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Authors: Opota O, Balmpouzis Z, Berutto C, Kaiser-Guignard J, Greub G, Aubert JD, Prod'hom G, Manuel O, Jaton K

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Visceral leishmaniasis in a lung transplant recipient: usefulness of highly sensitive real-time polymerase chain reaction for pre-emptive diagnosis

Onya Opota[1,a], Zisis Balmpouzis[2,a] , Carine Berutto[3], Julie Kaiser-Guignard[4], Gilbert Greub[1,5], John-David Aubert[2,3], Guy Prod'hom[1], Oriol Manuel[3,5,b], Katia Jaton[1b]

[1] Institute of Microbiology, University of Lausanne and University Hospital Center, Lausanne, Switzerland.

[2] Pneumology Service, University Hospital of Lausanne, Lausanne, Switzerland.

[3] Transplantation Center, University Hospital and University of Lausanne, Lausanne, Switzerland.

[4] Hematology Service, University Hospital and University of Lausanne, Lausanne, Switzerland.

[5] Infectious Diseases Service, University Hospital of Lausanne, Lausanne, Switzerland.

[a] O. O. and Z. B. contributed equally to this work.

[b] K. J. and O. M. contributed equally to this work.

Corresponding author:

Dr. Oriol Manuel,

Transplantation Center, University Hospital and University of Lausanne

Lausanne, Switzerland

0041 79 556 61 36

Oriol.Manuel@chuv.ch

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Short title: Leishmaniasis diagnosis in SOT

Abstract

We report the case of a lung transplant recipient in whom the diagnostic of visceral leishmaniasis (VL) was made by detection of parasites in a peripheral blood (PB) smear when the parasite load already reached 8.9 x 10^3 parasites per millilitres. We demonstrated that the VL diagnosis could have been done months before the development of symptoms by the use of *Leishmania*-specific real-time PCR, suggesting the usefulness a role of pre-emptive PCR-based diagnosis in transplant recipients at risk for VL.

Leishmaniasis is a severe vector borne-disease caused by a group of protozoan parasites of the genus *Leishmania*, transmitted to humans by the bite of infected female phlebotomine sandflies (1). There are 3 main forms of the disease: visceral leishmaniasis (VL), cutaneous leishmaniasis and mucocutaneous leishmaniasis. VL, the more severe form, is fatal if left untreated. An estimated 1.3 million new VL cases and 20,000 to 30,000 deaths occur annually with an increasing number of cases among immunocompromised patients (1-4). In solid-organ transplant (SOT) recipients, VL diagnosis is rare and often delayed; indeed 25% of VL final diagnostics may occur more than 1 month after symptoms onset (5). Polymerase chain reaction (PCR) for *Leishmania* has improved the initial diagnosis of VL both in immunocompromised and in immunocompetent patients, with a high sensitivity and specificity (70% - 100% and 95% - 100% respectively) (6). Additionally, highly sensitive specific real-time PCR allows the assessment of the response to treatment by monitoring the parasitic load in PB (7). However, the use of PCR for the assessment of *Leishmania* load in PB in asymptomatic immunocompromised patients at risk for reactivation has been insufficiently assessed. We report a case of a lung transplant recipient in whom early diagnosis of VL could have been done months before the development of symptoms by the use of *Leishmania* PCR, thus supporting the usefulness of this method for VL pre-emptive diagnosis.

Case report

A 61-year-old Swiss man underwent bilateral lung transplantation for idiopathic pulmonary fibrosis in June 2013. Additional pre-transplantation medical history included a past hepatitis B virus infection. After transplantation, the patient received the standard immunosuppressive regimen with oral prednisone, mycophenolate mofetil (MMF), and tacrolimus. In July 2014, he developed a persistent pancytopenia without any associated symptoms (unremarkable clinical examination, no hepatosplenomegaly and no lymphadenopathy). The initial tests excluded iron and vitamin deficiency. No splenomegaly was seen on the abdominal CT scan performed in January 2015. The PB smear showed no platelet aggregations, no evidence of microangiopathy and no schizocytes. A PCR assay performed in the PB was negative for

cytomegalovirus, Epstein-Barr virus, parvovirus B19, human-herpes virus 6 and hepatitis B virus. The serologic assays for hepatitis C virus (HCV) and human immunodeficiency virus (HIV) were negative. In April 2015, a bone marrow biopsy showed a normocellular bone marrow with hyperplasia of the erythroid lineage, a left shift in the granulocyte lineage, a normal percentage of blasts, a discrete lymphocytosis with a predominance of probably reactive T cells and no fibrosis. The cytological examination excluded a post-transplant lymphoproliferative disorder (PTLD) or a myelodysplastic syndrome. The microbiological analyses performed on the bone marrow remained all negative. Based on all these analyses a drug-induced pancytopenia was suspected and we decided to discontinue trimethoprim/sulfamethoxazole (used for *Pneumocystis* pneumonia prophylaxis) on September 2014 and later MMF on April 2015, and eventually replace tacrolimus by cyclosporine. Despite that, the pancytopenia worsened and a treatment with G-CSF stimulator was administered.

On June 2015 the examination of a PB smear showed an important number of macrophages and neutrophils containing intracellular organisms strongly evocating the amastigotes form of *Leishmania*. We also noticed a polyclonal hypergammaglobulinemia that was not present earlier. The definite diagnosis was made by real-time PCR detection of *Leishmania* DNA - 8.9×10^7 kinetoplastic DNA copies/ml - in PB, which corresponded to 8.9×10^3 parasites per ml, as the PCR targets the kinetoplastic DNA present in about 10,000 copies per parasite (Figure 1) (8). By PCR-restriction fragment length polymorphism (RFLP) based on the rRNA miniexon sequence (9) we could not distinguish between *L. infantum* and *L. donovani*. Treatment of liposomal amphotericin B was initiated at 3mg/kg per day intravenously for 5 days followed by another 6 doses of 4mg/kg once a week. Clinical and biological evolution was favourable with rapid improvement in total blood count and absence of parasite detection in PB approximately 3 weeks after amphotericin B introduction (Figure 1). A monthly follow-up by PCR did not detect any parasite DNA at 6 months after discontinuation of amphotericin B. MMF and trimethoprim/sulfamethoxazole were reintroduced on July 2015 and September 2015, respectively and no relapse of pancytopenia was observed.

We intended to determine the time of the initial infection based on travel history (the patient reported a trip to Greece July 2014, 13 months after transplant) and PCR on previous samples. The retrospective examination of the myelogram obtained 5 weeks before the diagnosis of VL revealed the presence of some parasites and the *Leishmania* PCR was positive with 7.2 x 10^5 kinetoplastic DNA copies/ml (Figure 1). As DNA extracted from the patient's blood samples collected after transplantation were available since the patient was routinely followed-up at the transplantation clinic of our hospital in a regular basis, we decided to retrospectively test them using the *Leishmania* specific PCR. Interestingly, the *Leishmania* PCR was already positive in samples collected more than 18 months before the first diagnosis, at a time were the patient was fully asymptomatic (Figure 1). However, our first appreciation that the infection was contracted during his trip in Greece was ruled out. We thus hypothesize that the infection occurred some years before transplantation, a period during which the patient reported several travels in endemic areas: Morocco in 2000, 3-4 trips to the south of France the last in 2007, Turkey in 2010, Andalusia in 2011, and Crete in 2012. The serology for *Leishmania* performed retrospectively on sera collected at the time of transplantation was positive for the recipient and negative for the donor, thus excluding a donor-derived infection.

Discussion

The diagnosis of VL in SOT recipients is challenging even in endemic areas; Clemente *et al.* reported that only one third of the patients presented the classic triad (fever, visceromegaly and pancytopenia) at time of diagnosis (5). VL is a rare opportunistic infection SOTs but should be included in the differential diagnosis of fever of unknown origin and/or pancytopenia. Two cases of VL in SOT recipients living in Switzerland have already been reported; a kidney transplant recipient after holidays in Spain and Tunisia (10) and a liver transplant recipient following a trip to Turkey (11). So far, only 7 cases have been reported in lung transplant recipients (3, 5). Noteworthy, because patients may be asymptomatic for long periods of time, a travel history in an endemic area may be easily overlooked. Our analysis demonstrates

that VL diagnostic could have been made up to 18 months earlier in the present case through the detection of *Leishmania* DNA using a highly sensitive *Leishmania* specific real-time PCR (5, 8). In particular PCR targeting kinetoplastic DNA which is present in up to 10'000 copies per parasite would allow a very early detection of infection in PB, a precise monitoring of the parasite burden and can be also used for the monitoring of the response to therapy including the detection of episodes of relapse. We thus recommend including PCR as a rapid and non-invasive screening method for VL diagnosis in at risk's SOT recipients.

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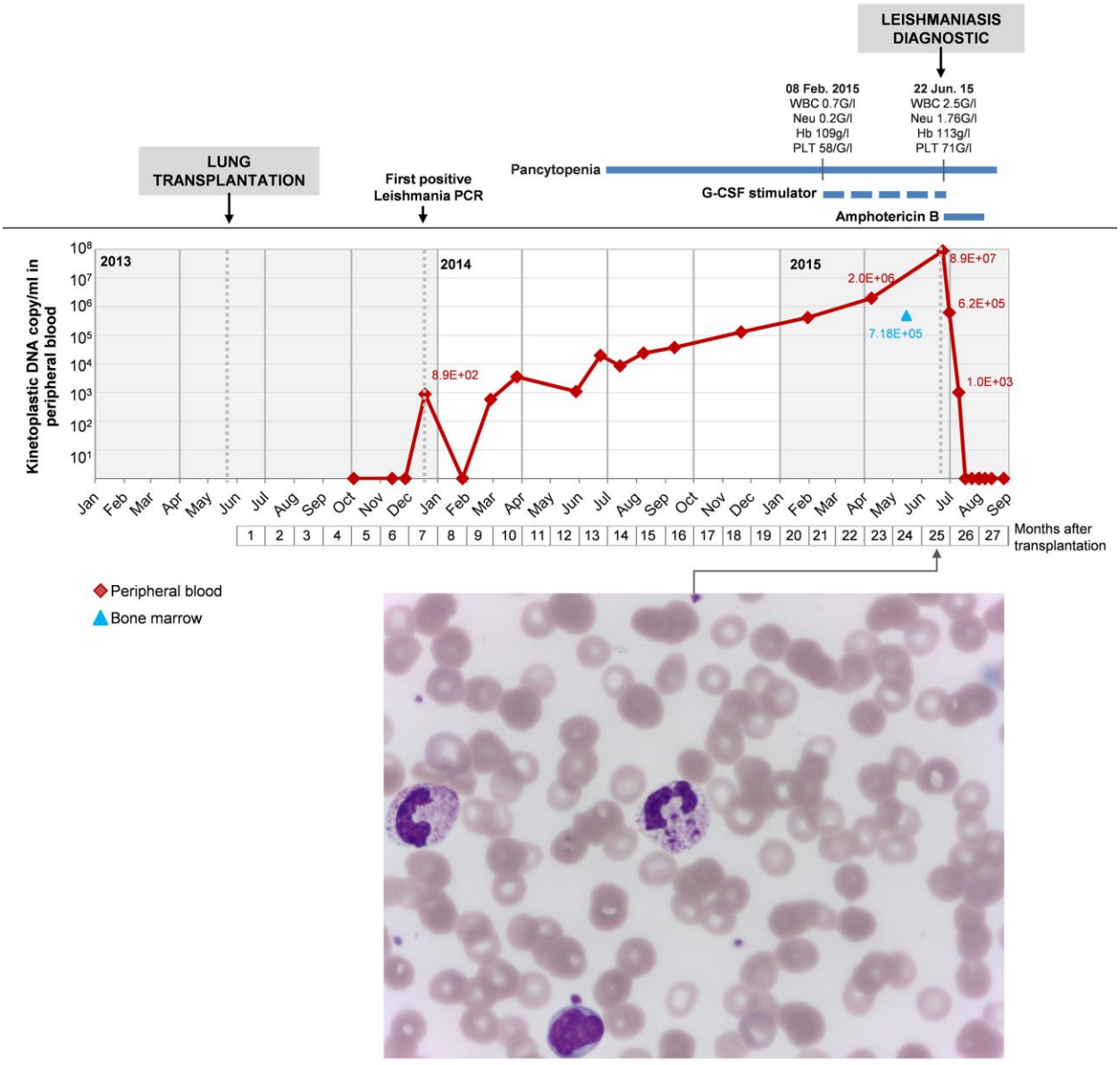
Figure 1: Kinetic of Leishmania DNA detection in peripheral blood and bone marrow using a specific real-time PCR prior and after the initial diagnostic of visceral leishmaniasis.

The main graphic represents Leishmania detection using a specific real-time PCR targeting a sequence of the kinetoplastic DNA. On June 22th 2015, a thin blood smear stained with Giemsa revealed several leucocytes infected with Leishmania sp. amastigotes. The diagnostic of leishmaniasis was made by real-time PCR detection of *Leishmania* DNA on this sample, 8.9 x 10^7 kinetoplastic DNA copies/ml, which corresponded to 8.9 x 10^3 parasites per ml as the PCR targets the kinetoplastic DNA present in up to 10,000 copies per parasite. The PCR performed retrospectively on a bone marrow puncture sampled in May 2015 was positive with 7.2 x 10^5 kinetoplastic DNA copies/ml. DNA from peripheral blood samples collected at the transplantation clinic of our hospital were retrospectively tested in our molecular diagnostic laboratory and revealed that the first positive PCR occurred in sample collected the 18th December 2013 (890 kinetoplastic DNA copies/ml), long before the beginning of the pancytopenia, which started in July 2014.


LEISHMANIASIS DIAGNOSTIC
08 Feb. 2015
WBC 0.7G/l
Neu 0.2G/l
Hb 109g/l
PLT 58/G/l
22 Jun. 15
WBC 2.5G/l
Neu 1.76G/l
Hb 113g/l
PLT 71G/l
LUNG TRANSPLANTATION
First positive Leishmania PCR
Pancytopenia
G-CSF stimulator
Amphotericin B
Kinetoplastic DNA copy/ml in peripheral blood
2013
2014
2015
8.9E+02
2.0E+06
8.9E+07
6.2E+05
7.18E+05
1.0E+03
Months after transplantation
Peripheral blood
Bone marrow

170