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ORIGINAL PAPER

Further evidences on a new diagnostic approach for monitoring human *Leishmania (L.) infantum chagasi* infection in Amazonian Brazil

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Abstract This was a prospective study carried out during a period over 2 years (May/2006–September/2008) with a cohort of 1,099 individuals of both genders, aged 1 year old and older, from an endemic area of American visceral leishmaniasis (AVL) in Pará state, Brazil. The object was to analyze the prevalence and incidence of human *Leishmania (L.) infantum chagasi* infection as well as the dynamics evolution of its clinical-immunological profiles prior identified: (1) asymptomatic infection (AI); (2) symptomatic infection (SI=AVL); (3) sub-clinical oligosymptomatic infection (SOI); (4) sub-clinical resistant infection (SRI) and; (5) indeterminate initial infection (III). The infection diagnosis was performed by using both the indirect fluorescent antibody test and leishmanin skin test with amastigotes and promastigotes antigens of *L. (L.) i. chagasi*, respectively. A total of 187 cases of infection were recorded in the prevalence (17%), 117 in the final incidence (6.9%), and 304 in the accumulated prevalence (26.7%), which provided the following distribution into the clinical-immunological profiles: AI, 51.6%; III, 22.4%; SRI, 20.1%; SOI, 4.3%; and SI (=AVL), 1.6%. The major finding regarding the dynamics evolution of infection was concerned to III profile, from which the cases of infection evolved to either the resistant profiles, SRI (21 cases, 30.8%) and AI (30 cases, 44.1%), or the susceptible SI (=AVL; 1 case, 1.5%); the latter 16 cases remained as III till the end of the study. These results provided the conclusion that this diagnostic approach may be useful for monitoring human *L. (L.) i. chagasi* infection in

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endemic area and preventing the high morbidity of severe AVL cases.

Introduction

Recently, the interaction between *Leishmania (L.) infantum chagasi* (Lainson and Rangel 2005) or *Leishmania chagasi* (Cunha and Chagas 1937), the etiological agent of American visceral leishmaniasis (AVL), and the human immune response has received reasonable attention in viewing of its importance regarding the clinical and immunological spectrum that may result from this interaction. In this way, a better understanding on the repertory of immune responses which can give rise to this clinical-immunological spectrum of human *L. (L.) i. chagasi* infection is also of interest. Taking these considerations into account, it has been suggested that the clinical spectrum may range from an asymptomatic stage of infection in resistant individuals, which have an efficient T-cell immune response (delayed-type hypersensitivity, lymphocyte proliferation, and high interferon-gamma response) towards to a symptomatic stage in susceptible ones, in which a specific immune-suppression of these T-cell responses may lead to classic AVL (Holaday et al. 1993; Vinhas et al. 1994). Nevertheless, between these two polar stages of infection, there are some individuals showing an intermediary immune-genetic profile which has been considered as a sub-clinical oligosymptomatic stage, in which the clinical and immunological features have not yet clearly defined (Pearson and Souza 1996; Costa et al. 1999).

In Brazil, although some studies have been carried out with the aim of a better understanding on the clinical-immunological spectrum of human *L. (L.) i. chagasi* infection, these investigations have, unfortunately, been based either on the specific antibody response, or on the delayed-type hypersensitivity response of infected individuals, which has raised some difficulties concerning a complete view of the immune response against infection (Badaró et al. 1986a; Gama et al. 2004; Jeronimo et al. 2000). In other words, these studies have generally used either a serological, such as the enzyme-linked immunosorbent assay, or a T-cell method, like the leishmanin skin test (LST), for diagnosing active *L. (L.) i. chagasi* infection, which has underestimated the possibility that some infected individuals living in endemic areas can express both immune responses, the humoral and T-cell responses, against infection.

Recently, however, we have shown the capacity of both indirect fluorescent antibody test (IFAT) and LST for diagnosing human *L. (L.) i. chagasi* infection in AVL-endemic area (Silveira et al. 2009a). This diagnostic approach was based in a high specificity of *L. (L.) i. chagasi* antigens used for IFAT (amastigotes) and LST (promastigotes), which also provided the identification of the largest clinical-immunological spectrum of human *L. (L.) i. chagasi* infection in Amazonian Brazil, consisted in the following profiles of infection: (1) asymptomatic infection (AI), (2) symptomatic infection (SI=AVL), (3) sub-clinical oligosymptomatic infection (SOI), (4) sub-clinical resistant infection (SRI) and, (5) indeterminate initial infection (III) (Crescente et al. 2009).

Regarding the above findings, we considered of interest to present further evidences on this diagnostic approach for monitoring human *L. (L.) i. chagasi* infection, which resulted from a prospective study over a 2-year period realized in AVL-endemic area in Cametá municipality, Pará state, Brazil; it reinforced the efficacy of this diagnostic approach, mainly for diagnosing the clinical-immunological profile III, which consists in early asymptomatic cases of infection but with potential for developing active AVL. The relevance in diagnosing early cases of infection for preventing the high morbidity of severe AVL cases is discussed here.

Materials and methods

Study area and population

This study was carried out in four small villages (Ajó, Vacaria, Vacajó, and Enseada) in Cametá municipality (01°56′S:54°45′W), northeastern Pará state, Brazil, which is situated on the border of the river Tocantins (Fig. 1). The climate is typically equatorial, with an average temperature of 28°C and high humidity. The annual rainfall in the region is of 2.500 mm or more, with the period from January to June forming the principal rainy season. Following extensive destruction of the primary forest, the area now consists mainly of plantations, with occasional patches of developing secondary forest. Approximately 70% of the inhabitants occupy wooden houses in the non-flood land, which is surrounded by a secondary forest, while the rest lives in the "várzea", an area of low vegetation which is flooded twice daily by waters of the river Tocantins. Thus, the climate and environment conditions of this area are very similar to those found in Barcarena municipality, Pará state, at about 150 km distant from this study area in Cametá municipality, where we have prior studied the transmission dynamics of human *L. (L.) i. chagasi* infection (Silveira et al. 2009a).

The population enrolled in this study consisted in a cohort of 1,099 individuals (92.2% of total population), being 596 males and 503 females aged between one (min.) to 84 (max.) years old, with a median age of 24.4 years old,

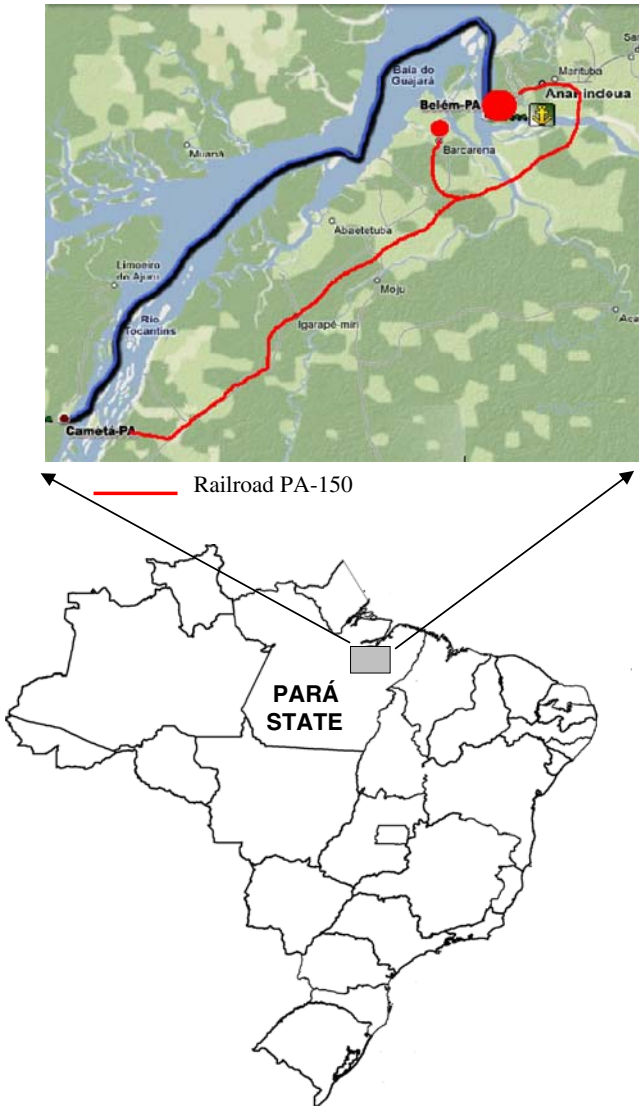

Fig. 1 Geographic localization of Cametá municipality, Pará state, Amazonian Brazil

characterizing to be a relatively younger population. When the study began, the number of inhabitants in the area was estimated to be 1,192 (Instituto Brasileiro de Geografia e Estatística 2004).

Study design

Regarding that the present study had been performed to analyse the prevalence and incidence of human *L. (L.) i. chagasi* infection as well as the dynamics evolution of its clinical-immunological profiles of infection, it was necessary to design a prospective study to follow-up a cohort (1,099 individuals) during a period of over 2 years (May/2006–September/2008). Thus, the IFAT and LST were chronologically used at the same time points in the prevalence and incidence surveys; i.e., for all individuals previously selected for the prevalence and, for the following two incidences, at 12 and 24 months, these tests were only performed on those individuals that were negative either in the prevalence or in prior incidence surveys. In this way, in cases of reactivity by LST alone, which represents a genetic characteristic of immunological resistance to infection (Jeronimo et al. 2007), these individuals were removed from subsequent LST surveys, similar to that proposed in a longitudinal study in Sudan (Zijlstra et al. 1994). Moreover, in cases revealing reactivity for both tests, the individuals were tested only by IFAT. Finally, in cases of reactivity by IFAT alone which, in contrary to LST, represents an immunological status of susceptibility to infection, the individuals remained under investigation by both tests, with the aim of analyzing the evolution of both immune responses. For a number of different reasons, such as holidays or travel, a loss of almost 5% (54 individuals) of original sample occurred over the 2-year follow-up period. In addition, the total population was also divided into three age groups; 1–10, 11–20, and ≥21 years old, consisting of 303, 252, and 544 individuals, respectively, with the aim of analyzing the age distribution of infection.

Clinical evaluation of infected individuals

All individuals presenting any type of immune reaction, either by LST and/or by IFAT, were clinically examined (a physical examination) in order to identify any signs and/or symptoms that could be recognized as classical features of AVL, as well as those symptoms (fever, asthenia, pallor, and slight hepatomegaly or spleenomegaly) prior associated to sub-clinical oligosymptomatic infection (Crescente et al. 2009); only cases with typical features of AVL received conventional antimony therapy, as recommended by the Brazilian AVL control program (Brasil 2003). The sub-clinical oligosymptomatic cases were, in principle, only followed-up during 2- to 3-month period to confirm their spontaneously clinical resolution, which has also been observed in a prospective study carried out in Maranhão state, Brazil (Gama et al. 2004).

Criteria for identification of human infection

Regarding that IFAT evidences, humoral response (susceptibility) and LST T-cell response (resistance; Awasthi et al. 2004), the definition of human *L. (L.) i. chagasi* infection case was assumed to be the reactivity to either one or both immunological tests. However, considering that human "HIV" co-infection could interfere with this diagnostic approach, it is important to state that up to the onset of the

study, no case of human "HIV" infection had been recorded in the study area by the Health Care Secretary of Cametá municipality.

Moreover, considering the importance in revealing the specificity of IFAT and LST, a scale of semi-quantitative results was used with scores varying from + to ++++, as follows: for IFAT, serological titers (IgG) with 80–160 and 320–640 received + and ++ and those with 1,280–2,560 and 5,120–10,240, +++ and ++++, respectively. For LST, exacerbated skin reactions (≥16 mm) were regarded as ++++, strongly positive (13–15 mm) as +++, moderately positive (9–12 mm) as ++, and weakly positive (5–8 mm) as + (Silveira et al. 2009a). Thus, it was assumed that serological reactions with 80 (IgG) titer and skin reactions forming indurations with ≥5 mm in diameter were regarded as positive cut-off for IFAT and LST, respectively (Lima et al. 2003; Silveira et al. 1991, 1998). In addition, combining the clinical status of infected individuals with this semi-quantitative scale of scores for LST and IFAT, it was possible to identify the following clinical-immunological profiles of infection: (1) AI (LST+/++++ and IFAT-), (2) SI=AVL, and (3) SOI with the same immune profile (LST− and IFAT+++/++++), (4) SRI (LST+/++++ and IFAT+/++), and (5) III (LST− and IFAT+/++; Crescente et al. 2009).

Immunological tests procedures

The proceedings for LST and IFAT were the same as those prior described (Crescente et al. 2009; Silveira et al. 2009a).

Data analysis

The data obtained were analyzed by Bio-Estat 4.0 software (Ayres et al. 2004) and the X^2 and binomial tests were used to determine the significance of differences between the clinical-immunological profiles of infection with a confidence interval of 95% (p value <0.05).

Results

Distribution of clinical-immunological profiles of human *L. (L.) i. chagasi* infection regarding the prevalence

The actual prevalence of infection was 17% (187 cases/1,099 individuals), which means 90 cases of infection diagnosed only by LST (AI profile), 54 by IFAT (SI profile, four; SOI profile, nine; and III profile, 41), and 43 by both LST and IFAT (SRI profile). The distribution of clinical-immunological profiles revealed a greater ($p<0.05$) frequency of AI profile (48.1%) over other profiles; SRI (23%), III (22%), SOI (4.8%), and SI=AVL (2.1%; Table 1), and showed that frequencies of SRI and III profiles were also higher ($p<0.05$) than those of SOI and SI, although there were no differences ($p>0.05$) between the frequencies of SRI versus III, and SOI versus SI profiles. These results have also shown that within the prevalence survey, the great majority (93%/174) of infected individuals was asymptomatic (AI, SRI, and III profiles).

Distribution of clinical-immunological profiles of human *L. (L.) i. chagasi* infection regarding the incidences

The first incidence (12 months) of infection was 7.2% (64 new cases/892 non-infected individuals from the prevalence), which represented 28 cases of infection diagnosed only by LST (AI profile), 21 by IFAT (SOI profile, four and III profile, 17), and 15 by both LST and IFAT (SRI profile). The distribution of these profiles revealed again a greater ($p<0.05$) frequency of AI profile (43.7%) over other profiles; III (26.6%), SRI (23.4%), and SOI (6.3%; Table 1).

Table 1 Frequency rates of clinical-immunological profiles of human *L. (L.) i. chagasi*-infection in the prevalence, incidence, accumulated prevalence and final evolution of infection in Cametá municipality, Pará state, Amazonian Brazil

Surveys	Clinical-immunological profiles				
	Number (%)				
	AI	SI	SOI	SRI	III
Prevalence (n=187 cases)	90 (48.1)	4 (2.1)	9 (4.8)	43 (23.0)	41 (22.0)
Incidence (12 months) (n=64 cases)	28 (43.7)	–	4 (6.3)	15 (23.4)	17 (26.6)
Incidence (24 months) (n=53 cases)	39 (73.6)	1 (1.8)	–	3 (5.7)	10 (18.9)
Final incidence (n=117 cases)	67 (57.3)	1 (0.8)	4 (3.4)	18 (15.4)	27 (23.1)
Accumulated prevalence (n=304 cases)	157 (51.6)	5 (1.6)	13 (4.3)	61 (20.1)	68 (22.4)
Final evolution (n=304 cases)	238 (78.3)	1 (0.3)	3 (1.0)	46 (15.1)	16 (5.3)

AI asymptomatic infection, *SI* symptomatic infection (AVL), *SOI* sub-clinical oligosymptomatic infection, *SRI* sub-clinical resistant infection, and *III* Indeterminate initial infection

There was no case of SI profile (=AVL) amongst new cases of infection within the first year of study. These findings have also shown that frequencies of III and SRI profiles were higher ($p<0.05$) than that of SOI profile, although there was no difference ($p>0.05$) between the frequencies of III versus SRI profiles ($p>0.05$).

The second incidence (24 months) of infection was 6.6% (53 new cases/763 non-infected individuals from prior incidence); this also represented 39 cases of infection diagnosed only by LST (AI profile), 11 by IFAT (SI profile, one and III profile, ten), and three by both LST and IFAT (SRI profile). The distribution of these profiles showed again a greater ($p<0.05$) frequency of AI profile (73.6%) over other profiles; III (18.9%), SRI (5.7%), and SI (1.8%; Table 1). Thus, there was no case of SOI profile amongst new cases of infection within the second year of study. These findings have also shown that frequency of III profile (18.9%) was higher ($p<0.05$) than those of SRI (5.7%) and SI (1.8%), and finally, that frequency of SRI profile was also higher ($p<0.05$) than that of SI.

In summary, these surveys have recorded a final incidence of 6.9% for both two years period with 117 new cases of infection which were classified in a decreasing order, as follows: AI profile recorded the greatest ($p<0.05$) frequency (57.3%), followed by III (23.1%), SRI (15.4%), SOI (3.4%), and SI=AVL (0.8%; Table 1). Thus, these results have shown again that the great majority (95.7%/112) of infected individuals in the incidence surveys were also asymptomatic (AI, III, and SRI profiles).

Distribution of clinical-immunological profiles of human *L. (L.) i. chagasi* infection regarding the accumulated prevalence

Following these three surveys (prevalence and two incidences), a total of 304 cases of human *L. (L.) i. chagasi* infection were recorded with an accumulated prevalence of 27.6%; AI profile was the most frequent (51.6%), followed by III (22.4%), SRI (20.1%), SOI (4.3%), and SI=AVL (1.6%) profiles (Table 1).

Age distribution of clinical-immunological profiles of human *L. (L.) i. chagasi* infection within the prevalence and incidence surveys

Regarding the prevalence (187 cases), it was noted that within the 1–10-year age group (23%/43 cases) there was no difference ($p>0.05$) amongst the frequencies of AI (30.2%/13), SRI (30.2%/13), and III (25.6%/11) profiles, which were higher ($p<0.05$) than those of SI (9.3%/4) and SOI (4.7%/2) profiles. Besides this, it was also observed that within the 11–20-year age group (23.5%/44 cases), there was no difference ($p>0.05$) between the frequencies of AI (47.7%/21) and III (34.1%/15) profiles, which were higher ($p<0.05$) than those of SRI (13.6%/6) and SOI (4.6%/2) profiles. At last, within the ≥21-year age group (53.5%/100 cases), it was noted that the frequency of AI profile (56%/56) was higher ($p<0.05$) than those of other profiles; SRI (24%/24), III (15%/15), and SOI (5%/5).

With regards to the incidence (117 cases), it was observed that most cases were recorded within the two smallest age groups; (1) in the 1–10-year age group (43.6%/51 cases) the AI profile presented a higher ($p<0.05$) frequency (58.8%/30 cases) than those of III (27.5%/14 cases), SRI (5.9%/3 cases), SOI (5.9%/3 cases), and SI=AVL (1.9%/1 case) profiles; (2) in the 11–20-year age group (37.6%/44 cases) the AI profile showed again a higher frequency (68.2%/30 cases) than those of SRI (18.2%/8 cases) and III (13.6%/6 cases) profiles.

Dynamics evolution of clinical-immunological profiles of human *L. (L.) i. chagasi* infection

The dynamics evolution of infection was based in the clinical-immunological profiles recorded in all three surveys, but only those from the prevalence and first incidence (12 months) were really follow-up, once those from the second incidence (24 months) could not be follow-up. Thus, regarding III profile (IFAT+/++ and LST−) as the earliest stage of infection, it was observed that amongst 68 (22.4%) cases recorded (41 in the prevalence and 27 in the incidence), 21 (30.9%) evolved to SRI profile, 30 (44.1%) to AI, one (1.5%) to SI (=AVL) and, 16 (23.5%) have conserved their original profile till the end of study. With regards to 61 cases (20.1%) of SRI profile (IFAT+/++ and LST+/++++), 43 in the prevalence and 18 in the incidence, it was noted that 47 (77%) changed to AI profile and, 14 (23%) have maintained their original profile; however, considering that 21 cases from III, eight from SOI, and three from SI (=AVL cases successful treated) profiles have also evolved to SRI profile, this provided a final evolution rate of infection of 15.1% (46 cases) to SRI profile. In relation to SOI profile (IFAT+++/++++ and LST−), which recorded 13 (4.3%) cases of infection (nine in the prevalence and four in the incidence), it was observed that eight (61.5%) cases developed to SRI, two (15.4%) to AI, and three (23.1%) have kept their original profile. Moreover, amongst five (1.6%) AVL (SI profile) cases (IFAT+++/++++ and LST−), four in the prevalence and one in the incidence, the antimony treatment carried out three (60%) cases to SRI profile, one (20%) to AI, and the last one (20%) although has retained its original immune response has also became clinically asymptomatic. Finally, with regards to 157 (51.6%) cases of AI profile (LST+/++++ and IFAT−), 90 in the prevalence and 67 in the incidence, it was noted that even these cases did not change their clinical status once they

represent the resistant immunological pole of infection, they also increased more 30 cases came from III, 47 from SRI, two from SOI, and one from SI (=AVL) profiles; thus, providing to AI profile a final evolution rate of infection of 78.3% (238 cases).

In summary, the AI profile was the unique profile which was benefited with the dynamics evolution of infection, evolving from 51.6% in the accumulated prevalence to 78.3% in the final evolution of infection. The other profiles have only decreased their frequency rates following the evolution of infection, as follows: SI (=AVL) from 1.6% to 0.3%, SOI from 4.3% to 1%, SRI from 20.1% to 15.1%, and principally, III from 22.4% to 5.3% (Tables 1 and 2).

At last, it is important to emphasize that throughout the study period there was not detected none case of cutaneous leishmaniasis amongst the individuals reacting either by LST and/or by IFAT, which reinforced the specificity of these immunological tests for diagnosing human *L. (L.) i. chagasi* infection. At the same time, there was no recorded none case of "HIV" infection amongst the individuals resident in this community.

Discussion

Initially, it should be highlighted the importance of AI profile in the context of this clinical-immunological spectrum of human *L. (L.) i. chagasi* infection once these results have clearly demonstrated that AI profile was the most frequent in all surveys analyzed, specially in the accumulated prevalence where it had 51.6% of infection, followed by III (22.4%), SRI (20.1%), SOI (4.3%) and, SI= AVL (1.6%) profiles. Thus, it is reasonable to assume that its high frequency might be the result of the small period of time developing between the initial stage of infection (III profile) and its final stage (AI profile), indicating that most III cases present a brief IgG antibody response (IFAT+/++) followed by rapid positive LST conversion (SRI profile), and finally, negative IFAT conversion (AI profile). This finding had prior been studied in AVL endemic area in Barcarena municipality, Pará state, at about 150 km distant from this study area in Cametá municipality, where AI profile corresponded to 73.2% of cases in the accumulated prevalence of infection (Silveira et al. 2009b). In this way, as T-cell hypersensitivity has been considered a genetic expression of T-cell immune response against human *L. (L.) i. chagasi* infection (Jeronimo et al. 2007), the present results have confirmed that the great majority of infected individuals in endemic area (final evolution of AI profile was 78.3%) are immune-genetic resistant against to infection. In addition, regarding that SRI profile (final evolution 15.1%) seems to represent a developing stage towards to the resistant AI profile, this rate can reach to 90% of all infection in endemic area.

With regards to SRI profile, which has been recognized as a new stage of infection in this diagnostic approach, its performance was similar to that found in previous study (Silveira et al. 2009b), with 23% in the prevalence and 15.4% in the final evolution of infection, suggesting to be more frequent amongst older cases of infection and with high possibility for developing towards to the resistant immunological pole of infection (AI profile). In this way, it was shown in the present study that 47 (77%) amongst 61 SRI cases have negatively converted the IFAT− and kept their T-cell hypersensitivity (LST+/++++ and IFAT−), assuming then the status of AI profile. Thus, as T-cell hypersensitivity expression is genetic controlled, it might be expected that just following a new case of infection (III profile) had converted its T-cell hypersensitivity (SRI profile), it will certainly develop to the resistant immunological pole of infection (AI profile). This is the reason we have not regarded SRI cases as a major target to follow-up in AVL control programs.

In relation to III profile, which has been regarded as the earliest stage of infection in this diagnostic approach, it was interesting to record its regular distribution in all surveys of

Table 2 Age distribution of clinical-immunological profiles of human *L. (L.) i. chagasi*-infection in the prevalence and incidence surveys in Cametá municipality, Pará state, Amazonian Brazil

Surveys	Number (%)	Clinical-immunological profiles				
		Number (%)				
		AI	SI	SOI	SRI	III
Prevalence (n=187 cases)						
1–10[a]	43 (23.0)	13 (30.2)	4 (9.3)	2 (4.7)	13 (30.2)	11 (25.6)
11–20	44 (23.5)	21 (47.7)	–	2 (4.6)	6 (13.6)	15 (34.1)
≥21	100 (53.5)	56 (56.0)	–	5 (5.0)	24 (24.0)	15 (15.0)
Incidence (n=117 cases)						
1–10	51 (43.6)	30 (58.8)	1 (1.9)	3 (5.9)	3 (5.9)	14 (27.5)
11–20	44 (37.6)	30 (68.2)	–	–	8 (18.2)	6 (13.6)
≥21	22 (18.8)	7 (31.8)	–	1 (4.6)	7 (31.8)	7 (31.8)

AI asymptomatic infection, *SI* symptomatic infection (AVL), SOI sub-clinical oligosymptomatic infection, *SRI* sub-clinical resistant infection, and *III* indeterminate initial infection

[a] Age groups (years old)

the present study, with 22% in the prevalence, 26.6% in the first incidence (12 months), 18.9% in the second incidence (24 months), 22.4% in the accumulated prevalence and, 23.1% in the final incidence, which might be reflecting an expressive regularity of the infection transmission in the endemic area of Cametá municipality, where the prevalence of infection (17%) was, coincidently, higher than that (12.6%) in prior study in Barcarena municipality (Silveira et al. 2009a). Besides this, regarding that final incidence of III profile was 23.1%, it is expected that about 2–3% of cases should require clinical follow-up in the endemic area once they have high potential for developing to the susceptible clinical forms of infection, i.e., SOI or SI (=AVL) profiles. Thus, these findings should be taking into account when developing AVL control programs.

The SOI and SI (=AVL) profiles have presented the smallest frequencies in all time-point surveys of this study, although SOI has shown an accumulated prevalence (4.3%) almost three times higher than that (1.6%) of SI. However, as similar to that found in the first study in Barcarena municipality (Silveira et al. 2009b), both profiles have shown an accumulated prevalence of only 5.9% of total cases of infection in endemic area of Amazonian Brazil, which differs from the northeaster region of this country; i.e., in Bahia state, for example, the sub-clinical oligosymptomatic form (=SOI profile) was recorded in 60% of 86 infected children below fifteen years old (Badaró et al. 1986b) and, in Maranhão state, the same clinical form was diagnosed in 17.4% of 189 children into the same age group (Gama et al. 2004). It should be highlighted, however, that in these two studies the diagnosis of disease was realized in children below fifteen years old, which might be providing to these children a greater susceptibility to develop symptomatic infection.

When distribution of clinical-immunological profiles was regarded by age, it was again demonstrated that AI profile was the most frequent in almost all age groups analyzed (1–10, 11–20, and ≥21 years age groups), either in the prevalence or in the incidence, with exception in case of the 1–10 years age group in the prevalence whose frequency (30.2%) was the same to that of SRI profile and, also, in case of the ≥21 years age group in the incidence whose frequency (31.8%) was also the same to those of SRI and III profiles. Thus, these results have provided to AI profile the status of the most frequent in all different age groups in both prevalence and incidence surveys.

On the other hand, when the frequency of infection was compared within the same clinical-immunological profile, the following findings deserve to be commented: (1) while in the prevalence there was an accumulative effect of AI profile with age, which may be interpreted as clear evidence that T-cell hypersensitivity increases with age (Pampiglione et al. 1975; Badaró et al. 1986a; Ali and Ashford 1993; Davies and Mazloumi Gavgani 1999), this, in fact, only reflects that older individuals (≥21 years old) have had a more exposure time to infection than younger people (1–10 and 11–20 years old); in the incidence, however, there was a decreasing effect of AI profile with age, which showed an important reduction of infection from the smallest age groups (1–10 and 11–20 years old with 30/44.8% cases each age group) towards to the older age group (≥21 years old with 7/10.4% cases), indicating that amongst the new cases of infection AI profile was principally represented by young children and adolescents; (2) all four AVL cases (SI profile) were recorded within the smallest age group (1–10 years old), confirming that AVL is typical of young children; (3) in SOI profile was found a similar distribution of cases amongst the different age groups, with 53.8% in the smallest age groups (1–10 and 11–20 years old) and 46.2% in the older age group (≥21 years old), which differs from the results found in prior study in Barcarena municipality where the mean age of SOI cases was 33 years old (Silveira et al. 2009b); (4) in SRI profile was observed in the prevalence a higher concentration of cases within the ≥21 years age group (55.8%) in relation to that found within the 1–10 and 11–20 years age groups (44.2% both), suggesting a higher presence of SRI profile amongst the individuals with older infection; however, in the incidence it was observed, in contrary, a higher concentration of cases within the 1–10 and 11–20 age groups (61.1%) in relation to that found within the ≥21-year age group (38.9%), which might be indicating a higher transmission of infection amongst younger individuals; (5) at last, it was shown in the incidence that III profile have had a higher concentration of cases within the 1–10 and 11–20 years age groups (74%) over that recorded within the ≥21 years age group (26%), suggesting again that transmission of *L. (L.) i. chagasi* to man is principally intra-domiciliary or peridomestic, where children and adolescents are particularly vulnerable (Lainson and Rangel 2003, 2005; Lainson and Shaw 2005; Silveira et al. 1997).

Regarding the dynamics evolution of infection, it should be emphasized the relevance of III profile in the context of this diagnostic approach due to its pivotal role in supplying other profiles of infection. In this way, it was shown that amongst 68 III cases recorded during this study 30 (44.1%) have evolved to AI and 21 (30.8%) to SRI profiles, which means almost 75% (74.9%) of III cases with evolution to the resistant immunological pole of infection strongly characterized by T-cell hypersensitivity. Besides these, one case (1.5%) had evolution to the susceptible immunological pole of infection (SI profile=AVL) and, 16 (23.5%) had not changed their original profile till the end of study. Thus, these results seem greatly to be in according with the proposal of this diagnostic approach (Silveira et al. 2009b), which has prior demonstrated that III cases may evolve,

depending on the genetic background of T-cell immune response (Blackwell et al. 2004; Jamieson et al. 2007), to either the resistant, SRI and AI, or the susceptible profiles, SOI and SI (Fig. 2).

Regarding the above prediction, we have also considered priority the evolution of the susceptible immunological profiles SOI and SI (=AVL) which, although have been identified with the same immune response (LST− and IFAT +++/++++), could be distinguished due, principally, to the fact that SOI cases had spontaneously developed to clinical cure in 10 (77%) amongst 13 cases recorded (eight have evolved to SRI and two to AI profiles; the last three have kept their original profile till the end of study), while typical AVL cases had required antimony therapy for developing to clinical cure (three cases have evolved to SRI, one to AI and, one had retained its original immune response but clinically asymptomatic). These observations are also in according with clinical evolution of SOI cases in Maranhão (Gama et al. 2004) and Ceará states (Holaday et al. 1993), where 33 cases in Maranhão and 12 in Ceará states have spontaneously healed. In addition, other study in Bahia state has demonstrated the capacity of SOI patients to product higher levels of IFN-γ in vitro culture of peripheral blood mononuclear cells (PBMC) than that of AVL (SI profile) patients (Bacellar et al. 1991), which might help to better understand the pathogenesis of these symptomatic forms of human *L. (L.) i. chagasi* infection.

Concerning the clinical-immunological profiles which have shown resistance against to infection, SRI and AI profiles, the impression left was that SRI seems to represent a development stage towards to AI profile once most SRI cases (77%) had negatively converted IFAT, assuming then the status of AI profile; this observation might help to explain the high frequency of AI profile which has been found in this and in prior study (Silveira et al. 2009b). On the other hand, a few AI profile cases from the prevalence showed transitory IFAT-conversion, which was interpreted as a result of a short-lived antigenic impulse produced by an abortive re-infection controlled by T-cell response. This possibility was based on the well-known ability of IL-10 to inhibit the production of INF-γ and macrophage activation, leading the infection towards to the Th2-type immune response (Bacellar et al. 2000; Blackwell et al. 2004). This might help to explain the low, transitory IFAT response found in a few AI cases. However, the clinical-immunological status of the great majority of AI cases remained unaltered, suggesting that AI profile is the end of line of infection (Fig. 2).

At last, the dynamics evolution of infection showed that only AI profile was benefited with this process, increasing its frequency from 51.6% in the accumulated prevalence to 78.3% in the final evolution of infection, whereas other profiles have had significant loss with this evolution process, such as: SI (=AVL) from 1.6% to 0.3%, SOI from

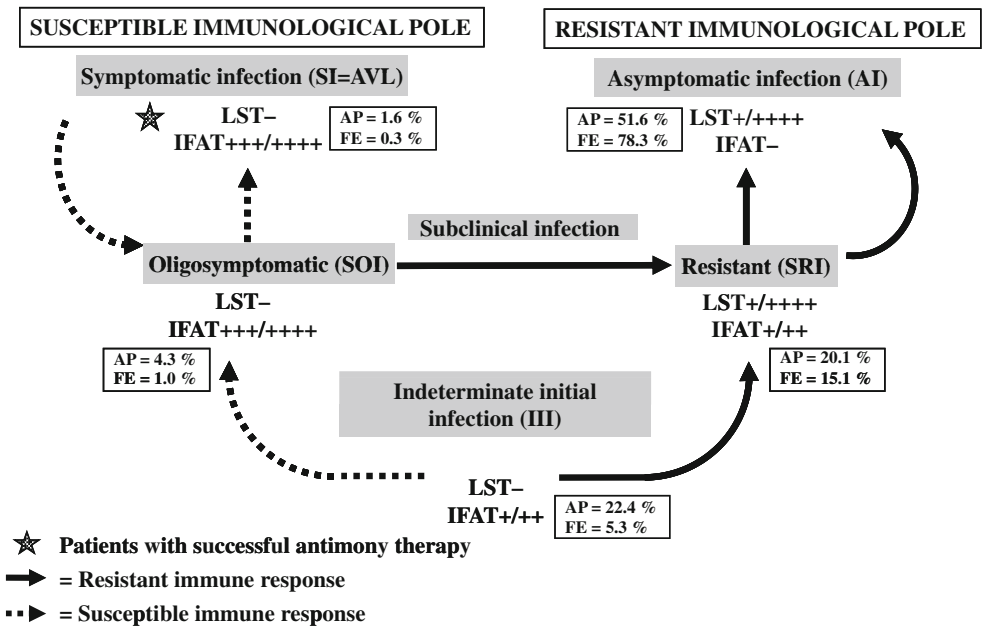

Fig. 2 Dynamics evolution of clinical-immunological profiles of human *Leishmania (L.) infantum chagasi*-infection in Amazonian Brazil. *IFAT* indirect fluorescent antibody test (IgG). *IFAT*++++ 5,120–10,240 (IgG). *IFAT*+++ 1,280–2,560 (IgG). *IFAT*++ 320–640 (IgG). *IFAT*+ 80–160 (IgG). *IFAT*– negative reaction. *LST* leishmanin skin test. *LST*++++ exacerbate reaction (≥16 mm). *LST*+++ strong reaction (13–15 mm). *LST*++ moderate reaction (9–12 mm). *LST*+ weak reaction (5–8 mm). *LST*– negative reaction. *AI* asymptomatic infection. *SI* symptomatic infection (=*AVL*). *SOI* sub-clinical oligo-symptomatic infection. *SRI* sub-clinical resistant infection. *III* indeterminate initial infection. *AP* accumulated prevalence. *FE* final evolution of infection

4.3% to 1.0%, SRI from 20.1% to 15.1%, and principally, III profile from 22.4% to 5.3%. Thus, it seems undoubted the relevance of these new clinical-immunological profiles (SRI and III) in promoting the evolution of infection, principally III which might help to prevent the high morbidity of severe AVL cases in endemic area.

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Conflicts of interest statement The authors have no conflicts of interest concerning the work reported in this paper.

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