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Short Communication

Identification of asymptomatic *Leishmania* infection in patients undergoing kidney transplant using multiple tests

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ABSTRACT

Objectives: In immunocompromised patients, asymptomatic *Leishmania* infection can reactivate, and evolve to severe disease. To date, no test is considered the gold standard for the identification of asymptomatic *Leishmania* infection. A combination of methods was employed to screen for *Leishmania* infection in patients undergoing kidney transplant (KT).

Methods: We employed polymerase chain reaction for the detection of parasitic DNA in peripheral blood, Western blot to identify serum immunoglobulin G and whole blood assay to detect cytokines/chemokines after stimulation of whole blood with parasitic antigen.

Results: One-hundred twenty patients residing in Italy were included in the study at the time of KT. Each patient that tested positive to at least one test was considered as *Leishmania* positive. Fifty out of 120 patients (42%) tested positive for one or more tests. The detection of specific cell-mediated response (32/111, 29%) was the most common marker of *Leishmania* infection, followed by a positive serology (24/120, 20%). Four patients (3%) harbored parasitic DNA in the blood.

Conclusion: Our findings underline the high prevalence of asymptomatic *Leishmania* infection in patients undergoing KT in Italy, who are potentially at-risk for parasite reactivation and can benefit from an increased vigilance. Understanding the clinical relevance of these findings deserves further studies.

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Introduction

Visceral leishmaniasis (VL) is a systemic infection caused by vector-borne protozoans; in southern Europe VL is caused by parasites belonging to the *Leishmania infantum* species [1]. Immunocompromised individuals, including transplant recipients, are at

risk for severe VL with high rate of relapses [2]. In transplant recipients, asymptomatic *Leishmania* infection can reactivate during the immunosuppressive therapy and promote the resurgence of clinically evident leishmaniasis; thus, screening asymptotically infected patients could be beneficial. To date, no test is considered the gold standard for the identification of asymptomatic *Leishmania* infection, as serological and molecular tests suffer from sub-optimal sensitivity [3]. In recent years, a whole blood assay (WBA) has been validated to assess the anti-leishmanial cell-mediated im-

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Table 1Rates of asymptomatic *Leishmania* infection among kidney transplant candidates, as evaluated by WB, real-time PCR and WBA.

Variables (No. of individuals)	Total pos ^a No. (%)	WB pos no. ^b (%)	PCR pos No. (%)	WBA pos No. (%)	P-value ^c
Total (120)	50 (41.7%)	24 (20.0%)	4 (3.3%)	32 (28.8%)	–
Sex					
Male (77)	33 (42.9%)	15 (19.5%)	2 (2.6%)	23 (29.9%)	0.723
Female (43)	17 (39.5%)	9 (20.9%)	2 (4.7%)	9 (20.9%)	

^a One-hundred twenty patients were screened by WB and PCR, 111 were screened by WBA. ^b 14 patients (58.3%) showed positivity to the p14 protein band only, seven (29.2%) to the p16 band only, and three (12.5%) to both bands. ^c χ^2 test was used to calculate P-value. No., number of individuals; PCR, polymerase chain reaction; Pos, positive; WB, Western blot; WBA, whole blood assay.

munity, by measuring cytokine/chemokine levels in plasma from blood cells stimulated with parasitic antigens [4,5].

In this study, a combination of methods was employed to screen for asymptomatic *Leishmania* infection in patients undergoing kidney transplant (KT); we carried out WBA to assess the parasitic cell-mediated immunity, Western blot (WB) to detect *Leishmania*-specific antibodies, and real-time polymerase chain reaction (PCR) to identify *Leishmania* kinetoplast (k)DNA.

Patients and methods

Between July 2019 and June 2021, KT recipients were enrolled at the Nephrology, Dialysis and Renal Transplant Unit (University Hospital of Bologna, Italy) and at the Nephrology, Dialysis, Transplantation Unit (University Hospital of Florence, Italy). Inclusion criteria were age ≥ 18 years, at least 2 years of residence in Italy, receiving KT, and no past VL. Blood samples were collected at the time of the transplant and processed within 24 hours.

An asymptotically, or latently infected individual with *Leishmania* is defined as someone from an endemic area that exhibits an immune response (either humoral or T-cell mediated) against *Leishmania* or has parasites or parasitic DNA in the blood but remains healthy [3]. Thus, serological screening was performed by employing the WB immunoglobulin (Ig)G kit from LDBIO Diagnostics® (Lyon, France) that detects antibodies against *L. infantum* antigens of 14 and 16 kDa (p14 and p16), shown to be highly specific for *Leishmania* infection [6]. The nucleic acids were extracted from whole blood by employing the PROMEGA Maxwell® 16 LEV Blood DNA kit on the Maxwell® 16 instrument (PROMEGA®, Madison, Wisconsin, USA) and real-time PCR was carried out targeting the minicircles of kDNA as reported [6]. WBA was performed by employing the Cytometric Bead Array Human Soluble Protein Flex Set (Becton Dickinson, Franklin Lakes, NJ, USA) and the BD FAC-SCanto® cytofluorometer (Becton Dickinson) as described [4–6].

Results

A total of 120 patients were enrolled in the study at the time of transplant. Clinical and demographic characteristics of the patients are reported in Table S1. Blood samples were screened for *Leishmania* infection; 24 out of 120 patients (20.0%) tested positive for specific IgG using WB (Table 1). Real-time PCR for *Leishmania* kDNA was positive in four patients (3.3%); three cases exhibited low parasitemia (<1 equivalent parasites/ml), while one patient presented a higher parasite count in blood (140 equivalent parasites/ml); a 30-month follow up of the last patient showed no development of VL. The WBA was performed on 111 out of 120 patients; 32 patients (28.8%) tested positive to interleukin (IL)-2 and/or IP-10 (Figure S1). Of these, 20 patients (62.5%) tested positive for IL-2 and 26 patients (81.3%) tested positive for IP-10; 14 patients (43.8%) tested positive for both soluble mediators.

Based on the above-mentioned definition of asymptomatic *Leishmania* infection, we considered as positive each individual that tested positive to at least one of the three tests; 50/120 (41.7%)

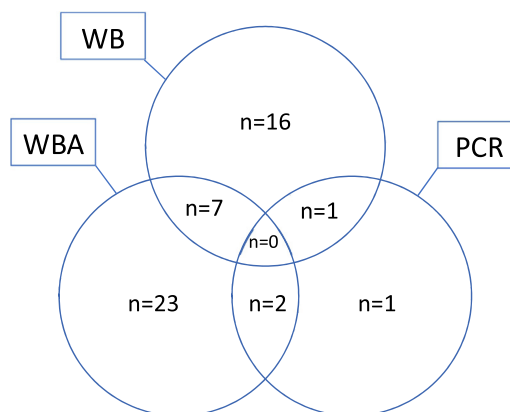


Figure 1. Venn diagram showing the agreement among PCR, WBA and WB in $n = 50$ transplant candidates that tested positive to at least one of the three methods. The overlap between the positive result/s in two or more tests is represented by shared regions in the diagram. PCR, polymerase chain reaction; WB, Western blot; WBA, whole blood assay.

patients had a positive result for at least one test (Table 1) with no difference of sex, age, origin, and underlying diseases between the *Leishmania*-positive and negative patients (Table S1). By evaluating the sensitivity of each test or test combination in relation to the operational definition of asymptomatic *Leishmania* infection, we observed that WBA exhibited the highest sensitivity as a single test (65.3%) and WB plus WBA was the best test combination with a sensitivity of 98.0% to detect asymptotically infected individuals (Table S2).

Seven out of 50 patients were positive for both WB and WBA, while only two patients and one patient were positive for WBA and PCR or WB and PCR, respectively; none was simultaneously positive for the three tests (Figure 1). Concordance between tests was evaluated; each test was in slight agreement with the others (Cohen's kappa coefficient of 0.02 for PCR/WBA and PCR/WB; 0.05 for WBA/WB). A weak correlation between tests was found by Pearson correlation coefficient (0.12 for PCR/WBA, 0.02 for PCR/WB and 0.03 for WBA/WB).

Discussion

To date, only few studies have been conducted to screen for *Leishmania* infection in KT candidates; a high prevalence (32%) of antibody against this parasitic infection has been reported in hemodialyzed patients in a VL endemic area of Brazil [7]. In contrast, we previously found a lower prevalence (16%) of asymptomatic *Leishmania* infection in 119 end stage renal disease patients in dialysis treatment [8]; the addition of a cell-mediated immunity assay (WBA) in the current study provides a boost in sensitivity, which greatly benefits the purpose of the screening.

In this study, WBA exhibited the highest sensitivity as single test (65%) and WB plus WBA was the best test combination with a sensitivity of 98% to detect asymptomatic *Leishmania* infection.

Nevertheless, WB and WBA were positive on two mostly separate groups of patients, with only seven subjects (14% of *Leishmania*-positive patients) showing positivity to both tests. This is in contrast with our findings in 145 individuals living in a *L. infantum*-endemic area, where a strong correlation and substantial agreement was observed between WB and WBA [6]. As a possible explanation, specific immune response against the parasite could have been impaired in patients undergoing KT, generating false negative results in WB and/or WBA and leading to a lower concordance between the two immunological tests in KT patients as compared to immunocompetent individuals. Divergent results obtained with WB and WBA may also be associated with the fact that *Leishmania*-specific antibody levels tend to fall away over time, whereas cell-mediated immunity is commonly retained for several years [9].

The detection of specific cell-mediated response was the most sensitive marker to detect *Leishmania* infection in patients undergoing KT and should be considered a valuable tool to identify latent leishmaniasis in this patient's group; the specific cell-mediated response against *Leishmania* was mainly detected by an increase of IP-10 (81% of cases). Our data are in line with recent findings in solid organ transplant patients [5] and suggest that a WBA panel for screening of *Leishmania* infection in KT candidates should include IP-10.

Only four individuals (3.4%) harbored parasitic DNA in the blood, one patient was positive to PCR only. This is consistent with earlier studies from southern Europe, which display the presence of *Leishmania* DNA in the blood in 0–22.0% asymptomatic immunocompetent individuals as reviewed in the study by Ortalli et al. [10]. Previous evidence has also shown discordant results between molecular tests and immunological methods to identify asymptomatic *Leishmania* infection [6].

Screening in transplant recipients is generally not recommended due to the lack of evidence about its benefits; nevertheless, a *Leishmania*-seropositive at the time of transplant would warrant close monitoring [11]. Increasing sensitivity of tests is crucial in a screening context, where the exclusion of a potentially at-risk individual is more harmful than the erroneous inclusion of a patient with no risk factors; as prophylaxis is not recommended for asymptomatic *Leishmania* infection in immunocompromised individuals [11], these patients are not exposed to the additional risk of anti-leishmanial therapy, but instead benefit from an increased vigilance.

In conclusion, we observed that 50 out of 120 patients (42%) undergoing transplant tested positive to one or more screening tests for *Leishmania* infection. Our findings indicate that asymptomatic *Leishmania* infection is highly prevalent in KT recipients in Italy and that WBA plus WB is the best test combination to detect asymptomatic leishmaniasis in this cohort of patients. However follow-up studies are needed to clarify the clinical relevance of these findings, in order to evaluate the reactivation rate in *Leishmania*-positive individuals as well as to identify biomarkers to predict clinical reactivation in asymptotically infected patients undergoing KT.

Declarations of competing interest

The authors have no competing interests to declare.

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Ethical approval

This study was approved by the Ethics Committee of CE-AVEC on 18/04/2018 (study no. 144/2018/Sper/AOUBO) and conducted in accordance with the principles of the Declaration of Helsinki on research in humans. All subjects agreed to participate by providing written informed consent.

Author contributions

Conception and design of the study: SV, AMDP, EC; Acquisition of data: AD, AMDP, MO, EB, FF, MB, LC, GC; Analysis and interpretation of data: AD, AMDP, MP, EB, GLM, LZ, AB, AVIM, EC, SV; Writing—original draft preparation: AD and SV; Writing—review and editing: AMDP, MO, EB MP, FF, MB, GLM, LZ, AB, LC, AVIM, EC, GC.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2023.11.012.

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