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Original article

The concurrent occurrence of *Leishmania chagasi* infection and childhood acute leukemia in Brazil

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ABSTRACT

Objective: This study investigated the co-existence of *Leishmania chagasi* infection and childhood leukemia in patients naïve to treatment; this has serious clinical and epidemiological implications.

Methods: The seroprevalence of *L. chagasi* antibodies prior to any treatment was investigated in children with clinical features of acute leukemia. Serological tests were performed in 470 samples drawn from under 14-year-old children from different regions of Brazil with clinical suspicion of acute leukemia. Acute leukemia subtypes were characterized by immunophenotyping using flow cytometry. Morphological analyses of bone marrow aspirates were systematically performed to visualize blast cells and/or the formation of *L. chagasi* amastigotes. Data analysis used a standard univariate procedure and the Pearson's chi-square test.

Results: The plasma of 437 children (93%) displayed antibodies against *L. chagasi* by indirect immunofluorescence assay and enzyme-linked immunosorbent assay tests. Of the 437 patients diagnosed from 2002 to 2006, 254 had acute lymphoblastic leukemia, 92 had acute myeloid leukemia, and 91 did not have acute leukemia. The seroprevalence of *L. chagasi* antibodies according to the indirect immunofluorescence assay test (22.5%) was similar in children with or without acute leukemia (p-value = 0.76). The co-existence of visceral leishmanasis and acute leukemia was confirmed in 24 children. The overall survival of these children was poor with a high death rate during the first year of leukemia treatment.

Conclusion: In the differential diagnosis of childhood leukemia, visceral leishmanasis should be considered as a potential concurrent disease in regions where *L. chagasi* is endemic.

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Introduction

Acute leukemia (AL) is the most common childhood malignancy. It is recognized as a disease with heterogeneous biological characteristics. Great progress has been made toward a cure and understanding the pathogenesis of AL.[1,2] An international survey of data that compared the relative frequencies of the different AL subtypes has demonstrated consistent frequencies among groups stratified according to age, gender, ethnicity, and social conditions.[3,4] To clarify the etiology of childhood leukemia, epidemiological studies have attempted to gain some understanding about the different rates of acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) in association with genetics, infections, and other environmental factors.[5] A recent population-based study of childhood leukemia demonstrated that substantial regional differences exist in the incidence of AL in Brazil, which warrants further studies.[4] These differences may be related to the underreporting of AL cases in some less-developed areas. In this context, visceral leishmaniasis (VL) or Kala-zar, a tropical disease caused by the intracellular protozoan parasite, *Leishmania infantum* (syn. *L. chagasi*), should be considered clinically as a co-morbid disease that can complicate the diagnosis of AL. The signs and symptoms of VL are very similar to those of some childhood types of AL. Affected children present splenomegaly, anemia, neutropenia, thrombocytopenia, and/or increased abnormal lymphocyte counts. Coagulation abnormalities have also been found in patients, often associated with disseminated intravascular coagulopathy.[6] Additionally, atypical cells and unusual blasts may be observed in bone marrow aspirates of patients with VL.[7,8] Thus, it is important to include VL in the differential diagnosis of AL in endemic areas.

In Brazil, VL frequently occurs in remote locations and endemic areas, and for the general pediatrician, AL is not the first disease to be investigated. Furthermore, VL treatment is often performed on the basis of clinical suspicion, because it is associated with high mortality in the absence of treatment.[9] The final diagnosis of AL is hampered by the complexity of clinical diagnoses, for instance, infections can stimulate the hypothalamus–pituitary–adrenal axis, leading to increased plasma cortisol levels that are sufficiently high to eliminate clonal leukemic cells,[10] thereby delaying decisions. In essence, AL and VL are serious diseases that require a rapid, proper diagnosis and adequate treatment to reduce childhood mortality. The present study investigated a series of samples from patients suspected of childhood leukemia at diagnosis, for the presence of *L. chagasi* antibodies and evaluated how the relationship between these two severe diseases can affect children.

Methods

Subjects

Serum samples from 785 children were selected for this study. The study population was enrolled throughout a multidisciplinary project that had been ongoing in the Pediatric Hematology-Oncology Program of the Research Center at the Instituto Nacional de Câncer (INCA), Rio de Janeiro, Brazil. Bone marrow (BM) aspirations and peripheral blood (PB) samples were sent for immunophenotyping-genotyping for a study on acute childhood leukemia during the period of 2001–2007. Complete epidemiological data have been described in detail elsewhere.[11] Biological samples (BM and PB) were first evaluated to determine the morphological characteristics of lymphoid and myeloid blast cells. Then, an algorithm of immuno-molecular testing was performed: (i) morphological characteristics of lymphoid and myeloid cells according to standard criteria, (ii) immunophenotyping of BM aspirates; (iii) DNA index (only in ALL) and (iv) identification of abnormal fusion genes according to leukemia subtypes.[12] The panel of monoclonal antibodies (MoAb) recommended by the European Group for the Immunological Characterization of Leukemias was applied to isolated mononuclear cells and analyzed by flow cytometry.[13] Briefly, the combination of fluorochrome-labeled MoAbs was used in triplet and/or quadruple staining experiments, using fluorescein isothiocyanate (FITC), phycoerythrin (PE) and PE-cyanine 5 (PECy5) and/or APC fluorochrome conjugates in each tube. Cell samples were analyzed by flow cytometry using a FACSCalibur device (Becton, Dickinson and Company, CA, USA) with the Cell-Quest and Paint-a-Gate computer programs.

1. Intracytoplasmatic – CD79b and/or CD22FITC/CD3PE/CD45PECy5 or APC; TdTFITC/aMPOPE/CD33/CD13 PECy5/CD45APC as initial screening;
2. Membrane surface according to screening results – if B-cell markers (CD79/CD22/TdT$^+$) were positive, then CD10FITC/CD19PE/CD45PECy5, CD34FITC/CD38PE/CD45PECy5, CD58FITC/CD10PE/CD19PECy5/CD45APC, SmIgFITC/CD20PE/CD19 PECy5 and CD4FITC/CD8PE/CD3 PECy5/CD45APC were performed. If T-cell markers (cCD3/TdT$^+$) were positive, then CD7FITC/CD33/13PE/CD45PECy5, CD34FITC/CD1aPE/CD45PECy5 and CD4FITC/CD8PE/CD3 PECy5/CD45APC were performed. Finally, a panel for anti-myeloid antigen cells was tested when myeloid morphology and/or intracytoplasmatic CD13/aMPO$^+$ were predominant. This panel consisted of CD34FITC/CD38PE/CD7PECy5/CD45APC, CD64FITC/CD14PE/CD33PECy5/CD45APC and CD15FITC/HLADrPE/CD7PECy5/CD45APC.

Cell surface antigens were considered positive when 20% or more cells showed fluorescence intensity greater than the negative control in the gate for CD45^{low} cells, while the cutoff for the cytoplasmic antigen aMPO was 10% in the gate for CD45^{low} cells. Cases with unusual positive markers were tested twice.

AL types were classified as B cell precursor ALL (Bcp-ALL), pro-B-cell, common B, and pre-B ALL; B-ALL; T-ALL, and AML accordingly.[11,13] Subsequently, RNA was processed for c-DNA; *MLL-AF4*, *TEL-AML1*, *E2A-PBX1* and *BCR-ABL1* were performed in the Bcp-ALL samples, whereas, the *SIL-TAL1* fusion and *HOX11L2* were performed in T-ALLs as has been described elsewhere.[14,15]

Patients with diagnoses that excluded ALL or AML and other malignant diseases were designated to the 'Non-leukemic Group'.