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Tacrolimus, a forgotten agent in kidney transplant leukopenia

Tacrolimus: agente esquecido na leucopenia pós-transplante renal

Pedro Azevedo[1], Cristina Freitas[1], Hugo Silva[1], Pedro Aguiar[1], Teresa Santos[1], João Cabral[2], Guilherme Rocha[1], Manuela Almeida[1], Sofia Pedroso[1], La Salete Martins[1], Leonídio Dias[1], António Castro Henriques[1], António Cabrita[1]

[1] Department of Nephrology, Centro Hospitalar do Porto – Santo António Hospital; Porto; Portugal
[2] Department of Urology, Centro Hospitalar do Porto – Santo António Hospital; Porto; Portugal

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ABSTRACT:

Leukopenia in kidney transplant patients is frequent, it causes potentially life-threatening complications, but it is often poorly characterized. Opportunistic infections, immunologic disturbances and drug-related toxicity are principal causes of single or multilineage cytopenias. Tacrolimus-induced leukopenia is a less recognized but frequent complication.

We describe one patient with leukopenia developing within seven months after renal transplant. After excluding other potential causes, tacrolimus was switched to cyclosporine, with recovery of white blood cell count.

Based on the clinical report, the authors reviewed causes of post-transplant leukopenia, focusing on the diagnostic investigation. Early diagnosis and interventions are fundamental to improve prognosis.

Key-Words: Leukopenia; renal transplantation, tacrolimus

RESUMO

A leucopenia no doente transplantado renal é comum, causa complicações com potencial risco de vida, mas é muitas vezes mal caracterizada. As infecções por agentes oportunistas, distúrbios imunológicos e toxicidade secundária a fármacos são as principais causas de citopenias de uma ou várias linhas. A leucopenia induzida pelo tacrolimus é uma complicação frequente, mas pouco reconhecida.

Descrevemos um doente com início de leucopenia sete meses pós-transplante renal. Após excluir outras potenciais causas, o tacrolimus foi substituído pela ciclosporina, com recuperação da contagem leucocitária.

Baseado no caso clínico, os autores fizeram uma revisão das causas de leucopenia pós-transplante e sua investigação. O diagnóstico e tratamento precoces são fundamentais na melhoria do prognóstico.

Palavras-Chave: Leucopenia, tacrolimus, transplante renal.

INTRODUCTION

Leukopenia refers to a low total white blood cell count and may be due either to lymphopenia and/or neutropenia. Most publications use leukopenia and neutropenia interchangeably, which may be a source of confusion[1]. Leukopenia is defined as leukocyte count inferior to 4.000/µL and neutropenia is defined as, at least, a neutrophil value ‹2000/µL[1].

The estimated incidence of leukopenia in kidney transplant recipients ranges from 10% to 55.5%, while neutropenia has been reported in between 4.9% and 37.5% of patients[2].

There are several factors that contribute to leukopenia after kidney transplantation. The most prevalent are drug toxicity, systemic infection and post-transplant lymphoproliferative disease[1]. In previous reports, the incidence of neutropenia in the first year post-transplant was significantly associated with mycophenolate mofetil (MMF)-tacrolimus (TAC) combination therapy[3].

Tacrolimus is a cornerstone therapy in preventing rejection in renal transplantation but has several side effects, such as new-onset post-transplant diabetes (8.4-9.8%)[4], hypertension (13-62%), hyperlipidemia (23%), neurotoxicity (7-32%)[5], electrolytic disturbances and nephrotoxicity[6].

Leukopenia related to the use of TAC is described in 13-48% of cases. Besides this high frequency, there are only few case-reports in the literature focusing the role of TAC as the culprit agent in leukopenia[3].

We report a case of post-transplant leukopenia in a renal transplant recipient under tacrolimus therapy, where tacrolimus seems to be the offending agent.

CASE REPORT

A 56-year-old man with end-stage renal failure due to IgA nephropathy underwent living donor renal transplantation, in May 2011. Initial immunosuppressive therapy was basiliximab, prednisolone, MMF and TAC. Both donor and recipient were CMV positive and no valganciclovir was used. There were no surgical or infectious complications, delayed graft function, neither acute rejection episodes. Peripheral blood count was normal and renal function remained stable at levels of plasmatic creatinine (Cr) of 1.2-1.4 mg/dL, for the first post-transplantation months. Maintenance therapy consisted in prednisolone, MMF, TAC, trimethoprim-sulphamethoxazol and ranitidine.

Seven months later, leukopenia (3760/µL) and neutropenia (1750/µL) were documented. Haemoglobin and platelet counts were normal and renal function remained stable (Cr: 1,23mg/dL).

Bacterial, fungal and viral infections [including herpes simplex virus (HSV), cytomegalovirus (CMV), Epstein-Barr virus (EBV), parvovirus B19 (PV B19), hepatitis virus and human immunodeficiency virus] were excluded. Peripheral blood immunophenotyping analysis revealed no abnormalities, particularly dysplastic changes or monoclonal proliferation, and bone marrow examination was normal.

Trimethoprim-sulphamethoxazol, MMF and ranitidine were discontinued as soon as leukopenia was confirmed, without improvement of leukocyte count.

Ten months after transplantation, leukopenia (2500/uL) and neutropenia (670/uL) progressively worsened and TAC was replaced by cyclosporine (CsA). After calcineurin-inhibitor conversion, white blood cell (WBC) count normalized. The patient remained asymptomatic with normal peripheral blood count. Two months later, MMF was reintroduced with persistence of normal WBC count.

DISCUSSION

Haematologic abnormalities are common in renal transplanted patients and can cause potentially life-threatening complications. Opportunistic (especially viral) infections, immunologic disturbances [such as haemophagocytic syndrome (HPS), graft versus host disease (GVHD), post-transplant lymphoproliferative disease (PTLD)] and drug-related toxicity are the main causes of single or multilineage cytopenias[1,7]. Cytomegalovirus, PV B19, EBV and HSV are common causes of infection-induced cytopenias in renal transplant recipients[1,7].

Cytomegalovirus is the most commonly recognized opportunistic pathogen, occurring in 20 to 60% of transplant recipients[8]. Cytomegalovirus seronegative recipients of seropositive grafts have the highest risk of recurrent, invasive and ganciclovir-resistant CMV infection[8]. Fever, constitutional symptoms, hepatitis, encephalitis, pneumonia and gastrointestinal bleeding are typical of CMV infection[9]. Leukopenia is present in as many as 20% of patients with active infection[9]. This virus causes myelosuppression by either directly infecting hematopoietic progenitor cells or stromal elements, interfering with the supportive microenvironment. Moreover, drugs used in prophylaxis, like valganciclovir, and treatment of CMV infection frequently cause leukopenia[10].

Parvovirus B19, which target specificity for human erythroid-lineage cells, infects the majority of humans and typically inhibits erytropoiesis[7]. Anaemia was the predominant laboratory abnormality (98.8%), but leukopenia (37.5 %) and thrombocytopenia (21%) were also reported by Albert *et al.* in their review of 91 transplanted patients (54% renal transplant patients) with PV B19 infection[11]. Bone marrow biopsy showing pathognomonic findings of giant pro-erythroblasts with prominent intranuclear inclusions confirm the diagnosis. Treatment consists in immunosuppressive dose reduction, red blood cell transfusion and, in the absence of clinical response, high-dose immunoglobulin[11].

Epstein Barr Virus (EBV) is a known causative agent of infectious mononucleosis, a usually benign disorder more prevalent in adolescents and young adults. The virus remains latent in B lymphocytes after primary infection[12]. Acute or recurrent infection may cause fever, organomegaly, lymphadenopathy and leukopenia. Treatment is based on immunosuppression reduction, ganciclovir and immunoglobulin[12].

Herpes simplex virus has a seroprevalence of 60% in the general population and an estimated incidence of 53% in renal transplant recipients[12]. Reactivation after transplantation is frequent but clinical disease is rare. Leukopenia is the most common haematologic manifestation of HSV infection, but other lines can be involved[7]. Oral or genital mucocutaneous lesions are typical of HSV infection. Other more serious systemic manifestations, such as HSV encephalitis are extremely rare, since introduction of acyclovir in the prophylaxis and treatment of HSV infection.

Our patient did not have fever, constitutional symptoms, organomegaly, lymphadenopathy or mucocutaneous lesions; microbiological examination was negative for CMV, PV B19, EBV, HSV acute infection, and bone marrow biopsy was normal. Opportunistic infections were excluded as potential causes for patient's leukopenia.

Acquired haemophagocytic syndrome (HPS) is a rare, aberrant immune response in reaction to a precipitating cause, such as opportunistic infections. It is a life-threatening systemic inflammatory disease in which there is haemophagocytosis by non-neoplastic macrophages in the bone marrow, liver, lymph nodes and spleen[13]. Karras *et al.* reported an incidence of 0.4% among RT patients with an average time of 52 days after transplant[13]. The clinical features include fever, hepatosplenomegaly, lymphadenopathy, skin rash, jaundice, dyspnoea, cachexia, and neurological dysfunction. The laboratory findings include pancytopenia, abnormal liver tests, elevated LDH and hypertriglyceridemia[14]. Bone marrow biopsy showing haemophagocytosis confirms the diagnosis. No specific therapy is recommended other than treatment of the precipitating cause and supportive care. The mortality rate is as high as 50%[14].

Graft versus host disease (GVHD) is caused by proliferation of allograft-associated lymphocytes in the immunosuppressed recipient, with subsequent immune-mediated attack by donor cells against host tissues. Usually, it occurs most frequently following small bowel and liver transplant, followed by lung and kidney transplantation. Graft versus host disease typically occurs with a median onset of 77 days after kidney transplant[7] and clinical presentation includes fever, rash, diarrhoea and pancytopenia[15]. Diagnosis is achieved by the demonstration of histological features of GVHD and confirmation of lymphocyte macrochimerism in the peripheral blood, marrow and affected tissues. In most reports, patients were treated by increasing immunosuppression and haematopoietic cytokines to support bone marrow function[15]. Prognosis is poor with reported mortality rates of 100% in lung, 75% in liver and 30% in other solid organ-transplant recipients[7].

As previously described EBV remains latent in B lymphocytes after primary infection. In the post-transplant period, immunosuppression provides expansion of B lymphocytes with increased risk of

post-transplant lymphoproliferative disorders[12]. Compared with other transplant populations, renal transplant recipients have the lowest risk of acquiring PTLD (1-3%), as they require lower dose of immunosuppression[7]. Post-transplant lymphoproliferative disease most commonly occurs in the first year post transplant and presents with fever, lymphadenopathy and haematologic manifestations. First-line treatment consists in the reduction or suspension of immunosuppressive therapy. Other therapeutic regimens, such as ganciclovir and interferon-alfa have shown no consistent results[16].

Immunologic disturbances were excluded in our patient because he was asymptomatic, with normal blood immunophenotyping and bone marrow histology. Bone marrow biopsy also excluded infiltrative diseases as other causes of leukopenia. Bone marrow aplasia and thrombotic microangiopathy (TMA) were less likely as the erythroid and megakaryocytic lineages have not been affected.

The diagnosis of drug-related cytopenias is difficult because there are no guidelines on management or specific tests to identify the culprit agent. If drug-related neutropenia is suspected, the diagnostic strategy involves discontinuing the most likely agent. This approach is not risk free, as it may trigger a rejection or an opportunistic infection.

MMF[18] and valganciclovir[10] are the most frequently drugs involved in leukopenia in renal transplant recipients. Antithymocyte globulin (ATG), rituximab, sirolimus, azathioprine and tacrolimus related leukopenia have been described[3,18]. Other non-immunossupessive drugs, such as trimethoprim/sulfamethoxazole, clopidogrel and ticlopidine are also relevant causes of pancytopenia in RT patients[19].

Mycophenolate mofetil-induced leukopenia was reported in 13-35% of patients[17]. The marrow effects of MMF are dose dependent and correlate with trough levels of the active metabolite, the mycophenolic acid. During lymphocyte proliferation, MMF reversibly and non-competitively inhibits the inosine monophosphate dehydrogenase, a rate-limiting enzyme for *de novo* purine synthesis and causes lymphopenia. The MMF effect is relatively selective to proliferating lymphocytes, but it is also associated with other reversible cytopenias, such as leukopenia and anaemia[7].

Co-administered agents with myelosuppressive effects, especially valganciclovir, increase the risk of leukopenia[10]. Leukopenia, usually reversible, is the most frequent side-effect of valganciclovir and is reported in 10-13% of cases[10]. Ganciclovir and valganciclovir can also lead to multilineage cytopenias or severe drug-related pancytopenia[7]. Kennedy *et al* suggest that the interaction of ganciclovir with MMF potentiates dysgranulopoiesis, contributing to leukopenia[20].

Sirolimus is an m-TOR inhibitor and causes leukopenia, anaemia and thrombocytopenia. Generally, these haematologic changes are dose-dependent and not clinically relevant. It seems that there is a direct anti-proliferative effect on bone marrow that explains leukopenia related to this drug[21].

Azathioprine-induced leukopenia is described in more than 50% of RT patients. After administration, azathioprine is rapidly converted to 6-mercaptopurine, which undergoes extensive metabolism and the end-products are excreted via urine[18]. Allopurinol inhibits the enzyme xantine-oxidase, a degradating enzyme of 6-mercaptopurine and increases azathioprine levels. Aditionally, renal dysfunction causes azathioprine end-products accumulation, with a higher risk of leukopenia[22].

Trimethoprim/sulfamethoxazole-associated cytopenias may be due to induction of folate deficiency, haemolysis in patients with unrecognized glucose-6-phosphate dehydrogenase deficiency or drug-induced haemolytic anaemia[7].

In our case, MMF, trimethoprim/sulfamethoxazole and ranitidine interruption did not lead to resolution of leukopenia and TAC-induced leukopenia was suspected.

Leukopenia exclusively linked with TAC is a common complication, described in 13-48% of cases, but it is rarely considered. The pathophysiologic mechanism has not been completely clarified and four mechanisms were proposed[3]. The best known mechanism involves pharmacokinetic interaction between TAC and MMF: TAC inhibits MMF glucoronidation and increases its bioavailability. Mycophenolate mofetil inhibits the rate-limiting enzyme of *de novo* purine synthesis during lymphocyte proliferation, causing leukopenia[7]. This may justify why,

in the *Symphony* trial, leukopenia was significantly more frequent with TAC-MMF than with CsA and MMF or sirolimus with MMF associations (13.4% *vs.* 10.2% *vs.* 10.3%, respectively)[5].

When immunosuppressive protocols include MMF-TAC and leukopenia develops, the reduction of the MMF dose is the rule and determination of mycophenolic acid levels may be indicated.

However, in our patient, leukopenia persisted after discontinuing MMF. A second mechanism[3] proposed that leukopenia is due to direct inhibition of myeloid cells by TAC, with a maturation arrest in myeloid precursor cells. Another hypothesis speculates the effect of TAC on mononuclear accessory cells, with production of cytokines causing greater death of neutrophils[3]. The last mechanism postulates the formation of autoantibodies against myeloid precursors of mature neutrophils, but such antibodies were not identified[3].

Independently of the pathophysiologic mechanism, in our case, conversion of TAC to CsA allowed the resolution of leukopenia and the reintroduction of MMF, with no use of granulocyte colony stimulating factor (G-CSF). There are no guidelines on the use of G-CSF for the treatment of leukopenia after RT, and G-CSF is associated with a reduction in time of agranulocytosis, antibiotic use and length of hospital stay, without side-effects, such as acute rejection[23]. Some authors suggest using C-GSF to allow maintenance of MMF in low dose, reducing the risk of acute rejection[1]. However, its use is not associated with reduction of mortality and definitive treatment of leukopenia requires a reduction or suspension of the culprit drug[23].

In a time where transplant programmes are increasing, our case may alert physicians to the need of systematic evaluation of cytopenias after transplantation. Attention to the time of onset must be paid because GVHD, HPS, and TMA are more likely to occur in the first few months and PTLD is most common in the first year after transplant. Drug-related cytopenia(s) should be considered and opportunistic infections should always be investigated as a potential precipitating cause of cytopenia(s), either as the direct agent of marrow suppression or as the trigger for HPS or immune cytopenia(s). Early investigation of pancytopenia by diagnostic bone marrow biopsy is warranted, because it is often the presenting symptom of life-threatening pathologies, such as GVHD, PTLD or unsuspected opportunistic infections, conditions that may have a better prognosis if early interventions are undertaken.

Conflict of interest statement: none declared

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Correspondence to:
Dr Pedro Azevedo
Department of Nephrology, Centro Hospitalar do Porto – Hospital de Santo António, Porto, Portugal
Largo Prof. Abel Salazar 4099 – 001 Porto
E-mail: pedronunesazevedo@gmail.com