Clinical Infectious Diseases

MAJOR ARTICLE

Chronic high level parasitemia in HIV-infected individuals with or without visceral leishmaniasis in an endemic area in North-West Ethiopia: potential superspreaders?

Johan van Griensven,[1,*] Saskia van Henten,[1] Aderajew Kibret,[2] Mekibib Kassa,[3] Hailemariam Beyene,[2] Saïd Abdellati,[1] Annelies de Hondt,[1] Wim Adriaensen,[1] Florian Vogt,[1,4,5] Myrthe Pareyn,[1] Koert Ritmeijer,[2] Ermias Diro [6, 7]

[1]Institute of Tropical Medicine, Antwerp, Belgium; [2]Medecins sans Frontières, Amsterdam, the Netherlands; [3]Leishmaniasis Research and Treatment center, University of Gondar, Belgium; [4]The Kirby Institute, University of New South Wales, Sydney, Australia; [5]National Centre for Epidemiology and Population Health, Australian National University, Canberra, Australia; [6]University of Gondar, Ethiopia; [7]Department of Internal Medicine, University of Washington, Seattle, United States of America

Background: HIV patients with recurrent visceral leishmaniasis (VL) could potentially drive *Leishmania* transmission in areas with anthroponotic transmission such as East-Africa, but studies are lacking. *Leishmania* parasitemia has been used as proxy for infectiousness.

Methods: This study is nested within the PreLeish prospective cohort study, following a total of 490 HIV infected individuals free of VL at enrollment for upto 24-37 months in North-West Ethiopia. Blood *Leishmania* PCR was done systematically. This case series reports on ten HIV-coinfected individuals with chronic VL (≥3 VL episodes during follow-up) for upto 37 months, and three individuals with asymptomatic *Leishmania* infection for upto 24 months.

Results: All ten chronic VL cases were male, on antiretroviral treatment, with 0-11 relapses before enrollment. Median baseline CD4 counts were 82 cells/μL. They displayed three to six VL treatment episodes over a period upto 37 months. *Leishmania* blood PCR levels were strongly

Corresponding Author: Johan van Griensven, jvangriensven@itg.be

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positive for almost the entire follow-up time (median Ct value 26 (IQR 23-30), including during periods between VL treatment. Additionally, we describe three HIV-infected individuals with asymptomatic *Leishmania* infection and without VL history, with equally strong *Leishmania* parasitemia over a period of upto 24 months without developing VL. All were on antiretroviral treatment at enrollment, with baseline CD4 counts ranging from 78 to 350 cells/µL.

Conclusion: These are the first data on chronic parasitemia in HIV-infected individuals from *L donovani* endemic areas. HIV patients with asymptomatic and symptomatic *Leishmania* infection could potentially be highly infectious and constitute *Leishmania* superspreaders. Xenodiagnosis studies are required to confirm infectiousness.

Key words: visceral leishmaniasis, Africa, PCR, transmission, superspreader, xenodiagnosis

Key points: HIV patients in North-West Ethiopia with asymptomatic and symptomatic *Leishmania* infection displayed chronic high level parasitemia for up to 37 months. These individuals could be highly infectious and constitute *Leishmania* superspreaders. Xenodiagnosis studies are required to confirm infectiousness.

BACKGROUND

Visceral leishmaniasis (VL) is a neglected tropical disease transmitted by the bites of sandflies. In East-Africa and the Indian subcontinent, VL is caused by parasites belonging to the *Leishmania donovani* species, with anthroponotic transmission [1,2]. In the Mediterranean region and Latin America, VL is caused by *L infantum* and transmission is predominantly zoonotic. Manifestations of VL include persistent fever, splenomegaly and pancytopenia. Without treatment, VL is usually fatal [1]. According to the World Health Organization (WHO), around 30,000 cases are estimated to occur annually at the global level [3]. East-Africa currently has the highest VL burden globally.

The ambition of VL elimination in East-Africa has recently been stated by WHO [4]. To inform an elimination strategy, a good understanding of which individuals are most likely to transmit the disease is essential. For most infectious diseases, infectiousness is largely driven by small sub-groups, sometimes referred to as super-spreaders [5,6]. HIV infected individuals have been put forward as potentially important sources of infection [7,8].

Even while on antiretroviral treatment (ART), HIV patients generally show a decreased parasitological response to VL treatment, with a subgroup of patients manifesting with repeated VL relapses [9,10]. Thus, there are concerns that such patients could remain infectious for many years. Whether HIV-infected individuals can be infectious without displaying clinical VL manifestations – *i.e* individuals with asymptomatic *Leishmania* infection (ALI) - is not well characterized for *L donovani* endemic areas. However, this could have huge implications, as such individuals would not seek VL care and could constitute hidden reservoirs of VL transmission.

In France, a chronic form of VL was described in 2010 in a case series of ten HIV infected patients with multiple VL relapses followed-up for up to 13 years [11]. PCR and blood culture results indicated continuous parasite circulation despite treatment and secondary prophylaxis, both during asymptomatic periods and the recurrent symptomatic VL episodes. This condition was labelled as active chronic VL; chronic due to the multiple VL episodes over many years and active due to the continuous blood parasite circulation. In France, VL is caused by *L infantum*, HIV coinfection rates are low and, like in all *L infantum* endemic areas, humans are generally only considered to play a minor role in transmission. In contrast, East-Africa is marked by high rates of HIV coinfection, with around 20% of VL cases coinfected with HIV in some parts of Ethiopia [9], and transmission is anthroponotic. Whether chronic parasitemia or active chronic VL also occurs in *L donovani* endemic areas, where it could have far-reaching implications, is not known.

In the PreLeish study, 490 HIV infected individuals were followed-up for up to two years in a VL endemic area in North-West Ethiopia, with the aim to identify early predictors of VL [12]. This study also provided a unique opportunity to monitor parasite circulation in HIV-infected individuals over time. In this case series, we report on ten HIV infected individuals with chronic VL, defined as those developing at least three VL episodes during follow-up. They represent 29% of all those developing VL in the PreLeish study. Additionally, we report on three HIV coinfected individuals with ALI. Both chronic VL and ALI cases displayed high levels of parasites in their peripheral blood for up to 37 months. These are the first data ever reported on chronic parasitemia in HIV-infected individuals from *L donovani* endemic areas, providing important information for VL elimination efforts.

METHODS

Study design, set-up and population

This is a case series nested within the PreLeish prospective cohort study [12], conducted between October 2017 and May 2021 in Abdurafi health center in the Amhara region, North-West Ethiopia. In the PreLeish study, 490 HIV infected individuals free of VL at the time of enrollment were followed up for up to 24 months. This included individuals with or without previous VL episodes. Individuals developing VL during the study period (incident VL cases) were followed-up until the end of the study.

In this case series, we included all participants from the PreLeisH study who developed at least three VL episodes (defined as chronic VL) during follow-up in the study, to identify individuals most likely to be potentially infectious for a prolonged period. The nine cases with two VL episodes during the study were hence not included in this case series. In addition, we included all three participants with ALI who displayed high parasitemia throughout the follow-up period, despite not having any VL episode before or during the study.

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Study procedures

A study visit was planned each time the study participants presented for their routine HIV consultation (usually every three to six months) and in between these scheduled visits if patients presented with symptoms compatible with VL. Besides a full clinical evaluation, blood and urine samples. For the first VL episode, *Leishmania* tests were also collected weekly until the end of treatment. After each VL episode, follow-up visits were scheduled at six and twelve months.

Study participants were evaluated for VL at each study visit and in case of clinical suspicion (fever >2 weeks with weight loss and/or splenomegaly) referred for routine diagnostic work-up. VL diagnosis was based on microscopic examination of spleen or bone marrow aspirates [13], and grading of the parasite load was done as reported before [14]. Treatment of VL relied on liposomal amphotericin B (AmBisome) infusions and oral miltefosine combination therapy for 28 days, or longer if indicated [15]. A VL treatment episode was defined as any follow-up visit at which VL treatment was started based on the routine VL diagnostic work-up. Study-specific *Leishmania* tests such as *Leishmania* PCR, urine antigen and serology results besides the rK39 RDT were not available to the physician at the time of the clinical visit. At the end of treatment, a parasitological test of cure was done by microscopy on tissue aspirates.

Laboratory assays and quality control

Leishmania tests were done at each study visit. This entailed *Leishmania* serological tests including the direct agglutination test (DAT, Institute of Tropical Medicine, Antwerp, Belgium), rK39 RDT (IT-LEISH, BioRad, USA), and rK39 ELISA (Serion *Leishmania* IgG ELISA, Serion Diagnostics, Würzburg, Germany). Tests were executed as reported before [16]. The rK39 RDT was reported as positive or negative. For the rK39 ELISA test, results were interpreted as follows: values <10 U/mL were negative, 10–15 U/mL were borderline positive, and ≥15U/mL were positive. A DAT titer ≥ 1/1600 was used to define positive tests, in line with previous studies [16].

In addition, two parasitological tests were done: real-time PCR targeting kinetoplast DNA (kDNA) on whole blood and a *Leishmania* urine antigen test (KAtex, Kalon Biological Ltd, Guilford England UK). The KAtex test is a semi-quantitative urine assay, with three levels of agglutination: 1+: weakly positive; 2+: moderately positive; 3+: strongly positive. Detection of *Leishmania* kDNA by qPCR was done as described before [17,18]. DNA was isolated from 300 µL whole blood using the Maxwell 16 LEV Blood DNA purification extraction kit (Promega, Leiden, The Netherlands) with the automated Maxwell 16 Instrument (AS1000, Promega). The reaction was run on a Rotor-Gene Q instrument (Qiagen, Venlo, The Netherlands). Results were expressed in cycle threshold (Ct) values. CD4 counts were done using the BD FACSCount (Becton Dickinson, USA), typically every six months. The full blood count, CD4 count and the rK39 RDT were performed locally at Abdurafi health center. Samples for DAT, rK39 ELISA, KAtex and PCR were transported in cold chain boxes to the Leishmaniasis Training and Research Center (LRTC) in Gondar for storage and further analysis in batch. In line with previous publications [16,18,19],

ALI was defined if at least one of the *Leishmania* tests was positive in an individual without symptoms/signs of VL such as persisting fever with weight loss and splenomegaly.

Data collection and quality control

The PreLeish study was conducted in a clinical research network that has been conducting VL clinical research in Ethiopia for over a decade, with support from the Clinical Trials Unit and the Clinical Reference Laboratory of the Institute of Tropical Medicine, Antwerp, Belgium. Study-related information was collected using a clinical and a laboratory paper case report form and subsequently transcribed into electronic case report forms, using the Macro data capture system. Data quality was monitored throughout the study, including on-site monitoring visits by the Clinical Trials Unit.

Statistical analysis

Only descriptive analysis was done. Continuous variables were summarized using medians and interquartile ranges (IQR). Categorical variables were summarized using frequencies and proportions. Statistical analysis was done using Stata 15 (STATACorp LP, USA).

Ethics

The study was approved by the institutional/ethics review board of ITM, the University of Antwerp, the university of Gondar, the Amhara Regional Health Bureau, Medecins sans Frontières and the Ethiopian research ethics review committee.

RESULTS

Characteristics of study participants

Amongst the 490 HIV-infected individuals followed-up in the PreLeish study, 34 developed VL during the study. Of these, 19 (56%) had multiple VL episodes during follow-up and ten (29%) had three or more VL episodes over a period of up to 37 months. These ten cases were included in this paper and are referred to as chronic VL cases.

All were male, with a median age of 30 years (IQR 28-37). Three were residing in the (VL-endemic) study area only temporary, three were stable residents having lived in the areas since birth and four were stable residents but born outside of the study area. Besides one being a farmer, all others were daily labourers (Table 1). ART was started at a median of 3.3 years (IQR 1.1-6.4) before enrollment. The median baseline CD4 count was 82 cells/µL (IQR 67-192) and below 200 cells/µL for eight. All but one had a history of VL, with a median of 2.5 (IQR 1-8) prior episodes.

For the ten chronic VL cases, follow-up ranged between 12-37 months (median 28 (IQR 19-32). Over this time, they had a median of four (IQR 3-4; range 3-6) VL treatment episodes, summing

to a total number of experienced VL treatments (combined before and during the study) of four to 14 (median 6.5 (IQR 4-12). For instance, case 5 had nine episodes prior to enrollment and experienced the 10th-14th VL treatment episode during the study, whereas case 9 had 11 previous episodes and had the 12th-14th VL treatment during follow-up.

Additionally, we included three HIV-infected individuals with persistently positive results on *Leishmania* blood PCR and urine antigen test, but without a VL history and without developing disease (VL) during the nine to 24 months of follow-up. They were all male, with the age ranging between 27 and 60 years. All three were stable residents, including two born in the study area. ART had been started within a year prior to enrollment, with baseline CD4 counts ranging from 78 to 350 cells/μL.

***Leishmania* markers over time**

An overview of the pattern of *Leishmania* markers over time is given in Table 2 for the five most illustrative cases (having visits during as well as in between VL treatment episodes), and in Table S1 for the other five. Overall, *Leishmania* PCR blood levels were strongly positive for most of the follow-up time (Table 2), with a median Ct-value of 26.0 (IQR 23.1-30.5). This included the period before VL onset, during and between the repeated VL treatment episodes. While all displayed an increase in the Ct levels (corresponding with lower parasite levels) of the *Leishmania* PCR by the end of treatment of the first VL episode, only four had an undetectable *Leishmania* PCR at the end of treatment. Similarly, the urine antigen tests were strongly positive on all but a few time points, with no to minimal decreases during treatment. In general, CD4 counts were low throughout follow-up. Three participants died (case 1, 5 and 7). For several participants (*e.g.* case 2, 3 and 5), aiming for parasitological cure was abandoned at some point in time. Case 2 (M18 visit), case 3 (M28 visit) and case 5 (M31 visit) were discharged after clinical improvement despite failing to achieve a negative test of cure; for case 2, treatment was not initiated at the subsequent visit with VL suspicion (M27) despite a 1+ positive diagnostic tissue aspirate, as parasitological cure was not achieved at the previous visit, the general condition was fair and starting VL treatment was not deemed to have clear clinical benefit.

Table 3 displays the *Leishmania* markers of the three cases with persistent ALI. While they had no VL history and VL was never diagnosed during follow-up, *Leishmania* PCR on blood and urine antigen tests were consistently and generally strongly positive during follow-up, over a period from nine to 24 months. The median PCR Ct-value was 24.7 (IQR 23.0-29.5). ALI case 1 underwent bone marrow aspiration at month 24, with negative results. CD4 counts were consistently below 200 cells/μL for two but not for ALI case 3. While platelets remained normal throughout follow-up, the levels of haemoglobin and white blood cells tended to be decreased, but only slightly and remained fairly stable throughout.

DISCUSSION

To our knowledge, this is the first description of HIV infected individuals from a *L donovani* endemic area with chronic high-level parasitemia. Markers were positive during but also between the repeated VL treatment episodes. In addition, we describe three HIV-infected individuals without VL before or during the study who displayed high levels of *Leishmania* parasitemia and antigenuria for 9-24 months, without developing VL. To the best of our knowledge, these are the first three such cases from both *L infantum* and *L donovani* endemic areas.

While the chronic VL cases described fit the entity of active chronic VL described in France [11], we did not perform culture so could not demonstrate that viable parasites could be recovered from the blood. Nevertheless, the persistent and high direct parasite markers in blood and urine indicate active replication since *Leishmania* DNA detected by PCR in the blood is degraded rapidly [20].

Xenodiagnosis studies performed in *L. infantum* endemic areas in VL cases with HIV or other immunosuppressive conditions found they were more infectious to sandflies than immunocompetent individuals and also demonstrated that HIV individuals with ALI were capable of transmitting the parasite [21,22]. Xenodiagnosis studies in HIV patients in *L donovani* endemic areas – where transmission is antroponotic – remain to be conducted, including HIV infected individuals with symptomatic and asymptomatic *Leishmania* infection.

A characteristic feature of superspreaders is that, even when relatively rare, they contribute to a relatively large part of transmission [5,6]. For example, a small number of highly infectious dogs have been shown to harbour close to 90% of *Leishmania* parasites [23]. In Ethiopia, a modelling study suggested that 3.2% of humans could contribute to 53-79% of the infected sandfly population [24]. In North-West Ethiopia, the HIV prevalence in VL cases remains at around 20% [9,25]. Amongst the 34 incident VL cases in the PreLeish study, ten (29%) developed at least three VL episodes over the 12 to 37 months of follow-up. Chronic VL cases could potentially be infectious for very prolonged periods, and given the repeated exposure to VL treatment, potentially also of drug-resistant parasites. These individuals are also highly mobile. This case series only included chronic VL cases (≥3 VL episodes in the study). However, several of the nine patients – not included in this study - with two VL episodes during follow-up also had persistent parasitemia, so this condition could be more common.

HIV-infected individuals with ALI and chronically high parasitemia were rather rare. Only three were identified with consistent parasitemia amongst the 412 individuals in the PreLeish study without VL treatment episodes and with at least three PCR measurements done during follow-up, hence detected in 0.7% of study participants. Even while few, they could constitute hidden reservoirs of VL transmission for a long time, as such individuals would not seek VL care. Further studies on the role of superspreaders are required to inform the East-African VL elimination initiative [4].

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While qPCR levels in peripheral blood generally correlated with infectiousness in studies from *L infantum* endemic areas [21,22], recent studies have suggested that the skin is a much underappreciated site of active parasite replication [5,23,26]. In (symptomatic) dogs, skin qPCR parasite loads were higher, even at levels similar to the spleen [23,27], and correlated well with infectivity during xenodiagnosis [23,27]. To assess this in humans, it could be useful to conduct studies using skin microbiopsy devices [28], which mimick the sandfly bite, in parallel with qPCR on peripheral blood and ideally xenodiagnosis. Reliable tools for infectivity would also be needed to assess whether reducing parasite load with treatment/prophylaxis has an impact on the transmission potential. While qPCR testing is currently not available at most health facilities in East African countries, highly accurate and simple loop-mediated isothermal amplification (LAMP) assay has been proposed as a molecular point of care test adapted to resource-constrained settings [29].

Strengths of our study include that it was conducted within a well-established clinical research network, with due attention to high-standard laboratory procedures and quality control, and oversight by a Clinical Trials Unit. There are a number of important limitations to acknowledge. As mentioned, xenodiagnosis studies would have strengthened the public health relevance of our findings. More frequent visits in between treatment episodes would have allowed more refined analysis of the evolution of *Leishmania* parasitemia. For the three individuals with ALI but high parasitemia, it remains unsure whether they developed VL after the study.

CONCLUSIONS

We describe ten cases of active chronic VL in HIV-infected individuals, displaying multiple VL episodes over a 37 months follow-up period. Both during and in between VL treatment, *Leishmania* PCR and urine antigen tests were consistently and generally strongly positive, indicating prolonged opportunities for transmission. We also report three cases without VL history but with persistently strongly positive *Leishmania* markers, which could act as hidden sources of infection. Both groups could act as superspreaders and constitute a major challenge for VL elimination in the area. If the infectiousness of these individuals is confirmed in future xenodiagnosis studies, simple biomarkers of infectiousness should be developed to identify the most infectious individuals, complemented by studies on interventions to reduce transmission from these individuals.

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Conflicts of interest: None to declare.

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Table 1. Sociodemographics and VL and HIV history of HIV infected individuals included in the case series, North Ethiopia 2017-2021

Chronic VL/HIV cases									
Case	Age (years)	Sex	Residence in study area (VL-endemic)	Occupation	Years of HIV	Years of ART	Previous VL episodes (N)	VL episodes in the study (N)	CD4 (cells/µL)

1	20	Male	Permanent, non-native	Daily labourer	5.4	5.1	6	6	90
2	28	Male	Temporary	Daily labourer	3.6	3.6	8	5	32
3	30	Male	Permanent, non-native	Daily labourer	6.5	6.4	3	4	50
4	31	Male	Permanent, non-native	Daily labourer	1.3	1.1	0	4	68
5	42	Male	Temporary	Daily labourer	3.9	2.8	9	5	302
6	31	Male	Permanent, native	Daily labourer	3.1	3.1	1	4	67
7	45	Male	Permanent, native	Daily labourer	13.0	12.4	11	3	192
8	28	Male	Permanent, native	Daily labourer	1	1	2	4	458
9	37	Male	Temporary	Farmer	0.1	0.1	1	3	79
10	25	Male	Permanent, non-native	Daily labourer	7.0	7.0	1	3	85
HIV patients with chronic asymptomatic *Leishmania* infection - ALI (PCR & KAtex +)									
1	27	Male	Permanent, native	Daily labourer	0.6	0.6	0	0	350
2	37	Male	Permanent, non- native	Daily labourer	0.2	0.2	0	0	78
3	60	Male	Permanently, native	Farmer	1.4	0.1	0	0	135

PCR: polymerase chain reaction; VL: visceral leishmaniasis; ART: antiretroviral treatment; KAtex: *Leishmania* urine antigen test

Table 2. Overview of *Leishmania* markers in five HIV infected individuals with chronic visceral leishmaniasis, North-Ethiopia 2017-2021

CASE 1[a]	M0	M3	M5/Tx	EOT	M9	M11	M14	M17	M22	M28	M35
rK39 RDT	+	+	+	+	+	+	+	+	+	+	+
rK39 ELISA	+	+	+	+	+	+	+	+	+	+	+
DAT	+	+	+	+	+	+	+	+	+	+	+
KATEX	3	2	3	3	0	2	3	3		2	3
PCR Ct	40,9	36,4	27,3	-	36,9	32,9	25,6	27,9		27,5	25,6
BMA									1		
SA	0		4	0	0	0	5	4		6	6
CD4	90		50		63		99			98	

CASE 2 [a]	M0	M3	M4/Tx	EOT	M7	M9	M12	M18	M27	M32
rK39 RDT	-	-	-	-	-	-	-	-	-	
rK39 ELISA	-	-	-	-	-	-	-	-	-	
DAT	+	+	+	+	+	+	+	+	+	
KATEX	3	3	3	2	3	3	3	3	2	
PCR Ct	37,5	31,6	27,2	-	23,4	31,2	24,7	26,9	21,3	
BMA	0		3	0	1		0		1	
SA								6		6
CD4	32		19		12	25		7	87	

CASE 3	M0	M3	M6/Tx	EOT	M10	M15	M18	M21	M28
rK39 RDT	+	+	+	+	+	+	+	+	+
rK39 ELISA	+	+	+	+	+	+	+	+	+
DAT	+	+	+	+	+	+	+	+	+
KATEX	0	2	3	3	3	3	3	3	3
PCR Ct	22,5	29	19,8	-	23,4	21,8	21,6	22	19,8
BMA			5	1		4		4	5
SA									
CD4	50		59		102	48	55	45	

CASE 4	M0	M3	M9/Tx	EOT	M13	M18	M19	M25	M28	M29
rK39 RDT	-	-	+	+	-	+	+	+	+	-
rK39 ELISA	-	-	+	+	-	+	+	-	-	-
DAT	-	-	-	-	-	-	**?**	+	+	+
KATEX	0	0	3	1	0	3	3	3	2	2
PCR Ct	-	-	25	-	41	24	23	24	29	24
BMA				0			4	2	0	1
SA			5							
CD4	68	367			116	10	25			7

CASE 5 [a]	M0	M2	M3/Tx	EOT	M8	M14	M15	M31	M37
rK39 RDT	+	+	+	+	+	+	+	+	
rK39 ELISA	+	+	+	+	+	+	+	+	
DAT	+	+	+	+	+	+	+	+	
KATEX	0	1	3	3	3	3	2	3	
PCR Ct	29	26,4	25,6	28,3	0	27	25,6	22,7	
BMA				0			5	5	
SA	0		6		6				4
CD4	302		95		146	167			

[a] individuals enrolled in the study after a negative test of cure (TOC)

Grey areas indicate time points with VL treatment; **White** areas indicate time points without VL treatment. Study related *Leishmania* tests such as rK39 ELISA, DAT, *Leishmania* PCR and urine antigen were not available to the physician at the time of the clinical visit.

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RDT: rapid diagnostic test; ELISA: enzyme-linked immunosorbent assay; DAT: direct agglutination test; PCR: polymerase chain reaction; SA: spleen aspirate; BMA: bone marrow aspirate; Tx: start of VL treatment; EOT: end of treatment; Ct: PCR cycle threshold value; VL: visceral leishmaniasis.

Table 3. Overview of Leishmania markers in three HIV infected individuals with chronic asymptomatic *Leishmania* infection (ALI) but persistently positive *Leishmania* markers in blood PCR and urine antigen, North-Ethiopia 2017-2021

ALI CASE 1							
	M0	M3	M12	M15	M18	M21	M24
rK39 RDT	+	+	+		+		+
rK39 ELISA	449	186	46		31		136
DAT	1/1600	1/3200	1/25600		1/204800		1/204800
PCR Ct	38,6	39,5	32,3	-	29,5		24,9
KATEX	0	2	2	0	3	1	3
BM							0
CD4	135		67		48		36
Hb	12,7	13	10,3	10,2	9,9		11,2
WBC	3,97	4,04	2,08	3,13	3		3,4
Platelets	273	285	220	274	271		263

ALI CASE 2					
	M0	**M3**	**M6**	**M9**	**M12**
rK39 RDT	+	+	+	+	+
rK39 ELISA	>800	>800	>800	>800	>800
DAT	1/204800	1/204800	1/204800	1/204800	1/204800
PCR Ct	23,4	26,4	24,4	22,8	**23**
KATEX	2	2	3	3	3
CD4	78		144		82
Hb	10,7	11,3	11,3	12	9,8
WBC	3,01	3,35	6,16	4,45	3,1
Platelets	226	251	249	228	212

ALI CASE 3				
	M0	**M3**	**M6**	**M9**
rK39 RDT	-	-	-	-
rK39 ELISA	-	-	-	-
DAT	1/204800	1/25600	1/51200	1/204800
PCR Ct	25,9	24,0	22,0	21,3
KATEX	2	2	3	0

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CD4	350		399	
Hb	11	10,2	11	8,2
WBC	2,13	2,5	4,98	2,8
Platelets	173	187	208	161

White areas indicate time points without VL treatment. Study related *Leishmania* tests such as rK39 ELISA, DAT, *Leishmania* PCR and urine antigen were not available to the physician at the time of the clinical visit.
RDT: rK39 RDT; ELISA: enzyme-linked immunosorbent assay, expressed in units/ml (positive if > 15 U/ml); DAT: direct agglutination test, expressed in titer (positive if titer ≥1/1600); PCR: polymerase chain reaction; SA: spleen aspirate; BMA: bone marrow aspirate; Hb: hemoglobine; WBC: white blood cells. Ct: PCR cycle threshold value.