia were significantly less likely to be immunosuppressed with mycophenolate mofetil than with azathioprine (p = 0.046) and to receive intravenous immune globulin for prophylaxis of cytomegalovirus disease (p = 0.048).

Table 2

Comparison of possible factors associated with the development of posttransplant haemolytic anaemia.

	no haemolytic anaemia (n = 65)	haemolytic anaemia (n = 16)	p value
Rhesus mismatch donor-/recipient[a]	3 (5)	2 (13)	0.28
Patients with at least one pregnancy[b]	16/33 (48)	7/11 (64)	0.40
Cytomegalovirus infection	13 (20)	5 (31)	0.32
Cytomegalovirus disease	8 (12)	4 (25)	0.24
Acute rejection episodes per patient (range)	1 (0–8)	1 (0–5)	0.71
Patients with ≥2 acute rejection episodes	24 (37)	3 (19)	0.14
Immunosuppression with mycophenolate mofetil[b]	29 (45)	3 (19)	0.046
Intravenous immune globulin prophylaxis	19 (29)	1 (6)	0.048

[a] Values are given as number of patients with percentages shown in parenthesis

[b] One patient receiving primary immunosuppression with cyclophosphamide instead of azathioprine was excluded from this analysis

Discussion

The present study demonstrates that otherwise unexplained haemolytic anaemia is a frequent disorder after lung transplantation. Whereas three haemolytic episodes could be ascribed to either cytomegalovirus disease or drug-related side-effects of ribavirin and valacyclovir respectively, there were 16 patients in whom there was no evident risk factor for haemolytic anaemia. Thus the cumulative incidence of haemolytic anaemia in our lung transplant recipients at risk was 20%. The disorder usually developed during the first year after transplantation and anaemia was of mild to moderate degree in most patients. There were no minor AB0 blood group incompatibilities and Coombs' test was negative in all cases tested for. Moreover, there was no evidence of possible

cyclosporine-associated microangiopathichaemolytic anaemia. One patient with persisting haemolytic anaemia developed a monoclonal post transplant lymphoproliferative disorder 10 months after the first evidence of haemolysis and died within 2 months due to progressive, generalised disease. Another patient had a polyclonal post transplant lymphproliferative disorder of the lung presenting as a pulmonary nodule, which was resected. Despite the fact that the patient showed no evidence of recurrence during the following years, haemolytic anaemia persisted for more than half a year after resection of the lymphoma.

In order to elucidate possible risk factors for the development of post transplant haemolytic anaemia we compared the group with haemolytic anaemia with the remaining lung transplant recipients at risk. We found that the patients suffering from haemolytic anaemia received immunosuppressive treatment with azathioprine significantly more often than with mycophenolate mofetil ($p = 0.046$). Since there is ample evidence that mycophenolate mofetil is a more potent immunosuppressive drug than azathioprine resulting in fewer acute rejection episodes after all kinds of solid organ transplantations [10, 14], the fact that treatment with mycophenolate mofetil protected against development of haemolytic anaemia may indicate a possible auto- or alloimmune pathogenetic mechanism for the development of this disorder. Moreover, we found that prophylaxis with intravenous immunoglobulin, which was routinely administered to patients who were seronegative for cytomegalovirus and had received a seropositive organ, also had a protective effect on the development of haemolytic anaemia. Investigating the possible immunomodulatory effects of this treatment [15] may add another piece of evidence for an immune-mediated mechanism in the development of post transplant haemolytic anaemia.

We are well aware of the limitations of the present study. The main problem is the lack of a widely accepted definition of haemolytic anaemia. Authors usually refer to haemolysis as a shortening of the red blood cell survival. With respect to laboratory methods the absolute reticulocyte count, the corrected absolute reticulocyte count, the reticulocyte production index and various calculations of red blood cell survival are suggested. As haemolysis was generally mild in our patients and because all of them received antiproliferative drugs such as azathioprine or mycophenolate mofetil, respectively, and ganciclovir (if either donor or recipient seropositive for cytomegalovirus, 84% of the cases), we decided to use the relative rather than the absolute reticulocyte counts. Thus, our approach might have overestimated to some extent the prevalence of haemolysis compared to the latter (i.e. the use of absolute counts), which in turn would lead to an underestimation of the prevalence of haemolysis. However, since in addition to an elevated reticulocyte fraction all patients had anaemia and elevated serum lactate dehydrogenase levels and other causes for these abnormalities, such as previous haemorrhage were excluded, we think that our arbitrary definition of haemolytic anaemia is quite appropriate in this special setting.

In conclusion, we found a high prevalence of mainly mild to moderate episodes of haemolytic anaemia usually developing during the first year after transplantation. A retrospective cohort analysis revealed that treatment with mycophenolate mofetil instead of azathioprine and prophylaxis for cytomegalovirus disease with intravenous immunoglobulin significantly reduced the prevalence of this phenomenon, indicating a possible auto- or alloimmune pathogenetic mechanism of post transplant haemolytic anaemia.

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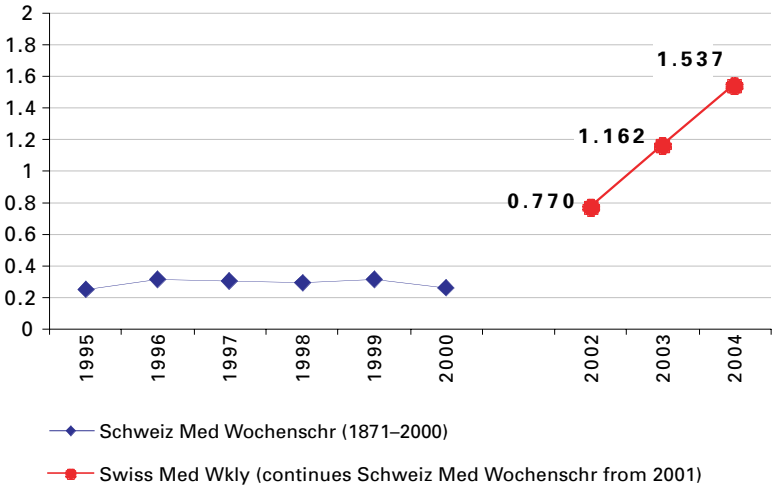

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