repiSalud
Repositorio Institucional en Salud

This is the peer reviewed version of the following article:

Carrasco-Antón, Nerea; Ibarra-Meneses, Ana Victoria; Carrillo, Eugenia; Fernández-Ruiz, Mario; Hernández-Jiménez, Pilar; Aguado, José María; Moreno, Javier; López-Medrano, Francisco. **An exploratory analysis of C-X-C motif chemokine ligand 10 as a new biomarker of asymptomatic Leishmania infantum infection in solid-organ transplant recipients**. J Infect. 2022 Apr;84(4):573-578.

which has been published in final form at:

https://doi.org/10.1016/j.jinf.2022.01.029

Title page

Title: An exploratory analysis of C-X-C motif chemokine ligand 10 as a new biomarker of asymptomatic *Leishmania infantum* infection in Solid-Organ Transplant Recipients.

Running title: CXCL10 in *Leishmania* infection of SOT recipients

Authors:

Nerea Carrasco-Antón[1a], Ana Victoria Ibarra-Meneses[1b], Eugenia Carrillo[b]*, Mario Fernández-Ruiz[a], Pilar Hernández-Jiménez[a], José María Aguado[a], Javier Moreno[2b], Francisco López-Medrano[2b]

[a]Unit of Infectious Diseases, University Hospital 12 de Octubre, Instituto de Investigación Hospital 12 de Octubre (imas12), Department of Medicine, School of Medicine, Universidad Complutense, Madrid, Spain. CIBERINFEC

[b]WHO Collaborating Centre for Leishmaniasis, Spanish National Center for Microbiology, Instituto de Salud Carlos III, Majadahonda, Spain. CIBERINFEC

[1]These first authors contributed equally to this article.

[2]These senior authors contributed equally to this article.

Nerea Carrasco-Antón. E-mail: nereacarrascoanton@gmail.com

Ana V. Ibarra-Meneses. E-mail: ana.ibarrameneses@gmail.com

Eugenia Carrillo. E-mail: ecarrillo@isciii.es

Mario Fernández-Ruiz. E-mail: mario_fdezruiz@yahoo.es

Pilar Hernández-Jiménez. E-mail: pilihj@hotmail.com

José M. Aguado. E-mail: jaguadog1@gmail.com

Javier Moreno. E-mail: javier.moreno@isciii.es

Francisco López-Medrano. E-mail: flmedrano@yahoo.es

Corresponding author contact information: Eugenia Carrillo, WHO Collaborating Centre for Leishmaniasis, Spanish National Center for Microbiology, Instituto de Salud Carlos III, Majadahonda, Spain. E-mail: ecarrillo@isciii.es.

Footnote Page

Current affiliations and addresses for authors whose affiliations have changed since completion of the study:

[1a]**Nerea Carrasco-Anton.** Internal Medicine-Sepsis Unit, IIS-Fundación Jiménez Díaz, UAM, Av. Reyes Católicos 2, 28040, Madrid, Spain.

[1b]**Ana V. Ibarra-Meneses.** Département de Pathologie et Microbiologie, Faculté de Médecine Vétérinaire, Université de Montréal, Canada; Research Group on Infectious Diseases in Animal Production (GREMIP), Faculty of Veterinary Medicine, University of Montreal, Canada.

Abstract—175 words

Objective: Sensitive and less laborious assays are needed to detect asymptomatic *Leishmania* among solid organ transplant (SOT) recipients. Using SLA-stimulated plasma from SOT recipients living where an outbreak of *Leishmania infantum* occurred, we examined potential biomarkers to identify asymptomatic *Leishmania* infections. Methods: Concentrations of cytokines/chemokines in plasma from whole blood stimulated with specific *Leishmania* antigen (SLA) were compared against infection status as determined by a currently used cell proliferation assay.

Results: Twenty-six percent (13/50) of the SOT recipients had a cell proliferation assay (CPA) indicating asymptomatic infection, and showed higher processed plasma C-X-C motif chemokine ligand 10 (CXCL10 or IP-10) concentrations than did non-infected subjects (median 2272.0 pg/ml [IQR-1570-2772] *vs*. 18.2 pg/ml [IQR 1-150.1]; $p<0.0001$). CXCL10 showed a sensitivity of 93% and a specificity of 95% compared to CPA. In addition, we demonstrated that the number of asymptomatic infections detected using CXCL10, decreased with distance from a park at the centre of the mentioned outbreak.

Conclusion: CXCL10 in plasma from SLA-stimulated blood could be a robust biomarker of asymptomatic *L. infantum* infection in solid organ transplant recipients.

Keywords: Solid-Organ Transplant recipients, *Leishmania*, leishmaniasis, biomarkers, asymptomatic infection, IP-10, CXCL10.

Text -2319 words-

Introduction

Visceral leishmaniasis (VL) is a potentially serious complication for recipients of solid organ transplants (SOT). The mortality attributable to such infection is thought to be around 3% and the relapse rate may exceed 25% (1, 2). After infection, progression to clinical VL depends on the balance between multiple factors that promote or prevent the multiplication and expansion of parasites in the body. The complexity of the response makes it difficult to predict the outcome of the infection, but it is known that most people infected with *Leishmania* remain asymptomatic (3). Up to date, there are only three relevant well-driven works studying the prevalence of asymptomatic leishmaniasis in SOT recipients (4), who can be at risk of developing clinical VL. Nowadays, there is not a formal recommendation for the treatment of SOT recipients with an asymptomatic *Leishmania* infection, although the beneficial impact of antiparasitic is widely discussed. There is no single universally accepted assay to identify asymptomatic infection. Conventional serological tests for leishmaniasis show limited sensitivity when used with immunocompromised patients (5). The detection of *Leishmania*-specific cell-mediated immunity, however, may offer a more accurate assessment, even indicating the prevalence of asymptomatic infection (asymptomatic subjects are those from an endemic area of VL with a detectable immune response, or parasitaemia, in the absence of signs or symptoms of active disease) (3, 5, 6). The Leishmanin Skin Test (LST) provides for such detection, and it has been used to study the prevalence of *Leishmania* infection in the field (7-9). However, its associated side effects, and the failure of its makers to follow the principles of good manufacturing practice, have caused its use in some countries to be abandoned. The cell proliferation assay (CPA), performed with peripheral blood mononuclear cell (PBMC) cultures stimulated with soluble *L. infantum* antigen (SLA),

can be used instead (10). However, while this can confirm asymptomatic *Leishmania* infection in SOT recipients, it is laborious and time-consuming (11). A further alternative is the whole blood stimulation assay (WBA), an easy, rapid test that can be used to monitor SOT recipients treated for VL, and for detecting asymptomatic *Leishmania* infection (12, 13). However, it relies on interferon-γ (IFN-γ) as a marker, and more sensitive and specific markers have recently been described (11). These include the Interferon-γ-induced protein 10 (IP-10 or CXCL10), the monokine induced by IFN-γ (MIG or CXCL9), and monocyte chemotactic protein 1 (MCP-1 or CCL2), all of which are produced at much higher concentrations in plasma from SLA-stimulated whole blood - at least that of immunocompetent patients (14-17). It is possible that they may also be useful for detecting asymptomatic infection in SOT recipients, who are of course immunosuppressed. The present work examines the chemokine profile of plasma from SLA-stimulated whole blood as a means of identifying asymptomatic *Leishmania* infection in SOT recipients.

In earlier work, it was found that symptomatic cases of VL among members of the general population, and among SOT recipients, were more numerous with increasing proximity to the semi-urban park at the centre of the 2009 outbreak in Fuenlabrada (Madrid, Spain) (18). The present work examines whether this relationship also holds true for SOT recipients with asymptomatic *Leishmania* infection as identified using a proposed test based on CXCL10 as a marker.

Methods

Ethics statement

This study was approved by the institutional Ethics Committee of the *Hospital Universitario 12 de Octubre*. All subjects gave their written, informed consent to be included.

Study design and setting

The study population included 50 adult patients (≥18 years of age) who had undergone SOT (kidney, liver or heart) at the *Hospital Universitario 12 de Octubre* (Madrid, Spain) (the reference centre for SOT in the Madrid Region's southwest) between 2005 and 2013, and whose usual place of residence was Fuenlabrada (where the above-mentioned outbreak occurred). Sample collection was performed between October 2012 and October 2013. None of these subjects had any symptoms of leishmaniasis or previous history of the disease.

Immunosuppression and prophylaxis regimens

Those SOT recipients at high risk of graft rejection received induction therapy with rabbit anti-thymocyte globulin (rATG) for 1-3 days after transplantation. Basiliximab was also administered to those at high risk of calcineurin inhibitor-related nephrotoxicity. A maintenance immunosuppressive regimen was followed, based on tacrolimus (0.1 mg/kg daily), mycophenolate mofetil (500-1000 mg twice daily) or mycophenolic acid (360 mg twice daily), and prednisone (0.5 mg/kg daily with tapering off after the first month post-transplantation). Supplementary Table 1 shows the treatment received by each patient.

Preparation of specific *Leishmania* antigen

Antigen extract from promastigote stationary phase parasite cultures (JPC strain, MCAN/ES/98/LLM-722) was used for the preparation of SLA for stimulation purposes as described by Aleka *et al* (19).

Laboratory analysis

Routine hematological parameters were determined.

144 **Cell proliferation assay to detect asymptomatic subjects**

145 All blood samples were subjected to CPA within 24 h of collection to determine which
146 of the SOT recipients had an asymptomatic infection. Peripheral blood mononuclear cells
147 were isolated from whole blood and resuspended in RPMI-1640 supplemented with 10%
148 fetal calf serum, and cultivated with SLA for 5 days (20). The lymphoproliferative
149 response of each subject was then determined by bromodeoxyuridine incorporation using
150 the Cell Proliferation Kit (GE Healthcare Life Sciences, UK), following the
151 manufacturer's instructions. Results were expressed in the form of a stimulation index
152 (absorbance of stimulated cells/unstimulated cells).

153 **Detection of new biomarkers in asymptomatic subjects**

154 To search for new biomarkers of asymptomatic infection, whole blood samples (9-10 mL)
155 were collected in heparinized tubes. Aliquots (500 μL) were incubated with 10 μg/mL
156 SLA or phytohemagglutinin (PHA-M) as a positive control. A third unstimulated tube
157 was used as a negative control. All tubes were incubated at 37°C for 24 h. After
158 centrifugation at 2000 *g* for 10 min, the plasma was collected and the concentrations of
159 the chemokines CXCL10, CXCL9 and CCL2 were determined using the BD Cytometric
160 Bead Array Human Flex Set (Becton Dickinson Biosciences, San Diego, CA, USA)
161 following the manufacturer's instructions, subtracting the background levels measured
162 inthe negative control samples (nonstimulated tube). IFN-γ, interleukin (IL)-2 and tumor
163 necrosis factor-α (TNF-α), which have been reported as markers of leishmaniasis
164 infection in other populations, were examined in the same way for comparative purposes.

165 **Serological and molecular tests**

166 Also, for comparative purposes, a routinely used enzyme-linked immunosorbent assay
167 (ELISA) was used to detect antibodies to SLA (11). Plasma samples were also subjected
168 to immunofluorescent antibody titer (IFAT) analyses using 2×10^5 *L. infantum*

promastigotes in PBS per well (MCAN/ES/98/LLM-722), as previously described (11). The threshold title for positivity was set at 1:80. The rK39 immunochromatographic test (rK39-ICT) was performed using the dipstick format Kalazar Detect Rapid test (InBIOS International, Seattle, WA). Antibody detection was performed with plasma samples according to the manufacturer's instructions. In addition, routinely used real-time PCR (qPCR) targeting the small subunit ribosomal RNA (SSUrRNA) genes of *Leishmania* was performed using DNA isolated from 200 µl of peripheral blood with a commercial extraction column, as described by Cruz *et al* (21).

CXCL10 profile and proximity to the park at the centre of the outbreak

To determine the relationship between asymptomatic infection as determined by CXCL10 (the marker that returned the clearest results in the above assays) and the distance between the semi-urban park where the outbreak begun, each SOT recipient's home was located on a map and the shortest linear distance to the border of the park measured using an online mapping tool (Google Maps, Google Inc., Mountain View, CA, USA).

Statistical analysis

Quantitative data are shown as medians with interquartile ranges (IQR). The normality of the distribution of continuous variables was assessed using the Shapiro-Wilk test. Biomarker concentrations were compared using the non-parametric Mann-Whitney U test. All tests were two-tailed. Significance was set at $p<0.05$. The area under the receiver operating characteristic (ROC) curve, diagnostic cut-off values, sensitivity and specificity, and the Youden J statistic (J = sensitivity + specificity - 1), were all calculated. All calculations were performed using SPSS v.20.0 (IBM Corp., Armonk, NY, USA) or Graph Pad Prism v.7.02 (GraphPad Software Inc., La Jolla, CA, USA).

Results

Clinical characteristics of the study cohort

Table 1 summarizes the demographic and clinical characteristics of the SOT recipients (40 [80%] had a kidney transplant, 8 [16%] a liver transplant, and 2 [4%] a heart transplant). Most recipients were male (76%). The mean age at transplantation was 50.2 ± 13.8 years. The mean hemoglobin was 13.4g/dL. The total number of leukocytes, neutrophils, and lymphocytes was 6.84, 4.52, and 1.46 x 10^3cell/ml, respectively. And the platelet count was 188 x 10^3cell/ml.

Capacity of the tested biomarkers to indicate asymptomatic *Leishmania* infection in SOT recipients

Fourteen of the 50 (28%) SOT recipients returned a positive CPA test (SI ≥3.44); these subjects were classified as having an asymptomatic infection (Fig. 1A). Those infected by *L. infantum* had significantly higher levels of IFN-γ, IL-2, TNF-α, CXCL9 and CCL2 ($p<0.0001$ for all) in plasma from SLA-stimulated whole blood compared to those with no asymptomatic infection (i.e., with a negative CPA result). However, those with asymptomatic infection had much higher CXCL10 concentrations that did non-infected subjects (Fig. 1B: median 2272 pg/ml [IQR 1441-3433] *vs*. 18.22 pg/ml [IQR 1-150.10]). The area under the ROC curve for the detection of asymptomatic infection by CXCL10 was 0.9644 (95%CI 0.91-1.00; $p<0.0001$); the cut-off was 762.5 pg/ml. Sensitivity was 93% (95%CI 77.23-99.15) and specificity 95% (95%CI 87.23-98.57) (Fig. 1C) - higher than for all other cytokines tested - and the Youden J value was 0.92 (Table 2).

Figure 1. (A) The cell proliferation assay (CPA) identified asymptomatic subjects (AS) and non-infected subjects (NI). (B) Production of CXCL10 in plasma from SLA-stimulated whole blood in both types of subject. Box-whisker plots show medians, interquartile ranges, and min/max values. ****$p<0.0001$. (C) Receiver operating

219 characteristic curve analysis showing the sensitivity and specificity of CXCL10 for
220 detecting asymptomatic subjects.
221
222 By way of comparison, no *Leishmania* DNA was detected in any blood sample from any
223 SOT recipient. IFAT and rK39-ICT tests detected anti-*Leishmania* antibodies in just one
224 asymptomatic subject, while ELISA did so in just four (Table 3).
225 **Spatial determinants of CXCL10 profiles**
226 In agreement with a previous finding that the incidence of VL is greater among SOT
227 recipients living <1000 m from the park than among those living further away, the present
228 subjects with a positive CPA result (indicative of asymptomatic *L. infantum* infection)
229 lived closer to the park than did those with a negative result (861 m ± 621 m *vs*. 1390 m
230 ± 581 m; p=0.04) (Figure 2). The home-park distance was then compared between SOT
231 recipients with and without a positive CXCL10 response in plasma from SLA-stimulated
232 whole blood according to the established cut-off value (≥762.5 pg/mL) (Table 2). As
233 expected, those with a positive CXCL10 response lived significantly closer than those
234 with a negative response (1011 m ± 581 m *vs*. 1380 m ± 784 m; p=0.0408). A significant
235 difference was also found with respect to IL-2 positivity (861 m ± 447 m vs. 1390 m ±
236 782 m; p=0.0247). No significant relationship was seen between the home-park distance
237 and a positive IFN-γ or CXCL9 response (1235 m ± 531 m vs. 1350 m ± 807 m; p=0.2531;
238 1235 m ± 531 m vs. 1350 m ± 827 m; p=0.2768).
239
240 **Figure 2.** Location of the SOT recipients' homes and relationship with CXCL10
241 (p=0.0408).
242
243 **Discussion**

In the present work, the detection of IFN-γ, IL-2, and CXCL9 in plasma from SLA-stimulated whole blood (i.e., in samples that returned a positive CPA result) yielded good sensitivity and specificity values (Table 2). However, CXCL10 was the most efficient biomarker (sensitivity 93%, specificity 95%) for identifying the asymptomatic population among the SOT recipients. A previous study performed in the same geographical area reported IL-2 to show good diagnostic accuracy in the detection of subjects with asymptomatic infection among blood donors and healthy volunteers (12, 22). To our knowledge, this is the first work to investigate the use of CXCL10 as a marker of *Leishmania* infection in SOT recipients. In work by other authors, the expression of CXCL10 in stimulated plasma was shown to reveal latent tuberculosis infection, and, in non-stimulated plasma from SOT recipients, it was reported to have potential as a biomarker of cytomegalovirus-induced inflammation (23, 24).

The present results reveal an inverse correlation between the home-park distance and the number of CXCL10-positive results. Those SOT recipients with an asymptomatic infection according to their CXCL10 result lived closer to the park than did those who were identified as not infected. In a previous paper, our group reported that living <1000 m from the park was an independent risk factor for SOT recipients developing clinical leishmaniasis (18). We also previously described the usefulness of IL-2 for establishing the true prevalence of asymptomatic infection in an immunocompetent population living in the area bordering the park (22). In that study, proximity to the park was measured as the distance from the latter to the primary healthcare centers where patients were attended to - not the home address of the subjects as it was in the present work.

The present study suffers from a number of limitations. The sample size is small, affecting the statistical power available for detecting differences between groups (although an intense effort was made to identify every single SOT recipient living in the affected area

during the study period; it is unlikely that any was missed). It is also possible that the subjects were asymptomatically infected before 2009, a period for when no such records are available. The nature of the 2009 outbreak (wild transmission cycle in contact with people) strongly suggests that natural immunity in the study area was little developed or absent (25).

In conclusion, CXCL10 detected in plasma from SLA-stimulated whole blood may act as a useful tool for identifying asymptomatic *L. infantum*-infection in SOT recipients. This finding might hold true in other endemic areas in which immunocompromised hosts act as a reservoir of disease. If so, determining CXCL10 concentrations in such plasma could be of much use in the control of leishmaniasis.

Acknowledgments

Thanks are owed to all the patients who took part in this study.

Funding

This study was co-funded by the World Health Organization (APW-2012/271093-O), and the Spanish Ministry of Science and Universities (via Proyecto Integrado de Excelencia [PIE] 13/00045). Additional funding was provided by the Instituto de Salud Carlos III via project PI18CIII/00028 and DTS16CIII/00010, and via the Red de Enfermedades Tropicales, Subprograma RETICS del Plan Estatal de I+D+I 2013-2016, which is co-supported by FEDER "Una Manera de Hacer Europa" funds via project RD16CIII/0003/0002. M.F.R. holds a "Miguel Servet" (CP18/00073) research contract from the Spanish Ministry of Science and Innovation, Instituto de Salud Carlos III. The funders had no role in the design of the study, data collection, or the interpretation of the results.

Conflict of Interest

The authors have declared no competing interest.

References

1. Clemente W, Vidal E, Girao E, Ramos AS, Govedic F, Merino E, et al. Risk factors, clinical features and outcomes of visceral leishmaniasis in solid-organ transplant recipients: a retrospective multicenter case-control study. Clin Microbiol Infect. 2015;21(1):89-95. doi: 10.1016/j.cmi.2014.09.002

2. van Griensven J, Carrillo E, Lopez-Velez R, Lynen L, Moreno J. Leishmaniasis in immunosuppressed individuals. Clin Microbiol Infect. 2014;20(4):286-99. doi: 10.1111/1469-0691.12556

3. Singh OP, Hasker E, Sacks D, Boelaert M, Sundar S. Asymptomatic Leishmania infection: a new challenge for Leishmania control. Clin Infect Dis. 2014;58(10):1424-9. doi: 10.1093/cid/ciu102

4. Ibarra-Meneses AV, Corbeil A, Wagner V, Onwuchekwa C, Fernandez-Prada C. Identification of asymptomatic Leishmania infections: a scoping review. Parasit Vectors. 2022;15(1):5. doi: 10.1186/s13071-021-05129-y

5. Varani S, Ortalli M, Attard L, Vanino E, Gaibani P, Vocale C, et al. Serological and molecular tools to diagnose visceral leishmaniasis: 2-years' experience of a single center in Northern Italy. PLoS One. 2017;12(8):e0183699. doi: 10.1371/journal.pone.0183699

6. Das S, Matlashewski G, Bhunia GS, Kesari S, Das P. Asymptomatic Leishmania infections in northern India: a threat for the elimination programme? Trans R Soc Trop Med Hyg. 2014;108(11):679-84. doi: 10.1093/trstmh/tru146

7. Schnorr D, Muniz AC, Passos S, Guimaraes LH, Lago EL, Bacellar O, et al. IFN-gamma production to leishmania antigen supplements the leishmania skin test in

identifying exposure to L. braziliensis infection. PLoS Negl Trop Dis. 2012;6(12):e1947. doi: 10.1371/journal.pntd.0001947

8. Custodio E, Gadisa E, Sordo L, Cruz I, Moreno J, Nieto J, et al. Factors associated with Leishmania asymptomatic infection: results from a cross-sectional survey in highland northern Ethiopia. PLoS Negl Trop Dis. 2012;6(9):e1813. doi: 10.1371/journal.pntd.0001813

9. Babuadze G, Alvar J, Argaw D, de Koning HP, Iosava M, Kekelidze M, et al. Epidemiology of visceral leishmaniasis in Georgia. PLoS Negl Trop Dis. 2014;8(3):e2725. doi: 10.1371/journal.pntd.0002725

10. Sassi A, Louzir H, Ben Salah A, Mokni M, Ben Osman A, Dellagi K. Leishmanin skin test lymphoproliferative responses and cytokine production after symptomatic or asymptomatic Leishmania major infection in Tunisia. Clin Exp Immunol. 1999;116(1):127-32. doi: 10.1046/j.1365-2249.1999.00844.x

11. Carrillo E, Carrasco-Anton N, Lopez-Medrano F, Salto E, Fernandez L, San Martin JV, et al. Cytokine Release Assays as Tests for Exposure to Leishmania, and for Confirming Cure from Leishmaniasis, in Solid Organ Transplant Recipients. PLoS Negl Trop Dis. 2015;9(10):e0004179. doi: 10.1371/journal.pntd.0004179

12. Ibarra-Meneses AV, Carrillo E, Sanchez C, Garcia-Martinez J, Lopez Lacomba D, San Martin JV, et al. Interleukin-2 as a marker for detecting asymptomatic individuals in areas where Leishmania infantum is endemic. Clin Microbiol Infect. 2016;22(8):739 e1-4. doi: 10.1016/j.cmi.2016.05.021

13. Singh OP, Sundar S. Whole blood assay and visceral leishmaniasis: Challenges and promises. Immunobiology. 2014;219(4):323-8. doi: 10.1016/j.imbio.2014.01.005

14. Alvar J, Alves F, Bucheton B, Burrows L, Buscher P, Carrillo E, et al. Implications of asymptomatic infection for the natural history of selected parasitic

342 tropical diseases. Semin Immunopathol. 2020;42(3):231-46. doi: 10.1007/s00281-020-
343 00796-y
344 15. Ibarra-Meneses AV, Moreno J, Carrillo E. New Strategies and Biomarkers for
345 the Control of Visceral Leishmaniasis. Trends Parasitol. 2020;36(1):29-38. doi:
346 10.1016/j.pt.2019.10.005
347 16. Ibarra-Meneses AV, Ghosh P, Hossain F, Chowdhury R, Mondal D, Alvar J, et
348 al. IFN-gamma, IL-2, IP-10, and MIG as Biomarkers of Exposure to Leishmania spp.,
349 and of Cure in Human Visceral Leishmaniasis. Front Cell Infect Microbiol. 2017;7:200.
350 doi: 10.3389/fcimb.2017.00200
351 17. Ibarra-Meneses AV, Sanchez C, Alvar J, Moreno J, Carrillo E. Monocyte
352 Chemotactic Protein 1 in Plasma from Soluble Leishmania Antigen-Stimulated Whole
353 Blood as a Potential Biomarker of the Cellular Immune Response to Leishmania
354 infantum. Front Immunol. 2017;8:1208. doi: 10.3389/fimmu.2017.01208
355 18. Carrasco-Anton N, Lopez-Medrano F, Fernandez-Ruiz M, Carrillo E, Moreno J,
356 Garcia-Reyne A, et al. Environmental Factors as Key Determinants for Visceral
357 Leishmaniasis in Solid Organ Transplant Recipients, Madrid, Spain. Emerg Infect Dis.
358 2017;23(7):1155-9. doi: 10.3201/eid2307.151251
359 19. Aleka Y, Ibarra-Meneses AV, Workineh M, Tajebe F, Kiflie A, Tessema MK, et
360 al. Whole Blood Stimulation Assay as a Treatment Outcome Monitoring Tool for VL
361 Patients in Ethiopia: A Pilot Evaluation. J Immunol Res. 2020;2020:8385672. doi:
362 10.1155/2020/8385672
363 20. Chamakh-Ayari R, Bras-Goncalves R, Bahi-Jaber N, Petitdidier E, Markikou-
364 Ouni W, Aoun K, et al. In vitro evaluation of a soluble Leishmania promastigote surface
365 antigen as a potential vaccine candidate against human leishmaniasis. PLoS One.
366 2014;9(5):e92708. doi: 10.1371/journal.pone.0092708

21. Cruz I, Millet A, Carrillo E, Chenik M, Salotra P, Verma S, et al. An approach for interlaboratory comparison of conventional and real-time PCR assays for diagnosis of human leishmaniasis. Exp Parasitol. 2013;134(3):281-9. doi: 10.1016/j.exppara.2013.03.026

22. Ibarra-Meneses AV, Carrillo E, Nieto J, Sanchez C, Ortega S, Estirado A, et al. Prevalence of asymptomatic Leishmania infection and associated risk factors, after an outbreak in the south-western Madrid region, Spain, 2015. Euro Surveill. 2019;24(22). doi: 10.2807/1560-7917.ES.2019.24.22.1800379

23. Qiu X, Tang Y, Yue Y, Zeng Y, Li W, Qu Y, et al. Accuracy of interferon-gamma-induced protein 10 for diagnosing latent tuberculosis infection: a systematic review and meta-analysis. Clin Microbiol Infect. 2019;25(6):667-72. doi: 10.1016/j.cmi.2018.12.006

24. Rollag H, Ueland T, Asberg A, Hartmann A, Jardine AG, Humar A, et al. Characterization of cytomegalovirus disease in solid organ transplant recipients by markers of inflammation in plasma. PLoS One. 2013;8(4):e60767. doi: 10.1371/journal.pone.0060767

25. Carrillo E, Moreno J, Cruz I. What is responsible for a large and unusual outbreak of leishmaniasis in Madrid? Trends Parasitol. 2013;29(12):579-80. doi: 10.1016/j.pt.2013.10.007

Legends

Figure 1. (A) The cell proliferation assay (CPA) identified asymptomatic subjects (AS) and non-infected subjects (NI). (B) Production of CXCL10 in plasma from SLA-stimulated whole blood in both types of subjects. Box-whisker plots show medians, interquartile ranges and min/max values. ****p<0.0001. (C) Receiver operating characteristic curve analysis showing the sensitivity and specificity of CXCL10 for detecting asymptomatic subjects.

Figure 2. Location of the SOT recipients' homes and relationship with CXCL10 (p=0.0408).