

Langerhans cell histiocytosis: 37 cases in a single Brazilian institution

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Objectives: To improve the level of 'definitive' diagnosis of Langerhans cell histiocytosis by immunohistochemical investigation of the CD1a surface antigen and to compare outcomes in respect to age, gender, stage of the disease, treatment response and level of diagnostic accuracy.

Methods: A retrospective study was carried out of 37 children and adolescents with possible Langerhans cell histiocytosis between 1988 and 2008. The diagnoses were revisited using immunohistochemical investigations for CD1a, S-100 and CD68 in an attempt to reach definitive diagnoses for all cases.

Results: Before the study, only 13 of 37 patients (35.1%) had a 'definitive' diagnosis; by the end of the study, this number rose to 25 patients (67.6%). All reviewed cases were positive for the CD1a antigen. Overall survival was 88.5%. Multisystem disease (Stage 2; n=19) and absence of response at the 6th week of therapy (n=5) were associated to significantly lower overall survival (p-value = 0.04 and 0.0001, respectively). All deaths occurred in patients with multisystem disease and organ dysfunction at diagnosis. Other potential prognostic factors were not significant. Reactivation episodes occurred in 75% of the patients with multisystem disease. Diabetes insipidus was the most common sequel (21.6%).

Conclusion: The level of diagnostic accuracy was increased through immunohistochemistry. The overall survival rate was similar to international multicentric studies. Multisystem disease and absence of response at six weeks of treatment were the most important unfavorable prognostic factors. The frequency of reactivation for patients with multisystem disease was higher than described in the literature, probably because maintenance chemotherapy was used only in two cases.

Keywords: Histiocytosis; Langerhans-cell; Pathology; Prognosis; Diabetes insipidus; Otitis

Introduction

Langerhans cell histiocytosis (LCH) is a rare disease that mainly affects children. Of unknown etiology, it is caused by clonal proliferation of Langerhans cells and may involve any organ or tissue in the body. The clinical manifestations are a result of this involvement and can range from isolated lesions that evolve into spontaneous remission to multisystemic and severe forms leading to death. The sites most commonly affected are the bones, skin and lymph nodes. Frequent recurrences can lead to significant morbidity and severe sequelae may therefore result. Depending on the clinical presentation, treatment can range from watchful waiting, surgical excision of lesions, steroid therapy, chemotherapy and bone marrow transplantation.⁽¹⁾

'Presumptive' diagnosis is considered when conventional histology of the lesions is characteristic; 'probable', when, in addition, two of the following histochemical reactions are positive: S100 protein, mannosidase, ATPase or peanut lecithin. A 'definitive' diagnosis is reached when, associated with a suggestive clinical picture, immunohistochemical investigation of suspected lesions test positive for the CD1a antigen or when Birbeck granules are found under electron microscopy.⁽²⁾ Recently, positive staining with Langerin (CD207) has also been considered a definitive diagnosis.^(1,3) The main risk factors related to death are multisystem disease, risk-organ involvement and the absence of response by the 6th week of therapy.⁽⁴⁻⁶⁾

Only 7 out of 33 patients included in a previous study from the same group of investigators had definitive diagnoses for the disease⁽⁷⁾ when, ideally, all cases should have one.^(4,5,8)

The main aim of the current study was to establish definitive diagnoses for those patients as well as the evaluation of four new cases. Disease outcomes were compared according to age, gender, disease stage, response at the 6th week of therapy and diagnostic certainty.

Methods

The study was retrospective (1988-2008) and analyzed all patients with ages at diagnosis of less than 18 years old between June 1988 and December 2008. The patients were diagnosed and treated at a single university hospital in Brazil. Clinical data were obtained through a review of medical records.

Criteria for histological diagnosis were based on the recommendations by the Histiocyte Society.⁽²⁾ 'Presumptive' diagnosis was established on conventional histological findings; 'probable' diagnosis, when these findings were associated with positive staining for protein S-100 by immunohistochemistry. A 'definitive' diagnosis was reached based on electron microscopy (presence of Birbeck granules) or CD1a positive immune staining. The paraffin blocks and slides from previous biopsies of all cases without definitive diagnoses were retrieved as were three cases for which a definitive diagnosis had previously been reached. Immunohistochemical investigations for CD1a, S-100, and CD68 were carried out for all available blocks. The corresponding histological slides were analyzed by two of the involved investigators.

Patients were retrospectively classified⁽⁶⁾ as:

- Stage 1: Only one organ or system involved. The involvement can be unifocal (1a) or multifocal (1b);
- Stage 2: Multisystem disease with more than one organ or system involved, with or without organ dysfunction.

Patients were retrospectively divided into 3 categories, according to the response observed at the 6th week of therapy:⁽⁶⁾

- "Better" response: total resolution or continued regression of disease;
- Intermediate response: stable disease or regression in some sites with new lesions in others;
- Lack of response ("worse"): disease progression.

Reactivation was defined as the appearance of new lesions or organ dysfunction after remaining stable for three months.⁽⁹⁾ The Kaplan-Meier method was used for survival analyses. Event free survival (EFS) was estimated considering both death and reactivation as events. Patients without any events on the day of statistical analysis (December 15, 2008) were censored. The Logrank test was used to compare survival curves. A p-value ≤ 0.05 was considered statistically significant.

The study was approved by the Universidade Federal de Minas Gerais Research Ethics Committee (Protocol ETIC 271/07).

Results

For the period of 1988-2008, 37 patients were followed up, 20 of which were female (54%). Age at diagnosis varied from 1 month to 16.9 years old (median 2.4 y). Time of follow up varied from 1 month to 20.9 years (median 6.2 y).

Table 1 - General characteristics of 37 children and adolescents with Langerhans cell histiocytosis

Characteristic	n (%)
Gender	
Male	17 (45.9%)
Female	20 (54.1%)
Follow-up time	1 month - 20.9 years
Median	6.2 years
Age at diagnosis	1 month - 16.9 years
Median	2.4 years
Definitive diagnosis	
Before study	13 (35.1%)
After study	25 (67.5%)
Clinical stage	
Single-system disease - unifocal	10 (27%)
Single-system disease - multifocal	7 (19%)
Multisystem disease	19 (51%)
Isolated lung involvement	1 (3%)
Response at the 6 th week of therapy	
"Better"	27 (72.9%)
"Intermediate"	4 (10.8%)
"Worse" (lack of response)	5 (13.5%)
Clinical status at the time of analysis	
In remission, still under follow-up	21 (56.7%)
In remission, discharged	8 (21.6%)
Lost to follow-up	4 (10.8%)
Active disease	1
In remission	3
Death	4 (10.8%)

The general epidemiological data of patients are summarized in Table 1.

Thirteen (35.1%) of the 37 patients had had 'definitive' diagnoses before the study. Of the 24 patients without a 'definitive' diagnosis, 11 (29.7%) had previous 'probable' diagnoses and 13 (35.1%) had 'presumptive' diagnoses. Of the 11 patients with 'probable' diagnoses, 5 samples were found for review. Of the 13 patients with 'presumptive' diagnoses, 7 samples were found. We also reviewed 3 randomly selected cases that previously had 'definitive' diagnoses, (2 with previous CD1a positivity and 1 with electron microscopy evidence). All 15 reviewed samples tested positive for CD1a.

At the end of the study, 25 patients (67.6%) had 'definitive' diagnoses and 31 (83.8%) had 'probable' or 'definitive' diagnosis (Figure 1).

The most frequent clinical manifestation at diagnosis was osteolytic lesions in 67.6% of the cases, followed by lymphadenomegaly and cutaneous or mucocutaneous lesions (45.9% each; Table 2). In relation to disease stage, 19 patients (51.4%) presented with multisystem disease (Stage 2), 6 of whom had dysfunctions of one or more risk-organs (lung, liver or hematopoietic system). Eighteen patients (48.6%) had single-system disease (Stage 1), including one with pulmonary involvement who was excluded from survival analysis because at the time of analysis this presentation had not yet been included in the

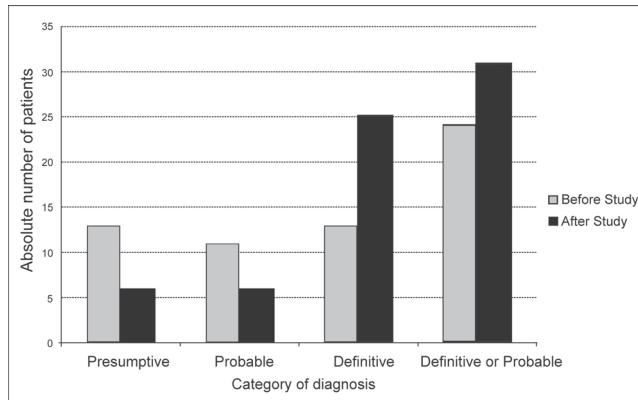


Figure 1 – Absolute number of patients by diagnostic category before and after the study

Table 2 - Clinic manifestations at the diagnosis of 37 children and adolescents with Langerhans cell histiocytosis

Clinical manifestation	Patients n (%)
Osteolytic lesions	25 (67.6%)
Lymphadenomegaly	17 (45.9%)
Cutaneous or mucocutaneous lesions	17 (45.9%)
Papular-crusted lesions on the scalp or inguinal region	9
Involvement of the gum or palate	3
Ulcerated lesions of the vulva	1
Nodular lesions of the lips	1
Others	3
Fever	12 (32.4%)
External auditory canal	9 (24.3%)
Hepatomegaly	9 (24.3%)
Hepatic dysfunction	4 (10.8%)
Splenomegaly	7 (18.9%)
<i>Diabetes insipidus</i>	4 (10.8%)
Pulmonary involvement as part of multi-system disease	3 (8.1%)
Pulmonary disease in isolation	1 (2.7%)
Involvement of the hematopoietic system	3 (8.1%)
Immunological alterations	2 (5.4%)

classification of the Histiocyte Society. Eleven patients (29.7%) had unifocal (Stage 1a) and seven (18.9%) multifocal disease (Stage 1b).

The most commonly used treatment initially was prednisone with vinblastine (17 out of 37 patients; 45.9%). Three patients were submitted to the treatment protocol proposed by the Histiocyte Society International Study Protocol (LCH III - Arm A). Of the 17 remaining patients, six received etoposide, three received prednisone alone, three underwent surgical resection, two received no treatment (watchful waiting) and three received other types of treatment. Only two patients received maintenance chemotherapy as part of the Histiocyte Society LCH-III protocol. A third patient, also treated with this protocol, had not reached the maintenance phase at the time of censoring for this report.

In relation to response by the 6th week of therapy, 27 (73%) had a "better" response, 4 (10.8%) an intermediate response and 5 (13.5%) a lack of response. It was impossible to evaluate the response in one case for lack of data. This patient was excluded from the survival analysis for this variable.

At the end of the study, 29 patients were in complete remission: eight had been discharged and 21 were still in follow-up. Four children had died. Clinical follow-up was lost for four patients: one was in remission at the time of his last visit (5 years from diagnosis), but had presented an episode of reactivation in the seventh month of observation. This event was considered in the analysis of EFS; for overall survival (OS), the patient was censored on that date. Two patients who dropped out were in continuous clinical remission and were censored at the date of their last visit with 4 and 5 years of follow-up. The fourth patient had not achieved clinical remission and abandoned her follow-up after one and a half years (Table 1).

OS for the whole group was 88.5% (95% confidence interval - 95% CI: 72.1-95.5%). As previously mentioned, four patients died. All deaths occurred among patients with multisystem disease and organ dysfunction at diagnosis. OS was significantly higher for patients with single-system (100%) when compared to those with multisystem disease (77.2%; 95% CI: 49.7-90.8%; p-value = 0.04; Figure 2a).

OS probability was significantly lower for patients with no response at the 6th week of treatment (30%; 95% CI: 1.2-71.9%) when compared to patients with 'better' (95.8%; 95% CI: 73.9-99.4%) or 'intermediate' response (100%; p-value = 0.0001; Figure 2b). There was no statistically significant difference in OS in terms of age younger or older than 2 years old (p-value = 0.14), age younger or older than 3 years old (p-value = 0.47), gender (p-value = 0.96) or degree of diagnostic certainty (p-value = 0.71).

The probability of EFS at 10 years for the whole group was 32.5% (95% CI: 17.9-48.1%). Of the 33 who entered into remission, 20 children (60.6%) had at least one reactivation episode. When only patients with multisystem disease are considered, a higher rate (75%) of reactivation was recorded. The majority of first reactivation episodes (60%) happened within the first two years of follow-up.

The probability of EFS was 47.1% (95% CI: 23-68%) for patients with single-system disease and 14.1% (95% CI: 26-35.1%) for patients with multisystem disease, a difference that was not statistically significant (p-value = 0.12). When patients with complete response were added to those with intermediate response, the probability of EFS was 38.9%. Patients unresponsive at the sixth week of treatment had 0% probability of EFS, a difference that was statistically significant (p-value = 0.0001). There was no statistically significant difference regarding gender (p-value = 0.8) or age younger or older than two years (p-value = 0.81).

There was no statistically significant difference in the probability of OS and EFS with respect to the degree

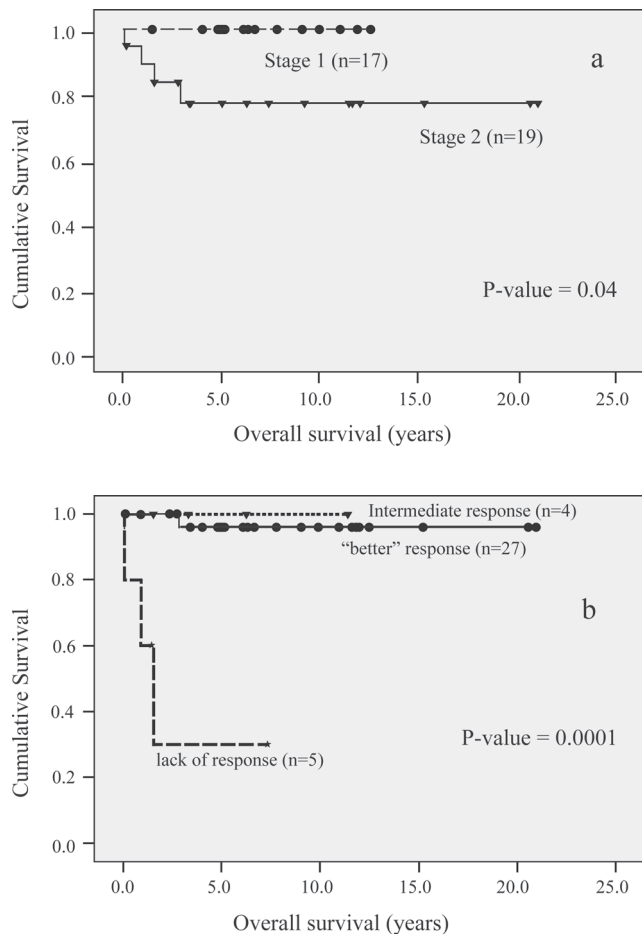


Figure 2 – Overall survival by groups (Kaplan-Meier's method); small marks over the curves represent *censored* patients;
 a: Curves according to disease stage at diagnosis; Stage 1 (n = 17): involvement of one organ or system with unifocal or multifocal lesions; Stage 2 (n = 19): multisystem disease (p-value = 0.04);
 b: Curves according to the response observed at the 6th week of therapy; 'better' (n = 27), intermediate (n = 4), or lack of response (n = 5); p-value = 0.0001

of diagnostic certainty (p-value = 0.71 and 0.39, respectively).

The presence of one or more permanent sequelae occurred in 15 of the 37 patients (40.5%) with diabetes insipidus, detected in eight cases (21.6%), being the most common. Of the 15 patients with sequelae, 9 (60%) had multisystem disease and of the total of patients with multisystem disease, 47.4% presented with at least one permanent sequel.

One patient developed acute promyelocytic leukemia two years and nine months after etoposide had been given as treatment for disease relapse. Prednisone with vinblastine without radiation was the initial treatment for this patient.

Discussion

Diagnostic confirmation of LCH is very important in particular in dubious cases – such as in cases in which the disease is confined to lymph nodes⁽¹⁰⁾ – and also to compare

results with those reported in international studies that only include patients with definitive diagnoses.^(4,5,8) Despite the limitations of a retrospective study, the main aim of the present study was attained with an increase in the proportion of patients with definitive diagnoses (from 35% to 67.5%). All the reviewed tests were positive for CD1a. This shows the importance of carrying out conventional histological examinations routinely by professionals with experience at diagnosing LCH. Moreover, this demonstrates how effective and practical the use of CD1a detection can be in confirming the disease: paraffin blocks obtained as early as 1988 stained positively, for instance. Langerin was not used because it was unavailable in Brazil when this study was carried out.

Some studies also include patients with 'probable' diagnoses, in addition to those with 'definitive' diagnoses.^(11,12) At the end of this study, 31 of the 37 patients (83.8%) had 'probable' or 'definitive' diagnoses. Of the 6 patients who remained with 'presumptive' diagnoses, 4 presented clinical manifestations highly suggestive of the disease, such as diabetes insipidus and multisystem disease. Lymphadenomegaly was the single clinical manifestation in two patients and only in these, a diagnosis of LCH might be considered dubious. For this reason, every case was included in the statistical analyses.

Clinical manifestations at diagnosis were very similar to international reports.^(13,14) Isolated or combined osteolytic lesions are by far the commonest reported manifestation and were recorded in two thirds of our patients. Lymphadenomegaly and cutaneous or mucocutaneous involvement are also very common and were present in almost half of our patients. A quarter of patients had involvement of the external auditory canal at diagnosis. This finding is relevant in the general pediatric practice as there is a hypothesis that LCH should be considered in children with persistent otitis refractory to usual treatments, associated with dermatitis of the outer ear.

The estimated OS was similar to that found in international multicenter studies.⁽⁴⁻⁶⁾ Likewise, bad prognostic factors in the present study were multisystem disease with risk-organ dysfunction and the absence of response by the 6th week of therapy.⁽⁴⁻⁶⁾ Results of the LCH II study showed that therapy intensification is of crucial importance for this type of patient.⁽⁶⁾

The majority of patients who had remission at some point presented at least one episode of disease reactivation. Most reactivation episodes occurred within the first two years of follow-up which is in agreement with previous reports.^(9,15) Also similar to reports in the literature, the majority of patients with permanent sequelae presented multisystem disease.^(9,16,17) The frequency of reactivations in the group with multisystem disease was considerably higher than that verified in international studies.⁽⁴⁻⁶⁾ A number of treatment protocols were used during the studied period, which makes it impossible to compare treatment of this study with others. Remarkably, however, only two patients received maintenance

therapy. With the added knowledge of more recent publications,⁽¹⁾ we can state that many patients who had LCH reactivation could probably have benefited from an increase in therapy intensity and/or duration to reduce reactivations, and possibly, sequelae.

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