Sensitivity and specificity of polymerase chain reaction in Giemsa-stained slides for diagnosis of visceral leishmaniasis in children


Departamento de Pediatria **Departamento de Clínica Médica ***Departamento de Patologia, Universidade Federal de Mato Grosso do Sul, Caixa Postal 102, 79080-190 Campo Grande, MS, Brasil *Laboratório de Imunopatologia, Instituto Oswaldo Cruz-Fiocruz, Rio de Janeiro, RJ, Brasil

The aim of this study was to evaluate the sensitivity and specificity of polymerase chain reaction (PCR) in the detection of *Leishmania* DNA in archived Giemsa-stained bone marrow slides for diagnosis of visceral leishmaniasis (VL), and to compare PCR with conventional diagnostic techniques, like direct microscopy and parasite culture. Specimens of archived Giemsa-stained bone marrow slides from 91 patients with VL and from 79 controls with other diseases or conditions were studied. PCR showed the highest sensitivity (92.3%) and had good specificity (97.5%). Direct examination detected 79.1% and culture 59% of positive samples. In addition, PCR was able to detect VL in 16 of 19 patients (84.2%) with negative microscopy. PCR in Giemsa-stained bone marrow slides is a suitable tool for confirming diagnosis in patients with VL and may be useful in the diagnosis of difficult cases. Slide smears are easily stored, do not require special storage conditions such as low temperatures, and can be easily mailed to centers where PCR is available, making it an excellent option for diagnosis in the field.

Key words: visceral leishmaniasis - polymerase chain reaction - diagnosis

Conventional methods for diagnosis of visceral leishmaniasis (VL), such as parasitological or serological tests, still have limitations in their use (Sundar 2003, da Silva et al. 2005). The identification of the causal agent of human leishmaniasis by these tests is difficult even in a well-equipped hospital and improved tools for this purpose are needed.

Polymerase chain reaction (PCR) has now revolutionized contemporaneous etiologic diagnosis of infectious diseases. The description of species-specific kisinetoplast DNA minicircles sequences (kDNA) (Kennedy 1984), with the possibility of amplification by PCR, made viable the utilization of the method for diagnosis of leishmaniasis in the early 1990s (Rodgers et al. 1990, Smyth et al. 1992). Currently, PCR techniques have been employed with a high level of accuracy by several researchers (Salotra et al. 2001, Cortes et al. 2004, De Doncker et al. 2005, Stark et al. 2006).

Various biological samples have been used for diagnosis of visceral or cutaneous leishmaniasis by PCR: bone marrow (Piarroux et al. 1994, Osman et al. 1997, Cortes et al. 2004), human (Lachaud et al. 2001, Cascio et al. 2002) and canine blood (Lachaud et al. 2002a), lymph nodes (Lachaud et al. 2002b),uffy coat (Lachaud et al. 2001), canine conjunctival or dermal scrapings (Lachaud et al. 2002b), and skin or mucosal biopsies (Pirmez et al. 1999, Stark et al. 2006). Moreover, PCR has made possible the use of dried or old materials: *Leishmania* DNA has been extracted and amplified from blood or bone marrow aspirates spotted on filter paper (Cortes et al. 2004, da Silva et al. 2004), paraffin-embedded tissues (Lanús et al. 2005), Giemsa-stained slides (Motazedian et al. 2002), and museum specimens (Costa 1998).

In Mato Grosso do Sul, Center-West region of Brazil, the Federal University Hospital (NHU-UFMS) situated in the capital Campo Grande is a reference center for diagnosis and treatment of VL, receiving patients from various cities of the state for diagnostic confirmation or exclusion. The aim of this study was to evaluate the use of PCR in VL diagnosis using archived Giemsa-stained bone marrow aspirate (BMA) slides, establishing its sensitivity and specificity, and to compare the performance of this retrospective diagnosis with direct microscopy and culture.

Ninety-one children from the state of Mato Grosso do Sul, admitted for diagnosis and treatment of VL at the NHU-UFMS from January 1998 to September 2004 were studied. Diagnosis of VL was based on a suggestive clinical presentation, associated with a positive BMA direct microscopy or culture or serology by indirect immunofluorescence antibody test (IFAT) ≥ 1:40; children with a suggestive clinical picture whose laboratorial tests were negative but who responded to a therapeutic trial with antimonials were included too. Controls consisted of 79 children or adults who had a BMA performed for diagnostic purposes and who did not have a confirmed leishmaniasis diagnosis but other diseases or conditions were detected. Table I shows the characteristics of the children with VL and of controls.

---

+ Corresponding author: brustoloni@uol.com.br
Received 24 November 2006
Accepted 29 March 2007
BMAs were collected for occasion of admission, by sternal puncture or more rarely from the posterior iliac crest. Four Giemsa-stained slides for each patient were analyzed by direct microscopic examination with a 10X eyepiece and a 100X oil objective. More than 1000 microscopic fields were examined, when necessary; before deciding for a negative result, the entire slides were viewed. The slides were then archived at room temperature in the sectors of Parasitology or Hematology. Cultures were performed with Novy-MacNeal-Nicolle (NNN) with Schneider’s insect medium, incubated at 24°C, and weekly examined by microscopy for the presence of parasites until eight weeks. IFA T, when possible, was performed with a commercial kit for the diagnosis of parasites until eight weeks. IFA T, when possible, was performed with a commercial kit for the diagnosis of visceral leishmaniasis from stored Giemsa-stained bone marrow slides. PCR allowed the retrieval of archival cases, since the good performance of the assay was reflected too in its ability to detect DNA of the parasite in 16 of 19 old materials for the extraction of DNA, some of them archived for as long as six years, the sensitivity achieved was highly satisfactory.

The present experiment suggests that it is possible to reach a retrospective diagnosis of visceral leishmaniasis from stored Giemsa-stained bone marrow slides. PCR allowed the retrieval of archival cases, since the test showed a high sensitivity and specificity.

The inclusion of patients in the absence of a gold-standard diagnosis may have important consequences when the sensitivity of a method is being evaluated. Analyzing only those children with a positive parasitological test (direct examination or culture: n = 77), PCR was positive in 73, thus increasing its sensitivity to 94.8% (86.5-98.3, 95% CI). Considering that we used dried, old materials for the extraction of DNA, some of them archived for as long as six years, the sensitivity achieved was highly satisfactory.

The inclusion of patients in the absence of a gold-standard diagnosis may have important consequences when the sensitivity of a method is being evaluated. Analyzing only those children with a positive parasitological test (direct examination or culture: n = 77), PCR was positive in 73, thus increasing its sensitivity to 94.8% (86.5-98.3, 95% CI). Considering that we used dried, old materials for the extraction of DNA, some of them archived for as long as six years, the sensitivity achieved was highly satisfactory.

The good performance of the assay was reflected too in its ability to detect DNA of the parasite in 16 of 19 patients (84.2%) for whom direct microscopy was negative.

The inclusion of patients in the absence of a gold-standard diagnosis may have important consequences when the sensitivity of a method is being evaluated. Analyzing only those children with a positive parasitological test (direct examination or culture: n = 77), PCR was positive in 73, thus increasing its sensitivity to 94.8% (86.5-98.3, 95% CI). Considering that we used dried, old materials for the extraction of DNA, some of them archived for as long as six years, the sensitivity achieved was highly satisfactory.

The good performance of the assay was reflected too in its ability to detect DNA of the parasite in 16 of 19 patients (84.2%) for whom direct microscopy was negative.
Table II

<table>
<thead>
<tr>
<th></th>
<th>Patients (n = 91)</th>
<th>Controls (n = 79)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n tested</td>
<td>n positive (%)</td>
</tr>
<tr>
<td>BMA microscopy</td>
<td>91</td>
<td>72 (79.1)</td>
</tr>
<tr>
<td>Culture</td>
<td>88</td>
<td>52 (59.0)</td>
</tr>
<tr>
<td>PCR</td>
<td>91</td>
<td>84 (92.3)</td>
</tr>
</tbody>
</table>

Polymerase chain reaction amplification products of bone marrow Giemsa-stained slides. MW: 100 bp DNA ladder size marker; PC: positive control; 1, 2, 3: patients with visceral leishmaniasis; 4, 5, 6: patients with other diseases; NC: negative control.

TABLE II

Bone marrow aspirate (BMA) microscopy, culture, and polymerase chain reaction (PCR) in patients with visceral leishmaniasis and controls

References


Motazedian H, Karamian M, Noyes HA, Ardehali S 2002. DNA extraction and amplification of *Leishmania* from archived,
Giemsa-stained slides, for the diagnosis of cutaneous leishmaniasis by PCR. Ann Trop Med Parasitol 96: 31-34.


