SENSITIVITY OF BONE MARROW ASPIRATES IN THE DIAGNOSIS OF VISCERAL LEISHMANIASIS

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Abstract. Bone marrow aspirates are believed to provide a safer but less sensitive method in the diagnosis of visceral leishmaniasis (VL) compared with splenic aspirates. We examined the effect of the number of fields and the time of observation on bone marrow smear sensitivity and compared it to our experience with spleen aspiration. Bone marrow smears of 98 patients and splenic aspirates from 120 patients were examined. Among 87 patients with VL, the sensitivity of bone marrow aspirates was 40.2%, 65.5%, 89.7%, 92%, and 95.4% at 1, 5, 20, 30, and 60 minutes, respectively. The sensitivity of spleen aspirate examination was 93% for 114 patients. One patient died of shock after spleen aspiration. A bone marrow smear is very sensitive if examined thoroughly, reaching a sensitivity similar to that of spleen aspirate. We propose that a bone marrow smear be established as the technique of choice for the parasitologic diagnosis of VL.

INTRODUCTION

Visceral leishmaniasis (VL), caused by species of the protozoa Leishmania, is present in many tropical and temperate countries, such as India, Sudan, and Brazil.1 In southern Europe, it is a major opportunistic infection in patients with acquired immunodeficiency syndrome.2 Protracted fever, wasting, pallor, and hepatosplenomegaly are common findings. Generally, fewer than 10% of treated patients die.3,4 Serologic or parasitologic methods and the polymerase chain reaction (PCR) are used for the diagnosis. However, serology may lack specificity due to asymptomatic infections.5,6 PCR is the best diagnostic method, but its use is limited to tertiary health centers.7,8,9

Parasitologic methods are highly specific. Smears or biopsies can be obtained from liver, lymph nodes, bone marrow, and spleen, but only smears from the marrow and spleen have been used routinely.10 Microbiologic culture of these specimens is usually very sensitive11 but not practical for early therapeutic, life-saving decisions. Therefore, because smears are simple to prepare, direct examination of these samples is usually the best diagnostic method in poorer areas where PCR is not available. However, risks associated with the aspiration technique and the time needed for examination of a bone marrow slide can make this diagnostic procedure difficult to implement.

Teresina is the city in which VL began as an urban epidemic in Brazil.12 Diagnosis in this city is performed through a combination of clinical data, serology, and direct parasitologic examination. During epidemics, when resources of clinicians are especially overstretched and the number of samples is greater than usual, the sensitivity of bone marrow smears decreases because less time is spent examining each film. For a limited time during a recent epidemic, physicians adopted spleen aspiration as the primary diagnostic technique in an effort to improve sensitivity. This study describes the sensitivity of bone aspiration as a function of the number of fields examined and time spent viewing the films, as well as a single hospital’s experience with fine-needle spleen aspiration during an epidemic of VL, and it compares the advantages and limitations of the two methods.

MATERIALS AND METHODS

The ethics committee of the regional medical board reviewed and approved this study. Patients were evaluated at an infectious disease hospital in Teresina during their first episode of VL. Bone marrow aspirates were taken from 98 patients suspected of having VL (BM group). In six of them, therapy with pentavalent antimony had been started up to seven days before the bone marrow aspirate was taken. Spleen aspirates were taken from 120 patients (SA group); 11 had initiated therapy up to four days before the procedure. Most patients underwent only one of the two diagnostic procedures. The final diagnosis of VL was reached as a result of the presence of typical signs and symptoms combined with parasitologic or serologic data or response to empirical therapy.

Bone marrow was taken from the sternum under local anesthesia. A 100× objective and an 8× ocular lens were used to examine Giemsa-stained slides under an oil-immersion objective. Each smear was carefully examined for at least one hour before being officially read as negative by a single observer. The examination time and the number of high-magnification fields studied were counted. If non-flagellar forms (amastigotes) of L. chagasi were found, the observer stopped the count and recorded the time spent on the examination and the number of fields studied.

Spleen aspiration was performed regularly at the hospital over a 10-month period following a technique previously described.1 Only patients without known bleeding disorders were selected for the procedure. A 20–22-gauge needle was inserted in the subcutaneous tissue over the area of a spleen, more then 3 cm below the costal margin. The needle was gently oriented at a 90° angle. During the inspiration of a crying child or an adult oriented to maintain it, the needle was then inserted into the spleen in a rapid movement (less than one second) while the patient inhaled. The quantity of the aspirate was usually quite small, so that several withdrawals with the syringe were required to obtain an adequate sample. The laboratory’s technical staff examined the Giemsa-stained smear. No special attempts were made to count the time or the number of fields of observation.

Sensitivity was calculated for several time intervals. Ninety-five percent confidence intervals (CIs) were calculated for proportions. Linear regression was used to test the correlation between the time of observation and the number of fields examined and to indicate the time taken per field examination. The Mann-Whitney test was used to compare the time of observation across different age groups and sex. Data were
analyzed with the Stata® statistical package (Stata Corp., College Station, TX).

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RESULTS

Among the 98 patients in the BM group, 84 (85.7%) had a diagnosis of VL in their medical records. After this study, the diagnosis of three additional patients was changed to VL, resulting in 87 patients (89%). The effect of time on sensitivity was as follows: 35 of 87 (40.2%) were diagnosed in 1 minute, 57 of 87 (65.5%) in 5 minutes, 78 of 87 (89.7%) in 20 minutes, (89.7%, 95% CI = 81–95%), 80 of 87 (92.0%, 95% CI = 84–97%) in 30 minutes, and 83 of 87 (95.4%, 95% CI = 89–99%) in 60 minutes. Figure 1 shows this time dependence.

One smear from a patient with VL inadvertently examined for more than one hour was positive, yielding the final sensitivity result of 84 of 87 (96.6%) for bone marrow smear examination. If the medical record diagnosis (before the study was performed) was used as the standard, the sensitivity in 60 minutes decreased to 80 of 84 (95%). Sixty-three patients (73%) were male. Most were children (mean age = 13.7 years); 51 (57%) of 87 patients were < 15 years old and 45 (52%) were ≥ 4 years old. The shortest time to obtain a positive result was four seconds. Seven (8.0%) patients with VL in this group died of causes unrelated to bone marrow aspiration.

The sensitivity according to the number of fields examined was 7 (8.0%) of 87 in the first field, 20 (23%) of 87 in 10 fields, 39 (44.8%) of 87 in 50 fields, 48 (55.2%) of 87 in 100 fields, 67 (77.0%) of 87 in 500 fields, 76 (87.4%, 95% CI = 79–94%) of 87 in 1,000 fields, 78 (89.7%, 95% CI = 81–95%) of 87 in 1,200 fields, and 82 (94.3%, 95% CI = 87–98%) of 87 in 1,500 fields. Figure 2 presents the relationship between sensitivity and the number of fields examined.

A strong correlation was found between the number of fields studied and the time of observation ($R^2 = 0.91$, $P < 0.0001$) and is shown in Figure 3. The linear regression coefficient of 1.07 indicates the regressed time in seconds spent per field examination. Parasites were identified more rapidly in children < 3 years old compared with those ≥ 3 years old (median = 60 versus 166 seconds; $P = 0.04$). Of the seven patient samples in which parasites were identified in the first field, six (86%, 95% CI = 42–100%), came from children ≤ 36 months old. Only 6 of 17 (35%, 95% CI = 14–62%) patients in this age group had smears with parasites identified after the examination ≥ 1,500 fields.

Of the 120 patients in the SA group, 114 (95%) were given a final diagnosis of VL. Among these, 74 (65%) were males.
and 64 (57%) were ≤ 4 years old with a mean age of 8.8 years. The sensitivity of spleen aspirates was 106 of 114 (93%, 95% CI = 87–97%). One patient (0.8%, 95% CI = 0–4.6%) who underwent the procedure died. This was a two-year-old girl who died of shock 50 minutes after the procedure. This death, in addition to the deaths of two other patients not included in this study (a 3-month-old boy and a 21-year-old man who died 10 and 2 hours, respectively, after the procedure), led to the elimination of splenic aspiration as a routine option for the diagnosis of VL at the hospital. Three patients (2.6%) in the SA group died, one as a result of the procedure and two of other causes.

**DISCUSSION**

The relationship between the sensitivity of bone marrow aspiration and the examination time and number of fields studied was not linear. In a minority of the patients, a large number of fields had to be viewed to identify amastigotes. In the majority, however, they were identified in the first few minutes, and a few patients, mostly small children, had parasites visualized in the first field that was studied. These groups reflect the distribution of parasite density in VL patients, and they can be divided into categories of scarcely, moderately, and heavily parasitized. In this study, young children comprised the majority of the heavily parasitized group and were rare in the least parasitized group. This finding is in accordance with the higher infectivity of children to the sand fly vector of the disease in the New World, *Lutzomyia longipalpis*, and confirms the idea that they are important reservoirs of VL.\(^\text{13}\)

The identification of amastigotes was an easy and rapid task in most cases for the well-trained observer, who took only a little more than one second per field. Less experienced technicians might take longer than the time observed in this study. Slower observers would examine fewer fields during the same time of observation as faster ones.

Adding the three subjects who had their diagnosis changed to VL after extensive bone marrow examination is appropriate for the purpose of this research. One reason is that the study intended to determine the best possible performance of bone marrow smear examination. In contrast, due to their size, spleen aspirates are rapidly examined in their entirety as a matter of routine. If bone marrow smears had been examined as thoroughly at the time of the epidemic as they were later when the study was performed, the diagnosis of VL would have been established in the three patients during their hospital stay, just as spleen aspirates were used to make the diagnosis of VL. Therefore, the analysis is appropriate, although it led to a small increase of sensitivity.

A rapid increase in sensitivity was observed as the examination time continued to increase up to 20 minutes. After this time, the increase was small, only 2% after an additional 10 minutes. Although the sensitivity increased to 95% at one hour, and to 97% beyond that time, which is the highest ever published for bone marrow examination,\(^\text{14–18}\) it seems impractical to take this long for the examination on a routine basis, especially during an epidemic. Similarly, 1,000 fields yielded a sensitivity of 89% and 1,500 fields increased the sensitivity to 93%. This 4% gain cost almost 10 minutes. Therefore, guidelines would set at least 1,200 high-magnification fields (20 minutes), with a sensitivity of 90%, as the minimum before determining that a sample was negative. Obviously, more time spent on the examination is recommended for negative smears from patients with a high pre-test probability, particularly if the laboratory staff is not hard-pressed for time. This threshold (1,200 high-magnification fields) must be clearly defined in proper guidelines, where the technique for performing the aspiration should also be described, as recently published.\(^\text{19}\) Aspiration should preferably be done from the iliac crests and, when the sternum is chosen, it should be done slightly laterally and from the first part of the bone or from the manubrium.

Patients with VL in the SA group were slightly younger than patients from the BM group, but the sex and age-group distributions were very similar in the two groups. More patients in the BM group died, but in none of these cases was the cause of death related to the bone marrow aspiration procedure. The reason for this increased mortality must be attributed to patient selection. Due to the higher risks associated with splenic aspiration, severely ill patients might have been selected for bone marrow aspiration, and therefore patients in this group could have had a higher chance of death from causes other than the procedure.

One advantage of spleen aspirates is the speed of the microscopic examination. The small volume of the fine-needle spleen aspiration offers only a very small area to be studied, so it takes only 1–2 minutes. The other advantage of spleen aspirates is the high sensitivity. Although spleen smears were not examined by the same well-trained researcher who examined the bone marrow smears, our hospital’s experience resulted in a sensitivity only slightly lower than the levels published elsewhere (approximately 95%).\(^\text{14,20,21}\) Therefore, the speed and sensitivity of the procedure would suggest on first glance that spleen aspirates would be the best parasitologic method for the diagnosis of VL.

For a long time, the indications for spleen aspiration in the diagnosis of VL have been debated due to concerns about the risk of serious bleeding.\(^\text{19}\) However, the decision of the hospital administration to eliminate the procedure was not based on emotions. Although fewer than 3% of the patients in the SA group died of VL, one of the three fatalities was due to the diagnostic procedure. Since the results in most of the patients who underwent spleen aspiration ended up confirming the clinical suspicion of VL, the medical personnel felt uncomfortable using a potentially fatal procedure for a disease whose clinical diagnosis is not difficult and whose mortality is relatively low, especially when less risky diagnostic alternatives are available.

The decision to choose a particular method for the parasitologic diagnosis of VL must be based on considerations of efficiency and risk. Bone marrow aspirates have a lower sensitivity if examined quickly. However, if they are studied for an appropriate length of time, they can achieve a sensitivity close to that of spleen aspiration. Published statistics relating to spleen aspiration show a lower death rate than we found at our hospital.\(^\text{20,21}\) One possible explanation is the higher proportion of small children with VL in Teresina, as compared with other areas where the method is frequently used. In both India and Kenya, areas with transmission of *L. donovani* and with the largest experience with spleen aspiration, the proportion of small children with VL is much lower than in areas where *L. chagasi* or *L. infantum* are transmitted.\(^\text{22}\) A historic review describes a rate of one death or less per 1,000 for...
A recent evaluation of the risk of bone marrow aspiration in England identified only one death in more than 50,000 biopsies. This comparison suggests that the risk of death is 50 times higher for spleen aspiration.

This study indicates that with its similar sensitivity and much lower mortality, bone marrow aspiration is the best option for the parasitologic diagnosis of VL, especially where small children are concerned. However, the full value of bone marrow aspirates will be achieved only if an adequate amount of time is spent on the examination, which can be difficult during epidemics or in places where work schedules are not followed strictly. It is possible that the use of a tagged antibody marker could reduce the amount of time needed for screening in the future. Finally, our good results regarding bone marrow aspiration must be confirmed elsewhere, particularly in settings in which clinicians are able to adhere to strict guidelines for the minimum number of bone marrow fields to be examined.

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