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Association of polymorphisms in NFκB1 promoter and NFκBIA gene with the development of antibodies against HHV-8 in HIV-infected individuals

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ABSTRACT

Human gammaherpesvirus 8 (HHV-8) is the etiologic agent of Kaposi's sarcoma, multicentric Castleman's disease and primary effusion lymphoma. Like other herpesviruses, the HHV-8 may exhibit latent or lytic cycle, both regulated by viral and host factors. Regarding host factors, we analysed the association of polymorphisms in *NFkB1* promoter (*NFkB1*-94 ins/del ATTG) and *NFκBIA* gene (*NFκBIA* 3′UTR A→G) with the development of antibodies against latent or lytic antigens from HHV-8. The ins/del [OR 7.9 (95% CI 3.3–19.1), $p < 0.001$], AG [OR 12.3 (95% CI 4.3–34.9) $p < 0.001$], GG [OR 9.4 (95% CI 3.2–27.9), $p < 0.001$], ins/del + AG [OR 94.5 (95% CI 9.6–924.4), < 0.0001], ins/del + GG [OR 50.4 (95% CI 5.2–482.2, $p < 0.0001$] and G allele [OR 3.3 (95% CI 2.0–5.6), $p < 0.001$] were strongly related with the presence of antibodies to lytic antigens. This is the first association of polymorphisms in *NFκB1* promoter and *NFκBIA* gene with the development of antibodies against HHV-8.

1. Introduction

Human gammaherpesvirus 8 (HHV-8), member of the *Herpesviridae* family and *Rhadinovirus* genus (ICTV, 2018), is the etiologic agent of all clinical forms of Kaposi's sarcoma (KS) (Bishop and Lynch, 2018). Since the HIV epidemic in the 1980s, KS has been studied mainly in people living with HIV/AIDS (PLHA), and currently is one of the most common cancers in this population (Torre et al., 2015). The HHV-8 is also related to multicentric Castleman's disease (MCD) (Sbenghe et al., 2012) and primary effusion lymphoma (PEL) (Christenson et al., 2015).

Like other herpesviruses, HHV-8 may exhibit two replication cycles, latency and lytic infection, both regulated by viral and host factors. The K9, K11/11.1, vGPCR, RTA and KCP viral proteins, for example, have been described during the lytic infection, whereas LANA, vCYC and vFLIP proteins have been associated with the viral latency (Douglas et al., 2010). Host factors, such as oxidative stress, hypoxia, unbalanced inflammatory cytokines and single-nucleotide polymorphisms (SNPs) in cytokines and growth factor, have been implicated in the regulation between lytic infection and latency (Stebbing et al., 2006; Aneja and Yuan, 2017).

The detection of HHV-8 by the innate immune response during both primary infection and/or reactivation from latency occurs via pattern recognition receptors (PRRs), mainly Toll-like receptors (TLR) 3, 4, 7, 8 and 9, NLRP1 and NLRP3 (nucleotide oligomerization domain-like receptors family, NLR), IFI16 [absent in melanoma 2 (AIM2)-like receptor family] and cGAS-STING (cytosolic DNA sensor) (Dittmer and Damania, 2016). Once stimulated, the PRRs may initiate the activation pathway of the nuclear factor of kappa light polypeptide gene enhancer in B-cells (NFκB), which results in the transcription of several genes, including inflammatory cytokines and anti-apoptotic proteins (Douglas et al., 2010). In addition, the NFκB pathway may also be activated by latent (e.g. K13) and lytic (e.g. K1, vGPCR) viral proteins, besides viral miR-K1 through 12 and host cytokines (e.g. TNF-α, IL-1 and interferon α/β) (Douglas et al., 2010; Pfeffer, 2011; Liu et al., 2017).

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Experiments performed with epithelial cells and fibroblasts suggested that NFκB would inhibit the HHV-8 lytic infection (Brown et al., 2003). In a cell line derived from PEL, Sgarbanti et al. (2004) showed that NFκB positively influenced the expression of HHV-8 lytic genes. Finally, when evaluating the participation of NFκB on lytic infection or latency in several cell lines, Grossmann and Ganem (2008) demonstrated that the influence of NFκB on the HHV-8 replication cycles is not universal, but is dependent of the cell type.

Although these findings have provided important insights into the role of NFκB in the HHV-8 life cycle, to our knowledge, there are no genetic studies investigating the influence of polymorphisms in *NFκB* and related-molecules on the development of antibodies against HHV-8. Here, we evaluated the association of polymorphisms in *NFκB1* promoter (*NFkB1*-94 ins/del ATTG polymorphism, rs28362491) and *NFκB* inhibitor alpha (*NFκBIA*) (*NFκBIA* 3′UTR A → G polymorphism, rs696) with the presence of antibodies to HHV-8 lytic or latent antigens.

2. Methods

2.1. Study population

The study was started with a total of 738 PLHA under antiretroviral therapy and accompanied at the Referral Service for Infectious and Parasitic Diseases, Hospital das Clínicas of the Federal University of Pernambuco, Brazil. General characteristics, such as age, gender, first and last counts of TCD4 (cell/mm^3), were obtained from medical records of patients in whom were detected antibodies against lytic or latent antigens from HHV-8. Ethnic classification in mulatto, black or white was also registered and was based on self-identification, according to the classification system of the Brazilian Institute of Geography and Statistic. The patients were more than 18 years-old and signed a free and informed consent form. The study was approved by the ethics committee, protocol number 5156215.5.0000.5208.

2.2. In-house ELISA for HHV-8 antibody

All participants were evaluated for the detection of IgG against HHV-8 antigens by whole-virus ELISA performed in-house. In the assays was used a crude antigen lysate from a mixture of TPA-induced (1 μg/mL for 5 days) and non-induced HHV8-infected BCBL-1. The treatment with TPA was used for the induction of HHV-8 lytic cycle. The cell mixture was washed in PBS (pH 7.2) and resuspended in PBS with sodium deoxycholate (1:50). The supernatant for viral preparation was obtained by sonication and centrifugation at 3,000 rpm for 10 min and stored at −80 °C.

Microplates (Nuncpolysorp, Waltham, MA, USA) were sensitized overnight with 100 μL of antigen diluted (1:100), previously titrated. The plates were washed with PBS with 0.05% Tween20 (PBST) and blocked with 5% skim milk (in PBS) for 1 h. Subsequently, serum samples, diluted to 1:200, were added and incubated for 1 h at 25 °C. The plates were washed four times with PBST, the anti-human IgG peroxidase conjugate (1:2000) (Dako, Waltham, MA, USA) was added and the plates were incubated at 25 °C for 40 min. After five washes, the plates were incubated with the tetramethylbenzidine substrate (Dade Behring, Deerfield, IL, USA) for 10 min at 25 °C. Finally, the reaction was stopped by sulfuric acid and the OD was measured in Asys Expert Plus microplate reader (Biochrom, Cambridge, UK) at 450/620 nm.

2.3. In-house IFA for HHV-8 antibody

In order to identify antibodies against lytic or latent antigens from HHV-8, indirect immunofluorescence was performed in BCBL-1 treated (20 ng/mL for 5 days) or not with TPA. The cells were washed with PBS (pH = 7.2), counted, and 10 μL of suspension, at a concentration of 10 cells/mL, were added to the slides, dried at room temperature and fixed with cold acetone for 10 min. After the fixation, the cells were incubated for 30 min at 28 °C with serum diluted to 1:40 and 1:80 for the detection of antibodies against lytic or latent antigens, respectively.

The slides were washed with PBS (pH = 7.2), dried at room temperature and a solution of 1% skim milk and 0.1% Triton X-100 was added. After 5 min, the solution was removed and the slides were incubated with anti-human IgG with fluorescein isothiocyanate (1:50) (Sigma-Aldrich, St Louis, MO, USA) for 30 min at 25 °C. The slides were washed again and evaluated in fluorescence microscope (Leica, Wetzlar, Germany). Lytic and latent cycles analyses were performed on different slides and in duplicate. Finally, non-TPA-treated and with punctate nuclear staining BCBL-1 cells were considered positive for anti-LANA antibodies, while fluorescence in approximately 20% of TPA-treated cells was considered positive result for lytic antigens.

2.4. DNA extraction and genotyping

Whole blood samples were collected in tubes containing EDTA and stored at −20 °C. Genomic DNA was extracted from 300 μL of the samples, using the Wizard Genomic DNA Purification kit (Promega, Madison, WI, USA), following the manufacturer's instructions. After extraction, the concentration and purity of the DNA samples were measured using the Thermo Scientific NanoDrop 2000 spectrophotometer. The study was performed with DNA samples with OD ratio (260/280 nm) between 1.8 and 2.0.

The N*FκB1*-94 ins/del ATTG and *NFκBIA* 3′UTR A→G polymorphisms were evaluated by PCR-RFLP method. For the *NFκB1*-94 ins/del ATTG polymorphism, were used the forward 5′-TGGGCACAAGTCGTT TATG-3′ and reverse 5′-CTGGAGCCGGTAGGGAAG-3′ primers. The forward 5′-GCTGAAAGAACATGGACTTG-3′ and reverse 5′-GTACACCA TTTACAGGGAGGG-3′ primers were used to determine the *NFκBIA* 3′UTR A→G polymorphism. The PCR was performed with Taq DNA Polymerase (Invitrogen, Carlsbad, CA, USA) with the following the conditions: 3 min at 95 °C for initial denaturation, 32 cycles using 95 °C for 30 s for denaturation, 52 °C for 30 s for annealing, 72 °C for 1 min for extension, followed by a final extension at 72 °C for 5min.

For identification of the *NFκB1*-94 ins/del ATTG polymorphism, the amplicon (281 bp) was digested with 1 unit of the PflMI (Van911) (Fermentas,Waltham, MA, EUA) restriction enzyme for 18 h at 37 °C, according to the manufacturer's instructions. The DNA samples with del/del genotype do not have the digestion site and thus remained undigested, 281 pb band. Samples with ins/ins have the PflMI (Van911) and showed 240 bp and 45 bp bands. Heterozygotes presented the three bands.

For the *NFκBIA* 3′UTR A→G polymorphism, the 424 bp amplicon was digested by the HaeIIIA (Fermentas, Waltham, MA, EUA) enzyme (1.0 U) at 37 °C for 18 h, according to the manufacturer's instructions. The AA genotype was not digested and presented the 424 bp band. Homozygotes from the G allele revealed two bands, 316 bp and 108 bp. Heterozygotes showed the three bands.

2.5. Statistical analysis

The χ^2 test was used to calculate whether genotypes distributions were in accordance with the Hardy-Weinberg equilibrium. The association of the alleles and single or combined genotypes on the latency or lytic infection was evaluated by the Fisher's exact test with Odds ratio (OR) and 95% confidence intervals (CI). Results with *p-value* < 0.05 were considered statistically significant. The Bonferroni test was applied to the significant *p-values* to correct the bias of multiple comparisons. The evaluation of the association of first and last TCD4 counts was performed by the Mann-Whitney test. The χ^2, Fisher and Mann-Whitney tests were conducted in the GraphPad Prism software v.6.07. The Bonferroni correction was performed in the Statistical Package for Social Sciences (SPSS) software v. 25.

Table 1
Study population.

Variable	Latent (n = 69)	Lytic (n = 63)	OR (95% CI)	*p-value*
Age	45.2 (± 11.9)[a]	43.4 (± 11.6)	–	–
Sex				
Male	22 (31.9%)	25 (39.7%)	Reference	–
Female	47 (68.1%)	38 (60.7%)	0.7 (0.3–1.4)	0.45[b]
Ethnicity[c]				
Mulattos	29 (42%)	41 (65.1%)	Reference	–
Black	24 (34.8%)	15 (23.8%)	0.4 (0.2–0.9)	0.06 [b]
White	16 (23.2%)	07 (11.1%)	0.3 (0.1–0.8)	0.03 [b]
First count of TCD4 (cell/mm^3)	249 (08–728)[d]	206 (19–1045)	–	0.46[e]
Last count of TCD4 (cell/mm^3)	641 (131–1674)[d]	576 (39–1336)	–	0.18[e]

[a] The results are expressed as mean (± SD).
[b] *p-value* obtained by χ^2 test.
[c] Based on ethnic self-identification, according to the classification system of the Brazilian Institute of Geography and Statistics; [d]Median (minimum-maximum).
[e] *p-value* obtained by Mann Whitney test.

3. Results

Of the 738 PLHA evaluated, 151 (20.4%) presented IgG for HHV-8. Among these, in 63 and 69 individuals were detected antibodies against lytic or latent antigens, respectively, and 19 had antibodies against both lytic and latent antigens and were excluded of the association analyses. In the individuals who presented antibodies to lytic antigens, 25 and 38 were male and female, respectively, with a mean age of 43.4 (± 11.6). Among the PLHA who presented anti-LANA antibodies, 22 and 47 were male and female, respectively, with a mean age of 45.2 (± 11.9). Regarding general characteristics, gender, age, first and last counts of TCD4 cells, there was no significant difference between the patients in whom were detected antibodies against lytic or latent antigens from HHV-8. Only the white ethnicity showed association with the HHV-8 cycle, precisely with the latency [OR 0.3 (95% CI 0.1–0.8), $p = 0.03$] (Table 1). However, no relation between ethnicity and the polymorphisms (*NFkB1*-94 ins/del ATTG or *NFκBIA* 3′UTR A→G) was observed (data not shown).

The *NFκB1*-94 ins/del ATTG and *NFκBIA* 3′UTR A→G polymorphisms were successfully genotyped in individuals who presented antibodies against lytic or latent antigens from HHV-8. The allele and genotype frequencies of both polymorphisms are described in Table 2. Regarding *NFκB1*-94 ins/del ATTG polymorphism, we observed an association between the ins/del genotype and the presence of antibodies to lytic antigens [OR 7.9 (95% CI 3.3–19.1), $p < 0.001$] and no alleles were associated with the development of antibodies against lytic or latent antigens. In the *NFκBIA* 3′UTR A→G polymorphism, the AG and GG genotypes were related with the presence of antibodies to lytic antigens, OR 12.3 (95% CI 4.3–34.9) and OR 9.4 (95% CI 3.2–27.9), respectively ($p < 0.001$). In addition, the G allele was also associated with the presence of antibodies against the lytic cycle [OR 3.3 (95% CI 2.0–5.6), $p < 0.001$] (Table 2). When combined, ins/del + GG [OR 50.4 (95% CI 5.2–482.2, $p < 0.0001$], ins/del + AG [OR 94.5 (95% CI 9.6–924.4), $p < 0.0001$], del/del + AG [OR 27.0 (95% CI 1.8–399.5), $p < 0.01$] and del/del + GG [OR 45.0 (95% CI 3.3–604.4), $p < 0.002$] were strongly related with the presence of antibodies to lytic antigens. Of all the results, only del/del + AG was no longer statistically significant after the Bonferroni correction (Table 3).

Finally, in all HHV-8 positive individuals, the distribution of the two polymorphic *loci* was in agreement with the Hardy-Weinberg genetic equilibrium [$\chi^2 = 3.28$ ($p = 0.06$) and $\chi^2 = 2.91$ ($p = 0.08$) for the *NFκB1*-94 ins/del ATTG and *NFκBIA* 3′UTR A→G polymorphisms, respectively].

4. Discussion

The *NFκB1*-94 ins/del ATTG polymorphism is a functional insertion/deletion in *NFκB1* gene promoter, which encodes the p50 subunit of NFκB (Karban et al., 2004; Koc et al., 2014). The ins/del variant encodes three genotypes: the wild-type homozygous insertion (ins/ins), homozygous deletion (del/del) and heterozygous variants (ins/del) (Karban et al., 2004; Koc et al., 2014). Although not yet investigated in viral infections or influencing the development of antibodies against viral antigens, the *NFκB1*-94 ins/del ATTG polymorphism has already been evaluated in cancer (Cheng et al., 2013; Gao et al., 2014; Wang et al., 2016) and deregulation of the immune response, such as ulcer colitis (Borm et al., 2005), rheumatoid arthritis, systemic lupus erythematosus (Orozco et al., 2005) and Hashimoto's disease (Koc et al., 2014).

In our study, only the ins/del genotype was associated with the presence of antibodies against HHV-8, precisely to lytic antigens. Although in some cell lineages the high expression of *NFκB* is related with the HHV-8 lytic cycle (Sgarbanti et al., 2004), in our study population, we believe that a moderate expression of *NFκB* may be also associated with the viral replication and thus with a greater development, and detection, of antibodies to lytic antigens. This hypothesis is supported by previous reports that the deletion and insertion of the four ATTG nucleotides may result in loss and increase of *NFκB1* promoter activity, respectively (Karban et al., 2004).

In addition, the ins/del variant showed association with hepatic cancer (Gao et al., 2014; Wang et al., 2016), Hashimoto's disease (Koc et al., 2014) and was negatively related to the risk for systemic lupus erythematosus (Gao et al., 2012). The ins/del genotype, however, showed no association with ulcer colitis (Borm et al., 2005) and rheumatoid arthritis (Orozco et al., 2005).

Table 2
Association of genotypes and alleles of *NFκB1*-94 ins/del ATTG and *NFκBIA* 3′UTR A→G polymorphisms with HHV-8 lytic and latent infections in PLHA.

NFκB1-94 ins/del ATTG	Latent n = 69 (%)	Lytic n = 63 (%)	OR (95% CI)	*p-value*[a]	*cp-value*[b]
ins/ins	37 (53.62)	14 (22,22)	Reference	–	–
ins/del	13 (18,84)	39 (61,91)	7.9 (3.3–19.1)	< 0.001	< 0.001
del/del	19 (27,54)	10 (15,87)	1.4 (0.5–3.7)	0.61	1.00
ins allele frequency	87 (63,04)	67 (53,17)	Reference	–	–
del allele frequency	51 (36,96)	59 (46,83)	1.5 (0.9–2.4)	0.10	–
***NFκBIA* 3′UTR A→G**					
AA	37 (53.62)	06 (09,52)	Reference	–	–
AG	17 (24.64)	34 (53,97)	12.3 (4.3–34.9)	< 0.001	< 0.001
GG	15 (21.74)	23 (36.51)	9.4 (3.2–27.9)	< 0.001	< 0.001
A allele frequency	91 (65,94)	46 (36.51)	Reference	–	–
G allele frequency	47 (34.06)	80 (63,49)	3.3 (2.0–5.6)	< 0.001	–

[a] *p-value* obtained by Fisher's exact test.
[b] Corrected *p-value* after Bonferroni test.

Table 3
Evaluation of combined genotypes of *NFκB1*-94 ins/del ATTG and *NFκBIA* 3′UTR A→G polymorphisms with HHV-8 lytic and latent infections in PLHA.

Combined genotypes	Latent (n)	Lytic (n)	OR (95% CI)	*p-value*[a]	*cp-value*[b]
ins/ins + AA	18	01	Reference	–	–
ins/ins + AG	11	09	14.7 (1.6–132.7)	0.008	0.16
ins/ins + GG	08	04	9.0 (0.9–93.9)	0.06	1.00
ins/del + AA	10	04	7.2 (0.7–73.6)	0.13	1.00
ins/del + AG	04	21	94.5 (9.6–924.4)	< 0.0001	< 0.0001
ins/del + GG	05	14	50.4 (5.2–482.2)	< 0.0001	< 0.0001
del/del + AA	09	02	4.0 (0.3–50.2)	0.53	1.00
del/del + AG	02	03	27.0 (1.8–399.5)	0.01	0.45
del/del + GG	02	05	45.0 (3.3–604.4)	0.002	0.025[c]

[a] *p-value* obtained by Fisher's exact test.
[b] Corrected *p-value* after Bonferroni test.
[c] After the distribution of the combined genotypes, this group showed little sample difference and was not considered for discussion.

We also no found association of alleles or homozygotes (ins/ins or del/del) with the development of antibodies against lytic or latent antigens from HHV-8. On the other hand, in ulcer colitis (Borm et al., 2005), colorectal cancer (Lewander et al., 2007), Graves' disease (Kurylowicz et al., 2007) and liver cancer (Gao et al., 2014) was observed a relation between clinical condition and del allele. Homozygous individuals for ATTG (ins/ins) would have increased risk of rheumatoid arthritis (López-Mejías et al., 2012) and liver cancer (Wang et al., 2016). Interestingly, the del/del genotype has already been associated with coronary artery disease in Han and Uygur women in China (Yang et al., 2014). In our study, however, there was no association between the *NFκB1*-94 ins/del ATTG polymorphism and the gender of the patients (data not shown).

The *NFκBIA* gene encodes I_KBα, an inhibitor of the NFκB1 protein (Koc et al., 2014). Numerous polymorphisms in *NFκBIA* gene, including *NFκBIA* 3′UTR A→G, have been investigated in several cancers types (Cheng et al., 2013; Li et al., 2017; Wang et al., 2016) and Hashimoto's disease (Koc et al., 2014). However, similar to the *NFκB1*-94 ins/del ATTG polymorphism, no study evaluated the influence of *NFκBIA* 3′UTR A→G polymorphism on the viral infection or development of antibodies against viral antigens.

In our study population, the G allele and the genotypes containing G (AG and GG) were both associated with the presence of antibodies to lytic antigens from HHV-8. Experiments carried out in cell culture indicated that miR-449a could bind strongly to the A allele and that the G allele would decrease mRNA stability or translation efficiency of *NFκBIA*. As consequence, in specific context, both alleles could reduce the expression of *NFκBIA* and consequently increase the NFκB levels (Song et al., 2011). It is possible that this participation of the A and G alleles in the NFκB activation dynamic may influence the replication of HHV-8 and therefore the development of antibodies against lytic antigens.

When the genotypes that were associated with the presence antibodies to lytic antigens were combined (ins/del + AG or ins/del + GG), it was possible to observe a strong synergism. Interestingly, the GG and ins/ins + ins/del were associated with an increased risk of colorectal cancer (Song et al., 2011) and ins/ins + AG has already been related to protection for Hashimoto's disease (Koc et al., 2014).

Several studies have reported the influence of NFκB on the HHV-8 replication (Brown et al., 2003; Sgarbanti et al., 2004; Blattman et al., 2014; Ehrlich et al., 2014). The participation of NFκB specifically in the lytic infection or latency, however, is still uncertain and probably depends on the cell lineage evaluated (Grossmann and Ganem, 2008). Here, as previously discussed, we believe that both NFκB1-94 ins/del ATTG and NFκBIA 3′UTR A→G polymorphisms, via specific mechanisms, influence the HHV-8 replication, and/or viral reactivation, and consequently favor the development and greater detection of antibodies against lytic antigens.

This possibility, in addition to the reports of greater seroreactivity of lytic antibodies compared to those against latent antigens (Camera Pierrotti et al., 2000; Hudnall et al., 2003; Chohan et al., 2004), contributes to explain the identification of antibodies to only one HHV-8 cycle; a virus whose infection naturally leads to the production of antibodies against both lytic and latent antigens (Duprez et al., 2005; Wakeham et al., 2015). Interestingly, other studies have also identified antibodies to lytic antigens in negative samples for anti-LANA antibodies, including in healthy individuals (Camera Pierrotti et al., 2000; Hudnall et al., 2003; Chohan et al., 2004).

Although IFAs are often more sensitive than ELISAs for the detection of antibodies against HHV-8 antigens (Müller et al., 2000; Juhász et al., 2001; Corchero et al., 2002), it is also worth noting the possibility of false positive or negative results, common to serological tests. Despite this observation, the OR values obtained in our studies strongly support the association of the polymorphisms with the greater development of antibodies against HHV-8 lytic antigens.

We believe that it is important that the relationship between the NFκB1-94 ins/del ATTG and NFκBIA 3′UTR A→G polymorphisms and the development of antibodies to HHV-8 be extended to phenotypic investigation. Since the KS, MCD or PEL pathogenesis depends of the regulated expression of viral and cellular proteins during the lytic infection and latency (Purushothaman et al., 2015), the *NFκB1*-94 ins/del ATTG and *NFκBIA* 3′UTR A→G polymorphisms may play a strong influence on the clinical evolution of PLHA HHV-8 coinfected.

In especial on KS, these polymorphisms could influence its development via NFκB activation, which is known to be important in chronic inflammation and KS development, mainly by inhibiting apoptosis and stimulating angiogenesis (Douglas et al., 2010). Other polymorphisms, such as in *IL-6* and *IL-13* genes, already have been related to KS and MCD development (Brown et al., 2006; Foster et al., 2000; Gazouli et al., 2004; Stone et al., 2011, 2013). In this perspective, we believe that the clinical impact of the *NFκB1*-94 ins/del ATTG and *NFκBIA* 3′UTR A→G polymorphisms should be further investigated in larger populations with HHV-8 related diseases (KS, MCD or PEL).

Finally, during the characterization of our study population, it was possible to identify an association between white ethnicity and anti-LANA antibodies. Although some reports have suggested association between black ethnicity and HHV-8 infection (Batista et al., 2009; Begré et al., 2016; Engels et al., 2007), we did not find other studies reporting relation between ethnicity and development of antibodies against HHV-8. This may be an interesting finding and future studies based on population genetics could provide additional insights into the real importance of this association.

Conflicts of interest

All authors have no potential conflict of interest to disclose.

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