Combination of everolimus with calcineurin inhibitor medication resulted in post-transplant haemolytic uraemic syndrome in lung transplant recipients—a case series

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Abstract

Background. Post-transplant haemolytic uraemic syndrome (HUS) is a rare but serious disease with a high mortality rate, when left untreated. Immunosuppressive drugs like calcineurin inhibitors as well as mammalian target of rapamycin inhibitors have been reported as causative agents for post-transplant HUS.

Methods. A retrospective observational study was performed in lung transplant recipients, who took part in an
interventional study, in two centres. Haemoglobin, platelets, creatinine and lactate dehydrogenase levels were monitored during routine follow-up and patients with deteriorating kidney function were screened for post-transplant HUS. All cases of post-transplant HUS were identified by clinical and laboratory findings. Outcome was recorded until 6 months after diagnosis.

**Results.** A total of 2188 visits in 512 lung transplant recipients (outpatients) were analysed. Out of those, 126 patients took part in an interventional study. In this study, 67 were switched to everolimus in combination with calcineurin inhibitors 4 weeks after transplantation, 59 patients remained on standard immunosuppression (calcineurin inhibitors, mycophenolate mofetil and prednisolone). Five cases of post-transplant HUS were identified in the everolimus group. None of the patients had evidence of gastrointestinal infection or preexisting renal disease. Post-transplant HUS was treated with therapeutic plasma exchange and methylprednisolone pulse therapy. Everolimus was discontinued in all five patients. This treatment regimen led to normalization of haemoglobin, platelets and improved renal function. Two patients developed end-stage renal failure and were maintained on haemodialysis. One patient died due to multiorgan failure. Improvement of renal function was seen in two patients. No further cases were recorded in patients without everolimus during the study period.

**Conclusions.** Our data should raise the awareness of post-transplant HUS in lung transplant recipients. Post-transplant HUS is a rare disease, but it is a serious cause of acute renal failure in lung transplant recipients treated with a combination of everolimus and calcineurin inhibitors.

**Keywords:** adverse effects; everolimus; lung transplantation; post-transplant haemolytic uraemic syndrome; thrombotic microangiopathy

**Introduction**

The term ‘thrombotic microangiopathy’ (TMA) describes a variety of pathological conditions characterized by thrombosis in capillaries, arterioles and arteries. The process leads to thrombocytopenia and symptoms, such as anaemia, purpura and renal failure.

Major categories of TMA are haemolytic uraemic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP). HUS is characterized by the appearance of several clinical and laboratory findings, such as microangiopathic haemolytic anaemia, thrombocytopenia and acute renal failure due to endothelial cell injury with consecutive platelet aggregation and development of intravascular microthrombi in the affected organs. In addition, several neurologic abnormalities and fever can be observed in some patients. The incidence of TMA is 11 cases/million population/year [1]. HUS can be classified into typical and atypical (tHUS and aHUS, respectively). tHUS is associated with Shiga toxin-producing enterohaemorrhagic *Escherichia coli*, predominantly in children. The causes of aHUS are variable and include drug toxicity, autoimmune diseases, pregnancy and postpartum and HIV Infection.

Furthermore, mutations of complement factors can be found in aHUS. Mutations of complement factor *H* (CFH), complement factor I (CFI), membrane cofactor protein (MCP) deficiency [2, 3], complement factor B (CFB) and C3, which promote alternative pathway activation have been reported. Also, in ~5% of patients with aHUS, mutations occur that impair the function of thrombomodulin [4].

Not only mutations in the complement system but also genetic polymorphisms are associated with HUS. These polymorphisms were found for CFH-related proteins (CFHR), C4b-binding protein (C4b-BP), MCP and CFH genes. Some drug-induced forms of post-transplant HUS can be triggered by immunosuppressive agents such as cyclosporine and sirolimus [5]. Young recipient age, older donor age, female gender and immunosuppressive regimens including tacrolimus or cyclosporine are predictors of post-transplant HUS after transplantation [6, 7].

The combination of sirolimus and cyclosporine seems to play a major role in the development of post-transplant HUS. The exact pathogenesis of this phenomenon remains unclear. Cyclosporine may increase platelet aggregation, but direct endothelial cell injury seems to be the most important step in this setting. In reference to everolimus, a similar mechanism is certainly possible, however, there is no evidence of an endothelium damaging or blood clotting effect [8]. Post-transplant HUS can be diagnosed based on clinical and laboratory findings, such as anaemia, thrombocytopenia, elevated lactate dehydrogenase (LDH), decreased haptoglobin and the presence of schistocytosis in peripheral blood smear. In addition, renal function parameters, blood pressure and urine excretion should be monitored.

So far, there are various reports of cyclosporine-, tacrolimus- or sirolimus-induced TMA. An increased risk of post-transplant HUS associated with the combination of cyclosporine and everolimus has not been reported in lung transplantation. However, two studies showed unexplained anaemia in liver- and kidney-transplanted patients treated with mammalian target of rapamycin (mTOR) inhibitors [9, 10]. Therefore, we retrospectively searched our database on lung transplant patients for a possible link between everolimus and HUS.

**Patients and treatment**

We report a series of five lung transplant recipients who were admitted to two tertiary care centres in a period of 12 months with a newly diagnosed severe impairment of renal function of unknown etiology. Two to twenty-four months prior to admission, the patients had undergone lung transplantation. The initial immunosuppressive regimen after lung transplantation in all these patients included cyclosporine, mycophenolate mofetil (MMF) and prednisolone. Four weeks after transplantation, patients were switched to everolimus in combination with a calcineurin inhibitor or remained on standard immunosuppression with cyclosporine, MMF and prednisolone.

Target trough levels of cyclosporine in conjunction with MMF were 200–250 ng/mL during the first postoperative year, 150–200 ng/mL during the second year and 100–150 ng/mL thereafter. Everolimus target trough levels were 5–7 ng/mL. Prednisolone was tapered in all recipients during the first 3 postoperative months to a maintenance dose of 0.1 mg/kg thereafter. The immunosuppressive therapy and patient characteristics are shown in Table 1. Furthermore, the patients were treated with anti-infective prophylaxis after lung transplantation including acyclovir, itraconazole and cotrimoxazole.

Disease was assessed on clinical and laboratory findings. Post-transplant HUS was assumed if haemolytic anaemia, thrombocytopenia, deteriorating renal function with elevated LDH, decreased haptoglobin and schistocytosis in peripheral blood smear were evident or renal biopsy
revealed the diagnosis of TMA [11]. Acute kidney injury was defined according to the AKI network [12] and occurred at a median of 60 days after transplantation. To obtain additional information about the underlying cause of the presented clinical and laboratory findings, renal biopsies were performed. After excluding other infectious and autoimmune diseases, drug-induced post-transplant HUS due to the immunosuppressants cyclosporine or everolimus were considered the most likely cause for renal impairment. Glucocorticoids, elimination of potential releasing factors and therapeutic plasma exchange (TPE)—which may be initiated even if there is uncertainty about the diagnosis of HUS—are established treatment options. All patients had their follow-up visits in specialized outpatient clinics. Clinical status, haemoglobin levels, platelets, serum creatinine and LDH were recorded at baseline and during follow-up. Liver function tests and differential blood count were performed. Estimated glomerular filtration rate (eGFR) was calculated and C-reactive protein levels were recorded during follow-up.

Statistics

Statistical analysis was performed using nonparametric Mann–Whitney U-testing. Data are presented as medians with range. A value of $P < 0.05$ was considered statistically significant.

Results

A total of 2188 visits in 512 lung transplant recipients (outpatients) were analysed. Of those, 126 patients took part in an intervention study. In 67 patients, immunosuppression was switched to everolimus in combination with a calcineurin inhibitor 4 weeks after transplantation and 59 patients remained on standard immunosuppression (cyclosporine, MMF and prednisolone). Five cases of post-transplant HUS were identified in the everolimus group ($5/67 = 7.5\%$) (Figure 1). No case of post-transplant HUS was observed during the period in either centre in patients not treated with everolimus ($n = 445$).

No patient had evidence of neurological abnormalities. Prior to diagnosis, two patients experienced mild headache. Laboratory values showed decreased haptoglobin and haemoglobin levels, thrombocytopenia, elevated LDH and schistocytosis in peripheral blood smear. After diagnosis of post-transplant HUS, three patients were treated with TPE and glucocorticoids. Immunosuppressive regimen was changed in all cases. Patient characteristics are shown in Table 1. Before transplantation, none of the patients had renal impairment. There was no record of diarrhoea in the patient charts. None of the patients had cytomegalovirus infections.

Of all the lung transplant recipients ($n = 512$), renal biopsies were taken from only 26 patients. Three of these 26 patients had biopsy-proven TMA. All of these three patients received everolimus. All of three biopsy-positive cases presented with a typical clinical picture of TMA. Light microscopy revealed intraluminal thrombi in arteries. Glomeruli were unremarkable, without thrombi or double contours of the basement membrane. No immune complexes were found on immunohistochemical examination of paraffin-embedded material. In standard histology, an acute TMA with arteriolar manifestations could be detected. Furthermore, moderate chronic calcineurin inhibitor vasculopathy with transmural arteriolar hyalinosis/vasculopathy was diagnosed (Figure 2).

In our first patient, eGFR using the chronic kidney disease epidemiology collaboration formula was 96 mL/min/1.73m$^2$ at the time of transplantation. Before discontinuation of everolimus, laboratory investigations showed hemolytic anaemia (7.5 g/dL), thrombocytopenia platelet counts (145.500/μL), elevated serum creatinine (204 μmol/L) and reduced creatinine clearance (29.5 mL/min) and elevated serum-LDH (317.5 U/L). No schistocytes could be detected in peripheral blood smear after change in immunosuppression. Urine abnormalities could not be detected. For each individual, the time course is shown separately in Figure 3a–d.

Two of the five patients suffered from hypertension at diagnosis. All patients developed acute kidney injury and increased serum creatinine levels [median 75.5 μmol/L (range 59–94 μmol/L) and 140.0 μmol/L (range 98–148 μmol/L) before and 30 days after lung transplantation, respectively; $P < 0.05$]. Six months after lung transplantation, median creatinine levels were significantly increased to 202.0 μmol/L (range 181–256 μmol/L) ($P < 0.005$). At the time of the diagnosis, median calcineurin inhibitor levels were 122.5 ng/mL (range 39–261 ng/mL) with median everolimus levels of 6.65 ng/mL (range 3.5–19.6 ng/mL). After lung transplantation, median time to development of post-transplant HUS was 105 days (range 74–730 days). In one patient, post-transplant HUS was diagnosed 7 months after first signs of HUS were obvious. Two patients required dialysis treatment. Two patients recovered from HUS; two did not recover and remained dialysis dependent. One patient deceased 3 days after diagnosis of post-transplant HUS due to cardiovascular collapse and unsuccessful resuscitation. In two cases, renal function returned to normal baseline levels and hypertension was controlled by antihypertensive therapy.

In our first patient, intravenous corticosteroid pulse therapy (500 mg prednisolone for 3 days with following tapering scheme) was performed. The immunosuppressive regimen was switched to MMF under continuation of cyclosporine and prednisolone. Everolimus was discontinued. Subsequently, platelet counts increased and renal function improved within days (Figure 3a) but was not maintained during follow-up.

Discussion

This is the first case series of lung transplant recipients with post-transplant HUS treated with everolimus in combination with calcineurin inhibitors.

Drug-induced HUS represents 13% of all HUS cases [1]. It is a rare adverse reaction to calcineurin inhibitors and mTOR inhibitors, e.g. sirolimus. While the complete pathophysiological process, which leads to post-transplant HUS remains unknown, increased platelet aggregation and endothelial cell damage seem to be pivotal. Both are known side effects of calcineurin inhibitors. In several reports, cyclosporine and tacrolimus have been linked to post-transplant HUS [7, 13–17]. Furthermore, single cases of sirolimus-induced HUS or TTP have been reported [18–20]. Sirolimus-induced downregulation of vascular endothelial growth factor (VEGF) seems to be the most important step in this setting [21].
Monoclonal antibody against VEGF leads to profound thrombotic glomerular injury. Eremina et al. showed that the local deletion of VEGF in renal podocytes in adult mice resulted in a profound thrombotic glomerular injury. The injury seems to be a direct targeting of VEGF through bevacizumab [22].

Moreover, the combination of sirolimus and calcineurin inhibitors increases the risk for HUS or TTP through dual activities on endothelial cell death and repair [23].

To our knowledge, there are no reported everolimus-induced cases of post-transplant HUS in lung transplant recipients. In the EVTAC trial on haematopoietic stem cell transplantation, TMA due to combination of cyclosporine and everolimus has recently been described. Hachem et al. showed that the combination of cyclosporine and sirolimus causes post-transplant HUS in up to 14.7 cases/100 patient-years, which is the highest incident compared to other medications [18].

In nonrenal solid organ recipients, end-stage renal disease occurs in 10% within 10 years [24–27].

Most of these patients develop chronic kidney disease due to calcineurin inhibitor nephrotoxicity after transplantation. Lefaucheur et al. observed that among cystic fibrosis patients who underwent lung transplantation, nearly all had calcineurin inhibitor (CNI) toxicity (93.3%) and nearly half of all transplanted patients developed TMA (46.7%). In all cases of TMA, nephrotoxicity lesions typical for CNI were found. Interestingly, none of them developed thrombocytopaenia or signs of intravascular hemolysis.

This shows that deterioration of renal function without haematological signs underestimates post-transplant HUS in solid organ transplantation. Routine renal biopsies in lung transplant recipients might have revealed more cases of post-transplant HUS. If nephrologic consultation is made early, the diagnosis of the renal lesion and therapeutic options can save renal function [28].

It is noteworthy to mention that post-transplant HUS under mTOR inhibitors alone (without calcineurin inhibitors) has been reported. It seems that sirolimus is an independent risk factor for post-transplant HUS. In a cohort of renal transplant recipients in the United States, the

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**Table 1.** Clinical data, pretreatment and outcomes

<table>
<thead>
<tr>
<th>Patient/Age at LTx/ gender/underlying disease</th>
<th>Immunosuppressive regimen before TMA</th>
<th>Time from transplantation to occurrence</th>
<th>Treatment</th>
<th>Immunosuppressive treatment after post-transplant HUS</th>
</tr>
</thead>
<tbody>
<tr>
<td>145/f/emphysema</td>
<td>CsA, Ever, Pred</td>
<td>Day 120</td>
<td>Plasma exchange</td>
<td>CsA, Pred, MMF</td>
</tr>
<tr>
<td>236/m/alpha 1 antitrypsin deficiency</td>
<td>CsA, Ever, Pred</td>
<td>Day 74</td>
<td>Change of medication</td>
<td>Tac, Pred, MMF</td>
</tr>
<tr>
<td>342/m/emphysema</td>
<td>CsA, Ever, Pred</td>
<td>Day 90</td>
<td>Plasma exchange</td>
<td>CsA, Pred, MMF</td>
</tr>
<tr>
<td>454/m/fibrosis</td>
<td>CsA, Ever, Pred</td>
<td>Day 360</td>
<td>Change of medication</td>
<td>CsA, Pred, MMF</td>
</tr>
<tr>
<td>551/f/emphysema</td>
<td>CsA, Ever, Pred</td>
<td>Day 302</td>
<td>Plasma exchange</td>
<td>CsA, Pred, MMF</td>
</tr>
</tbody>
</table>

LTx, lung transplantation; CsA, cyclosporine; Ever, everolimus; Pred, prednisolone; Tac, tacrolimus; f, female; m, male. MMF, mycophenolate mofetil.
incidence of TMA was 4.9/1000 patient-years for de novo TMA. The highest risk for post-transplant HUS was within the first 3 months among younger recipients, female gender and initial use of sirolimus [29].

Everolimus is a novel derivative of sirolimus with immunosuppressive and antiproliferative properties and works similarly as an mTOR inhibitor. It is approved in Europe to be used as an immunosuppressive agent after heart and kidney transplantation and can be combined with corticosteroids alone or may be part of a triple therapy combined with calcineurin inhibitors and corticosteroids.

All our patients initially received an immunosuppressive triple therapy including cyclosporine, MMF and prednisolone without signs of post-transplant HUS. After 4 weeks, MMF was replaced by everolimus. At a mean of 2 months after switch from MMF, post-transplant HUS could be diagnosed by histology and/or laboratory findings.

After intravenous corticosteroid pulse therapy and courses of TPE as well as switching everolimus to MMF under continuation of cyclosporine and prednisolone, post-transplant HUS resolved quickly in 80% of the cases.

TPE was performed in all patients with a rather abrupt change in renal function or when subsequent renal biopsy showed TMA. All of those patients had at least a temporary improvement of renal function. The theory behind this is the removal of pathophysiologically important factors like angiopoietin-2 [30, 31]. In one patient, TPE was not performed due to the late diagnosis of the disease. In one patient, TPE was not performed because renal tissue showed signs of irreversible damage.

In contrast to our renal biopsy programme in renal transplant recipients [32], there is no uniform protocol to identify patients with other solid organ transplants for renal biopsy. Hence, the renal biopsies were performed at the discretion of the attending nephrologist.

In a study by Le Quintrec, 24 renal transplant recipients who developed de novo TMA were screened for CFH, MCP and CFI mutations to investigate whether there is an association between protein complement regulatory mutations and risk of de novo TMA post-transplantation. In this study, 67% patients who develop de novo TMA had CNI-based immunosuppressive regimens and the onset of the disease occurred during the first 3 months post-transplantation. The mean time between transplantation...
and onset of de novo TMA was 205 days (range 1 day to 6 years). Mutation of complement regulatory factors was found in 29% of these patients [33].

We postulate that the combination of everolimus and cyclosporine has a high potential to induce TMA. Increased target levels of calcineurin inhibitors early after lung transplantation seems to predispose patients to post-transplant HUS in patients treated with everolimus.

We postulate that the combination of everolimus with calcineurin inhibitors is more likely to trigger post-transplant HUS.

Even without haematological signs, a more liberal policy of renal biopsies can identify post-transplant HUS in patients with isolated renal dysfunction.

Patients should be closely monitored for CNI levels and haematological and renal disturbances and preemptive therapy started and mTOR discontinued in this situation.

Limitations

Our analysis has imminant limitations. Although the results were obtained within patients prospectively and randomly assigned to different immunosuppressive regimens, we did not perform a prospective study to analyse the occurrence of post-transplant HUS in lung transplant recipients. The sample size is limited, yet the number of lung transplant patients included in the analysis represents nearly half of the total number of lung recipients in Germany per year (126/270). Further, no screening for mutations of complement regulatory factors, such as CFH, MCP or CFH, was performed because the patients had no underlying renal disease before lung transplantation. As a last point, we do not have complete data on possible confounders such as data on parvovirus in all of our patients.

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