Diagnostic Performance of Filter Paper Lesion Impression PCR for Secondarily Infected Ulcers and Nonulcerative Lesions Caused by Cutaneous Leishmaniasis

Andrea K. Boggild, Ana Pilar Ramos, Braulio Mark Valencia, Nicolas Veland, Flor Calderon, Jorge Arevalo, Donald E. Low, and Alejandro Llanos-Cuentas

Tropical Disease Unit, Division of Infectious Diseases, UHN-Toronto General Hospital, Toronto, Canada; Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia (UPCH), Lima, Peru; Departamento de Bioquímica, Biología Molecular y Farmacología, Facultad de Ciencias, Universidad Peruana Cayetano Heredia, Lima, Peru; Laboratories Branch, Ontario Agency for Health Protection and Promotion, Etobicoke, Canada; Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Canada; and Hospital Nacional Cayetano Heredia, Lima, Peru

Received 5 December 2010/Accepted 13 December 2010

We compared traditional cutaneous leishmaniasis diagnostic methods to filter paper lesion impression (FPLI) PCR for secondarily infected ulcers and nonulcerative lesions. The sensitivity and specificity of FPLI PCR for secondarily infected lesions (n = 8) were 100%. In primarily nonulcerative lesions (n = 15), the sensitivity of FPLI PCR was inferior to that of pooled-invasive-specimen PCR (72.7% versus 100%) (P = 0.10). FPLI PCR is sensitive, specific, and unlike invasive procedures, can be used in secondarily infected ulcers. Invasive specimen collection is superior in nonulcerative lesions.

Gold standard diagnosis of cutaneous leishmaniasis (CL) involves the identification of parasites microscopically or by culture, which involves obtaining specimens invasively (6, 11, 18). Scrapings and aspirates are common clinical specimens, the sensitivity of which ranges from 40 to 75% for culture (1, 2, 4, 5, 7) and to >90% for PCR (3, 15, 17, 18, 20, 22). Obtaining invasive specimens causes discomfort, requires technical expertise, carries risks of bleeding and infection, is difficult to perform with children and in remote field settings, and is contraindicated in the case of secondary bacterial infection due to risks of bacteremia or complicated soft tissue infection (18). In addition, it poses risks to health care workers of body fluid exposure via needlestick injury and necessitates sharps biohazard procedures. Thus, less invasive, more simple and sensitive diagnostic procedures are desirable.

Filter paper lesion impression (FPLI) PCR is a sensitive, tolerable, noninvasive diagnostic approach to CL (5). However, it has not been evaluated for secondarily infected ulcers, which account for 10 to 15% of ulcers in CL (14). As incising secondarily infected ulcers to obtain invasive specimens prior to a course of antibiotics is contraindicated, FPLI PCR may provide rapid diagnosis of CL in secondarily infected ulcers rather than waiting until clearance of bacterial infection to perform the diagnostic evaluation.

We compared methods for diagnosing CL, including culture and PCR of lesion aspirates and scrapings, Giemsa-stained lesion smears, and leishmanin skin tests (LSTs), to noninvasive FPLI PCR for both secondarily infected and uninfected ulcers and for predominantly nonulcerative lesions.

The study was conducted at the Leishmania Clinic of the Instituto de Medicina Tropical Alexander Von Humboldt (Hospital Nacional Cayetano Heredia) in Lima, Peru, between January and April 2010, following Institutional Review Board approvals. Patients presenting to the Leishmania Clinic for evaluation of skin lesions were eligible for enrollment following informed consent. We included patients referred for suspected CL and, also, patients whose ulcers had clinical evidence of secondary bacterial infection, including heat, redness, swelling, tenderness, and exudation. Patients with secondarily infected ulcers were treated empirically with a 5-day course of clindamycin and ciprofloxacin. We excluded patients undergoing treatment for CL.

FPLIs were collected and processed as described previously (5). For secondarily infected ulcers, FPLIs were collected at enrollment and then after oral antibiotics, at which time invasive specimen collection was performed. Skin scrapings for Giemsa-stained smears, lesion aspirates for culture, and LSTs (21) were performed as described previously (4, 5). Lancets and aspirates were stored for PCR as described previously (5).

Isolation of DNA from FPLIs, aspirates, and lancets was performed as described previously (5, 19). Kinetoplast DNA (kDNA) PCR was performed using a HotStar Taq Plus DNA
polymerase kit (Qiagen, Germany), and the conditions were as described previously (5, 12). Two pairs of primers were used as described previously (5, 12). Amplicons were visualized as described previously (5, 12). Two pairs of primers were used as described previously (5, 12). Amplicons were visualized as described previously (5, 12). Two pairs of primers were used as described previously (5, 12). Amplicons were visualized as described previously (5, 12). Two pairs of primers were used as described previously (5, 12). Amplicons were visualized as described previously (5, 12). Two pairs of primers were used as described previously (5, 12). Amplicons were visualized as described previously (5, 12). Two pairs of primers were used as described previously (5, 12). Amplicons were visualized as described previously (5, 12). Two pairs of primers were used as described previously (5, 12). Amplicons were visualized as described previously (5, 12). Two pairs of primers were used as described previously (5, 12). Amplicons were visualized as described previously (5, 12). Two pairs of primers were used as described previously (5, 12). Amplicons were visualized as described previously (5, 12). Two pairs of primers were used as described previously (5, 12). Amplicons were visualized as described previously (5, 12). Two pairs of primers were used as described previously (5, 12). Amplicons were visualized as described previously (5, 12). Two pairs of primers were used as described previously (5, 12). Amplicons were visualized as described previously (5, 12). Two pairs of primers were used as described previously (5, 12). Amplicons were visualized as described previously (5, 12).

Species identification by PCR-restriction fragment length polymorphism (PCR-RFLP) following kDNA PCR employed three assays targeting different sequences specific to *Leishmania* subgenus *Viannia* species, including *L. (V.) braziliensis*, *L. (V.) peruviana*, and *L. (V.) guyanensis*, the principal causative species in Peru, as described previously (5, 9, 10, 16). Assays targeted the mannose phosphate isomerase (*mpi*) gene (16, 23), cysteine proteinase B (*cpb*) gene (10, 16), and the heat shock protein 70 (*hsp70*) gene (9, 16). PCR-RFLP analysis of *mpi*, *cpb*, and *hsp70* PCR products was performed as described previously (5).

Lesions were defined as CL when 2 of 5 tests (LST, lesion smear, culture, PCR of aspirates or scrapings, or PCR of FPLIs) were positive. To demonstrate superior performance of FPLI PCR compared to LST (the only other test applied at enrollment) for secondarily infected ulcers, 8 lesions were required, yielding a target enrollment of ~80 lesions. Descriptive statistics were calculated and compared using 2-tailed *t*-testing or Yate’s corrected Chi-square analysis. Differences in sensitivities were compared using the z-test. SigmaStat 2.03 software (SPSS, Inc., Chicago, IL) was used. Significance was set at a *P* value of <0.05.

Sixty-five patients with 85 skin lesions were enrolled. Clinical evidence of secondary bacterial infection was present in 8 lesions from 6 patients. Most lesions were purely ulcerative (*n* = 70; 82%), with fewer having a verrucous or nodular appearance (*n* = 8 [9%] and *n* = 7 [8%], respectively) with minimal ulceration. Using the composite standard (*≥*2/5 tests positive), 67 lesions (79%) fulfilled criteria for CL. The performance characteristics of the assays employed are described in Table 1. PCR of invasively collected specimens and FPLIs was more sensitive than LST, smear, and culture (*P* < 0.001). PCR of FPLIs was equally as sensitive as PCR of pooled invasive specimens (*P* = 0.314), aspirates (*P* = 0.396), and scrapings (*P* = 0.274).

The performance characteristics of assays for secondarily infected ulcers are summarized in Table 2. There was 100% concordance between the results for FPLIs collected at enrollment (i.e., during active secondary bacterial infection) and those collected after 5 days of antibiotics. FPLI PCR of secondarily infected ulcers demonstrated sensitivity and specificity of 100%. LST, the only other test applied at enrollment of patients with secondarily infected ulcers, was less sensitive than FPLI PCR (*P* = 0.011). FPLI PCR for secondarily infected ulcers had a level of performance comparable to that of post-antibiotic-invasive-specimen PCR (*P* = 0.889) but was superior to culture (*P* = 0.014) (Table 2).

The performance characteristics of assays in purely ulcerative and primarily nonulcerative lesions are summarized in Table 2. FPLI PCR and pooled-invasive-specimen PCR for ulcers showed comparable sensitivities (*P* = 0.957) (Table 2). FPLI PCR for ulcers was superior to smear (*P* < 0.001), culture (*P* < 0.001), and LST (*P* = 0.004) (Table 2). For primarily nodular or verrucous lesions, FPLI PCR trended toward inferiority compared to pooled-invasive-specimen PCR (*P* = 0.10) (Table 2). PCR of invasive specimens was superior to culture (*P* < 0.001), LST (*P* = 0.002), and smear (*P* = 0.003) for nonulcerative lesions (Table 2). Conversely, FPLI PCR demonstrated no such superiority compared to culture (*P* = 0.116), LST (*P* = 0.161), or smear (*P* = 0.253) for nonulcerative lesions (Table 2).

Of 36 kDNA-positive filter papers with definitive RFLP results (62%), species identification was as follows: *L. (V.) braziliensis*, 15 lesions; *L. (V.) peruviana*, 9 lesions; *L. (V.) guyanensis*, 9 lesions; *L. (V.) lainsoni*, 1 lesion; and *L. (V.) braziliensis/L. (V.) peruviana* hybrid, 2 lesions.

We have demonstrated in a clinical evaluation of secondarily infected and uninfected ulcers of CL that FPLI PCR offers diagnostic sensitivity and specificity comparable to those of invasive-specimen PCR and is superior in performance to LST, smear, and culture. FPLI PCR can therefore be used to diagnose CL in secondarily infected ulcers at the point of presentation prior to a course of antibiotics. This finding is important since, in some areas of Peru, it is common traditional practice to apply vegetable (e.g., garlic) or chemical corrosive (e.g., battery acid) substances which predispose to secondary bacterial infection of lesions. This finding is also significant for patients in whom treatment cannot be delayed or who must travel to an urban center for diagnosis, as invasive diagnostic testing occurs after several days of antibiotics if secondary bacterial infection is present. That FPLIs are noninvasive, easy

---

### Table 1. Analysis of 5 diagnostic tests used in the evaluation of 65 patients with 85 lesions suspected to be cutaneous leishmaniasis

<table>
<thead>
<tr>
<th>Assay</th>
<th>No. of lesions with indicated result</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>[% (95% CI)]</td>
<td>[% (95% CI)]</td>
<td></td>
</tr>
<tr>
<td>LST*</td>
<td>33</td>
<td>30</td>
<td>60.7 (49–72.4)</td>
<td>83.3 (66.1–100)</td>
<td>93.9</td>
</tr>
<tr>
<td>Smear</td>
<td>33</td>
<td>49</td>
<td>49.3 (37.3–61.3)</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>kDNA PCR of invasive specimens</td>
<td>64</td>
<td>21</td>
<td>92.5 (86.2–98.8)</td>
<td>88.9 (74.4–100)</td>
<td>96.9</td>
</tr>
<tr>
<td>kDNA PCR of lesion aspirates</td>
<td>55</td>
<td>30</td>
<td>80.6 (71.1–90.1)</td>
<td>94.4 (83.8–100)</td>
<td>98.2</td>
</tr>
<tr>
<td>kDNA PCR of lesion scrapings</td>
<td>54</td>
<td>31</td>
<td>79.1 (69.4–88.8)</td>
<td>94.4 (83.3–100)</td>
<td>98.1</td>
</tr>
<tr>
<td>kDNA PCR of noninvasive specimens</td>
<td>60</td>
<td>25</td>
<td>86.6 (78.4–94.8)</td>
<td>88.9 (74.4–100)</td>
<td>96.7</td>
</tr>
<tr>
<td>Culture</td>
<td>22</td>
<td>63</td>
<td>32.8 (21.6–44)</td>
<td>100.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

* a LST, leishmanin skin test. Two individuals did not undergo leishmanin skin testing.
  b Includes pooled analysis of lesion aspirates and scrapings.
  c Includes filter paper lesion impressions.
  d CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value.
TABLE 2. Analysis of 5 diagnostic tests used in the evaluation of secondarily infected and uninfected ulcers, as well as purely ulcerative and nonulcerative lesions suspected to be cutaneous leishmaniasis

<table>
<thead>
<tr>
<th>Assay</th>
<th>Result (%) (95% CI)</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>kDNA PCR of noninvasive specimens</td>
<td>85.7 (59.8–100)</td>
<td>72.2 (58.7–74.1)</td>
<td>80.4 (64.3–86.9)</td>
</tr>
<tr>
<td>kDNA PCR of lesion aspirates</td>
<td>80.4 (70.0–90.9)</td>
<td>82.5 (75.8–89.3)</td>
<td>85.7 (80.8–88.3)</td>
</tr>
<tr>
<td>kDNA PCR of invasive specimens</td>
<td>72.2 (59.6–84.7)</td>
<td>72.2 (59.6–84.7)</td>
<td>65.9 (53.3–78.3)</td>
</tr>
<tr>
<td>FPLI PCR of lesion scrapings</td>
<td>80.4 (70.0–90.9)</td>
<td>82.5 (75.8–89.3)</td>
<td>85.7 (80.8–88.3)</td>
</tr>
<tr>
<td>FPLI PCR of lesion aspirates</td>
<td>80.4 (70.0–90.9)</td>
<td>82.5 (75.8–89.3)</td>
<td>85.7 (80.8–88.3)</td>
</tr>
<tr>
<td>FPLI PCR of invasive specimens</td>
<td>72.2 (59.6–84.7)</td>
<td>72.2 (59.6–84.7)</td>
<td>65.9 (53.3–78.3)</td>
</tr>
</tbody>
</table>

We thank Eduardo Gotuzzo, Ana Luz Quispe, Milena Alba, and Carmen Medina of the Instituto de Medicina Tropical Alexander von Humboldt (UPCH) for logistical support.

This study was funded by the Ontario Association of Medical Laboratories (SGP-09-002). A.K.B. was supported by a Detweiler traveling fellowship through the Royal College of Physicians and Surgeons of Canada during the study period. Personnel and facility fees for the Arevalo molecular laboratory (N.V. and J.A.) were supplied by the Institutional Collaboration Framework Agreement 3 from the Belgian Development Cooperation. No author has a commercial or other interest which would pose a conflict with publication. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

B.M.V., A.P.R., and F.C. contributed to data collection and were responsible for enrolling patients. N.V. and J.A. conducted the molecular analyses and contributed to data interpretation and writing the manuscript. A.L.C. and D.E.L. contributed to study design, implementation, and data interpretation. A.K.B. contributed to study design, data collection, analysis, and interpretation and was primarily responsible for writing the manuscript. All authors critically appraised the manuscript.

REFERENCES