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

3

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- 22 **Key words:** Leishmaniasis, lung transplant, pre-emptive diagnosis, real-time polymerase chain reaction,
23 kinetoplastic DNA, solid organ transplant.
- 24 **Short title:** Leishmaniasis diagnosis in SOT

25 **Abstract**

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

32

33 Leishmaniasis is a severe vector borne-disease caused by a group of protozoan parasites of the genus
34 *Leishmania*, transmitted to humans by the bite of infected female phlebotomine sandflies (1). There are 3
35 main forms of the disease: visceral leishmaniasis (VL), cutaneous leishmaniasis and mucocutaneous
36 leishmaniasis. VL, the more severe form, is fatal if left untreated. An estimated 1.3 million new VL cases
37 and 20,000 to 30,000 deaths occur annually with an increasing number of cases among
38 immunocompromised patients (1-4). In solid-organ transplant (SOT) recipients, VL diagnosis is rare and
39 often delayed; indeed 25% of VL final diagnostics may occur more than 1 month after symptoms onset
40 (5). Polymerase chain reaction (PCR) for *Leishmania* has improved the initial diagnosis of VL both in
41 immunocompromised and in immunocompetent patients, with a high sensitivity and specificity (70% -
42 100% and 95% - 100% respectively) (6). Additionally, highly sensitive specific real-time PCR allows the
43 assessment of the response to treatment by monitoring the parasitic load in PB (7). However, the use of
44 PCR for the assessment of *Leishmania* load in PB in asymptomatic immunocompromised patients at risk
45 for reactivation has been insufficiently assessed. We report a case of a lung transplant recipient in whom
46 early diagnosis of VL could have been done months before the development of symptoms by the use of
47 *Leishmania* PCR, thus supporting the usefulness of this method for VL pre-emptive diagnosis.

48

49 **Case report**

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

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

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

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

98

99 **Discussion**

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

108 that VL diagnostic could have been made up to 18 months earlier in the present case through the detection
109 of *Leishmania* DNA using a highly sensitive *Leishmania* specific real-time PCR (5, 8). In particular PCR
110 targeting kinetoplastic DNA which is present in up to 10⁷000 copies per parasite would allow a very early
111 detection of infection in PB, a precise monitoring of the parasite burden and can be also used for the
112 monitoring of the response to therapy including the detection of episodes of relapse. We thus recommend
113 including PCR as a rapid and non-invasive screening method for VL diagnosis in at risk's SOT recipients.

114

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155

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

158 The main graphic represents Leishmania detection using a specific real-time PCR targeting a sequence of
159 the kinetoplastic DNA. On June 22th 2015, a thin blood smear stained with Giemsa revealed several
160 leucocytes infected with Leishmania sp. amastigotes. The diagnostic of leishmaniasis was made by real-
161 time PCR detection of *Leishmania* DNA on this sample, 8.9×10^7 kinetoplastic DNA copies/ml, which
162 corresponded to 8.9×10^3 parasites per ml as the PCR targets the kinetoplastic DNA present in up to
163 10,000 copies per parasite. The PCR performed retrospectively on a bone marrow puncture sampled in
164 May 2015 was positive with 7.2×10^5 kinetoplastic DNA copies/ml. DNA from peripheral blood samples
165 collected at the transplantation clinic of our hospital were retrospectively tested in our molecular
166 diagnostic laboratory and revealed that the first positive PCR occurred in sample collected the 18th
167 December 2013 (890 kinetoplastic DNA copies/ml), long before the beginning of the pancytopenia,
168 which started in July 2014.

169

