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Predictors of an unsatisfactory response to pentavalent antimony in the treatment of American visceral leishmaniasis

Indicadores de resposta insatisfatória ao antimônio pentavalente no tratamento da leishmaniose visceral americana

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Abstract *Although treatment of visceral leishmaniasis with pentavalent antimony is usually successful, some patients require second-line drug therapy, most commonly with amphotericin B. To identify the clinical characteristics that predict an inadequate response to pentavalent antimony, a case-control study was undertaken in Teresina, Piaui, Brazil. Over a two-year period, there were 19 cases of VL in which the staff physicians of a hospital prescribed second-line therapy with amphotericin B after determining that treatment with pentavalent antimony had failed. The control group consisted of 97 patients that were successfully treated with pentavalent antimony. A chart review using univariate and multivariate analysis was performed. The cure rate was 90% with amphotericin B. The odds ratio for the prescription of amphotericin B was 10.2 for children less than one year old, compared with individuals aged over 10 years. Patients who presented coinfection had an OR of 7.1 while those on antibiotics had an OR of 2.8. These data support either undertaking a longer course of therapy with pentavalent antimony for children or using amphotericin B as a first-line agent for children and individuals with coinfections. It also suggests that chemoprophylaxis directed toward bacterial coinfection in small children with VL may be indicated.*

Key-words: *Visceral leishmaniasis. Antimony. Amphotericin B. Therapy.*

Resumo *Embora o tratamento da leishmaniose visceral com antimoniato pentavalente seja normalmente bem sucedido, alguns pacientes requerem uma terapêutica de segunda linha, habitualmente com anfotericina B. Para identificar as características clínicas que possam indicar uma resposta inadequada ao antimônio pentavalente, realizou-se um estudo caso-controle em Teresina, Piauí, Brasil. Em um período de dois anos ocorreram 19 casos de LV para quem os médicos de um hospital prescreveram o tratamento de segunda linha com anfotericina B após determinarem falha com o uso de antimônio pentavalente. O grupo controle foi constituído por 97 pacientes que tiveram o tratamento com antimônio bem sucedido. Foi feita uma análise de prontuários, utilizando-se análise univariada e multivariada. A porporção de cura após o uso de anfotericina B foi 90%. A razão de odds para a prescrição de anfotericina B foi 10,2 para crianças com menos de um ano, quando comparadas com pessoas com mais de 10 anos. Pacientes que tinham tido co-infecção tinham uma RO de 7,1 e aqueles que tinham utilizado antibióticos tinham uma razão de odds de 2,8. Estes dados estão de acordo com o uso mais prolongado de antimônio pentavalente para crianças ou com a recomendação de anfotericina B como droga de primeira escolha para crianças e para pessoas com co-infecções. Sugerem também que se pode indicar a quimioprofilaxia para infecções bacterianas em crianças menores com LV.*

Palavras-chaves: Leishmaniose visceral. Antimônio. Anfotericina B. Terapêutica.

First-line therapy for visceral leishmaniasis (VL) still consists of the parenteral administration of salts of pentavalent antimony (Sb^V), 90 years after it was first used successfully for this purpose[2]. Only recently**,** after the first trials with miltefosine**,** has a potentially superior alternative been identified for Indian kala-azar[10 21]. Treatment generally achieves cure rates greater than 90 percent[11 12]. Those patients who survive the initial treatment but are not cured are treated with a second-line drug. The two major choices are amphotericin B and pentamidine[3]. Each of these is problematic: although amphotericin B is affordable, it may entail a prolonged

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and difficult course of treatment[24 26], and pentamidine has serious side effects[23].

Since the transformation of VL into an urban disease in Brazil, it has been defined by epidemics involving large numbers of patients in multiple cities[7 12]. In an epidemic in the city of Teresina, nearly one thousand people were treated with pentavalent antimony during a two-year period, and a small percentage of these patients subsequently received therapy with amphotericin B. The indication for a second-line agent was based upon clinical parameters, although parasitologic criteria to define a treatment failure had been proposed, the risks observed in Teresina associated with splenic aspiration to obtain a parasite count rendered these impractical. We attempted to identify the clinical predictors of a failure to respond to treatment so that we could determine which patients would benefit from a change in the initial therapeutic regimen.

PATIENTS AND METHODS

Data was gathered about all patients with VL in Teresina. A case-control study was designed involving the cases of 19 patients treated at the Hospital of Infecto-Contagious Diseases over a two-year period for whom second-line therapy with amphotericin B was prescribed. Only one patient who was treated with amphotericin B in Teresina had not been originally diagnosed and treated in this hospital, and this patient was excluded from the study.

The controls were 97 patients chosen among those who were admitted to the hospital for treatment of VL who survived the therapy and did not require the use of amphotericin B within 12 months of the initial treatment. They were admitted and treated during the same time period as the cases and were selected at random. Of the 100 controls initially selected, three died during the initial treatment phase and were therefore excluded. Consequently, this analysis only refers to patients who survived the initial treatment of VL. None of the controls required treatment with amphotericin B during the two years following the study.

The diagnosis criteria for the cases and controls were the presence of clinical signs of VL together with a positive serology, with or without the demonstration of the presence of the parasites in the body. Cases were defined as those patients whom the physicians at the hospital prescribed amphotericin B; this decision was based on the persistence of signals or symptoms, notably significant splenomegaly, after the completion of a course of pentavalent antimony and also on the recurrence after an apparent cure. The two principal methods of administering pentavalent antimony (Glucantime®) at the hospital have been courses consisting either of 40 daily doses of 20mg/kg/day up to a daily maximum of 850mg (as recommended by the World Health Organization[27]) or of 30-45 doses on alternating days, divided into 2-3 series of 15 applications of the same dose with 10-day intervals in between the series (an old dosing schedule still used in Teresina by some physicians based on early suggestions of treatment for Indian VL[20]). The total dose of amphotericin B was most frequently less than the maximum dose of 50mg/kg and was discontinued upon clear resolution of splenomegaly.

Statistics such as the total number of patients with LVA and the number of deaths were obtained from the files of the National Health Foundation, which had determined the place of origin of all patients in Teresina who received free treatment with pentavalent antimony. Clinical information was obtained from hospital files and from supplemental information provided by relatives of patients treated with amphotericin B. The clinical data and laboratory results considered were those that preceded the initial treatment with pentavalent antimony. The dimensions of the liver and spleen were not always recorded. The patients' levels of hepatic enzymes or blood urea, nitrogen and creatinine were usually not present in the records. Only occasionally could the platelet count be determined. All patients were submitted to a direct examination of amastigotes in the bone marrow, but only rarely was culture of the material performed.

The number of doses of pentavalent antimony administered to each patient was obtained from two databases: 1) hospital charts from admissions during which pentavalent antimony or amphotericin B was used, and 2) the records of medication distribution from the sector of epidemiologic vigilance (SEV) of the hospital. Frequently the number of doses was not recorded in the hospital chart, or the dose of pentavalent antimony given to a patient was not registered with the SEV because patients would receive the medication directly from the nursing staff when they were allowed to leave the hospital on a day pass and the SEV was closed.

Due to the National Health Foundation's monopoly on the distribution of pentavalent antimony, the restriction of the treatment of VL to only three hospitals in the city, and the fact that patients to whom amphotericin B is prescribed to treat VL are normally referred to this hospital (or used under the advice of a hospital's physician), the total number of patients in Teresina treated with amphotericin B and the total number of patients with VL in the city were known with a high level of accuracy.

The following clinical data obtained during the initial hospital admission were analyzed: sex, age, duration of fever, weight loss, presence of cough, diarrhea, abdominal distention, bleeding episodes, edema, pallor, icterus, or pulmonary rales, splenic size, hematocrit and white blood cell count; analysis of parasites in the bone marrow, results of an indirect immunofluorescence test for *Leishmania*, use of blood transfusions, presence of coinfections, use of antibiotics, and type and amount of Sb^{V} used. Chi-square test and Fisher's exact test were used in the univariate analysis. For continuous variables,

the Mann-Whitney test was used. The odds ratios were calculated in a univariate analysis, and a logistical multivariate regression was undertaken using the Stata® Statistical Software, College Station, Texas.

RESULTS

Despite an exhaustive search in two different sources, we were not able to obtain reliable data about the number of doses of pentavalent antimony used by each patient; frequently the number of registered doses was less than the minimum recommended, or each source had different information about a patient's use of the drug. This made it difficult to compare the two modes of prescribing pentavalent antimony used in the hospital: daily use and use on alternate days. Although the data do not permit us to determine whether some patients classified as non-responders received fewer than 40 doses, it would have been highly unlikely at this hospital. On the other hand, some patients not treated with amphotericin B received more than 45 doses of pentavalent antimony, and at least two patients received more than 100.

Of the 20 patients treated with amphotericin B, 19 were treated at this hospital and 16 were from Teresina. During the period of the study there were 941 patients with VL who were residents of Teresina. The prevalence of unsatisfactory response to pentavalent antimony among the patients from Teresina was therefore 1.7% (16/941). There was one death (5.3%) among the 19 non-responders who were treated at the hospital (OR = 1.8, $p<0.05$); this was an adult with diabetes mellitus who died of presumed sepsis. The single non-responder who was treated at another facility also died; this was an infant diagnosed with VL at three months who died during a splenectomy and still had parasites in the bone marrow after treatment with amphotericin B. Another adult patient required two courses of amphotericin B (almost 70mg/kg) but was cured. Another patient had AIDS but remained free of parasites after treatment with amphotericin B before dying of causes unrelated to VL. At least three non-responders required at least 50mg/kg of amphotericin B (which is the maximum recommended per series) and seven more patients required 40-50mg/kg. Since all the patients who did not die during treatment were cured, the cure rate in this population was 90% (18/20).

Only age and the occurrence of coinfection during the first hospital admission clearly were predictors of an unsatisfactory response. This relationship held true for all age groups of less than 10 years. It was strongest for infants below one year of age: the odds ratio was 10.2 in relation to persons older than 10 years of age. For children younger than 2, the *odds ratio* (OR) was 6.7; less than 5 was 5.9, and under 10 was 5.4. Table 1 shows the data for infants below 1, 2, 5 and 10 years of age compared with others aged 10 and over.

Of the 17 infections observed in the study population, 14 were diagnosed as pneumonia. The chance of an unsatisfactory response to treatment with pentavalent antimony and the need for treatment with amphotericin B was much greater among the patients who had had a coinfection, with an odds ratio of 7.1. Table 1 also summarizes this data. The use of antibiotics at hospital admission was only moderately associated with an unsatisfactory response. Multivariate logistical regression confirmed age and the presence of coinfection as independent predictors of an unsatisfactory response to pentavalent antimony.

Table 1 - Univariate analysis of the risk of therapeutic failure with pentavalent antimony for visceral leishmaniasis with age, the incidence of coinfections, and the use of antibiotics during the initial phase of treatment.

Risk factor	Exposed/non-exposed cases	Exposed/non-exposed controls	*Odds ratio*	Confidence interval (95%)	p Value
Age < 1 year old*	5/8	8/57	10.2	2.2 - 47.1	<0.01
Age < 2 years old*	9/12	22/71	6.7	1.8 - 25.0	<0.01
Age < 5 years old*	14/17	39/88	5.9	1.7 - 20.3	<0.01
Age < 10 years old*	16/19	48/97	5.4	1.6 - 18.5	<0,01
Coinfection	8/19	9/97	7.1	2.3 - 21.8	<0.001
Antibiotic usage	8/19	20/97	2.8	1.0 - 7.7	<0.05

* compared to individuals ≥ 10 years old.

DISCUSSION

The rate of treatment failure with pentavalent antimony in immunocompetent patients with VL in the New World of less than 2% found in this study is acceptable when compared with the proportion of failure of treatment identified in India and Kenya[19,22]. Since some patients needed a much larger number of doses of pentavalent antimony than was recommended and achieved a cure, the current requirement in Brazil to declare a failure to respond after administering 40 doses may need to be modified in some situations (National Health Foundation, Brazil, unpublished). This is important because amphotericin B has many side effects and, as was confirmed in this study, may be required in doses of up to 50mg/kg, which can represent up to 4 months of inpatient treatment when administered on alternate days. Thus, the advent of an easy to use oral drug such as miltefosine may completely change the recommendations for treatment of VL[10].

The great difficulty of the study to establish a definition of therapeutic failure or success is a problem which needs to be resolved by a parasitologic examination[6], but this approach was not feasible for two reasons. First, because bone marrow examination at the hospital has a very low sensitivity (approximately 50%) a negative result cannot rule out the presence of parasites. Second, the staff physicians at the hospital are reluctant to perform splenic biopsy due to the risk of death, especially because during the time that the study took place up to 1/5 of the deaths by VL at the hospital were associated with splenic biopsy. Therefore, until more modern, rapid, and secure methods such as PCR or QBC® are routine[14 15], the clinical criteria for a therapeutic failure ought to continue to be firmly based on the dimensions of the spleen and on other clinical data[18 26].

The response to amphotericin B was high, but still inferior to those that have been described in the literature, and the dose necessary to satisfy the recommendations for cure was more than has been recommended[24 26]. This probably is due to the special characteristics of the patients in this study who had already failed previous therapy: one of the two patients who did not respond to amphotericin B died before the study was complete and had a coexisting illness that predisposed him to infections, and the other patient was very young.

For unclear reasons, age appears to be a risk factor in VL in two other circumstances: the risk of developing illness after infection[1] and the infectivity for the vector *Lutzomyia longipalpis*[8]. Since the risk of unsatisfactory response is five to ten times greater in children, the principal victims of the illness in periods of epidemics, special attention should be given to the treatment of VL in children. If the criteria were imprecise, such as that based upon the persistence of splenomegaly, a greater duration of treatment of children and closer follow-up could be good options. These data also indicate that one should exercise caution with new therapeutic agents until they have been studied better in children. Establishing amphotericin B as the first-line drug in very small children should be considered[16 17 26] keeping in mind that the risk of unsatisfactory response to pentavalent antimony is ten times greater than in individuals older than 10 years of age. Another reason to consider this option is that the probability of death is also greater in small children in treatment with pentavalent antimony[19].

The presence of coinfection during the initial treatment of VL is another strong predictor of therapeutic failure. Because the progression to cure is related to an efficient cellular response[5], it is likely that patients with VL have so many coinfections due to immunosuppression. Typically, patients with VL develop a poor cellular immune response with low production of g-interferon and IL-2 by T-cells[4] which simultaneously may predispose to a poor response to the specific therapy and to opportunistic infections to certain pathogens. Therefore, this work shows a link between immunologic deficiency in VL (causing therapeutic failure) and the common presence of bacterial infections in patients with VL. If this is confirmed in the future by observations with clearer definitions of therapeutic failure or by cohort studies, it will mean that infections that occur in patients with VL will be interpreted as opportunistic infections rather than simply coinfection. Additionally, the lower risk of therapeutic failure in patients who were on antibiotics compared to the overall group with coinfections could suggest, as proposed for malnourished children[9], an option for the use of chemoprophylaxis directed toward opportunistic bacterial infections, aiming to prevent therapeutic failure in the most susceptible group, the small children under one or two years of age.

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