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IgG intrathecal synthesis in HIV-associated neurocognitive disorder (HAND) according to the HIV-1 subtypes and pattern of HIV RNA in CNS and plasma compartments

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Abstract

We hypothesized that humoral immunity stimulation in the CNS in HIV-1C patients would be lower than that in HIV-1B due to a defective Tat chemokine dimotif (C30C31) that might influence cellular trafficking and CNS inflammation. Sixty-eight paired CSF and blood samples from people with HIV (PWH), free of CNS opportunistic infections, were included, HIV-1B (n=27), HIV-1C (*n*=26), and HIV negative (*n*=25). IgG intrathecal synthesis was assayed using quantitative and qualitative methods. IgG oligoclonal bands (OCB) in CSF were observed in 51% of PWH, comparable between HIV-1B and HIV-1C, as well as the medians of IgG intrathecal synthesis formulas. The group with HIV infection aviremic in CSF and blood showed 75% of

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Authors' contributions: SM de Almeida participated in the conceptionand design of the study; patient recruitment; acquisition, statistical analysis and interpretation of clinical and laboratorial data; and drafting, revisionand finalization of the manuscript. He had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Anonymized data from the current study will be made available at the request of qualified investigators if approved by our Research Ethics Board.

I Rotta participated in the patient recruitment; acquisition, laboratorial analysi, and interpretation of clinical and laboratorial data; and revisionand finalization of the manuscript.

B Tang participated in the statistical analysis, and revisionand finalization of the manuscript.

F Vaida participated in the statistical analysis

S Letendre participated in the conception and design of the study.

RJ Ellis participated in the conception and design of the study; and revisionand finalization of the manuscript.

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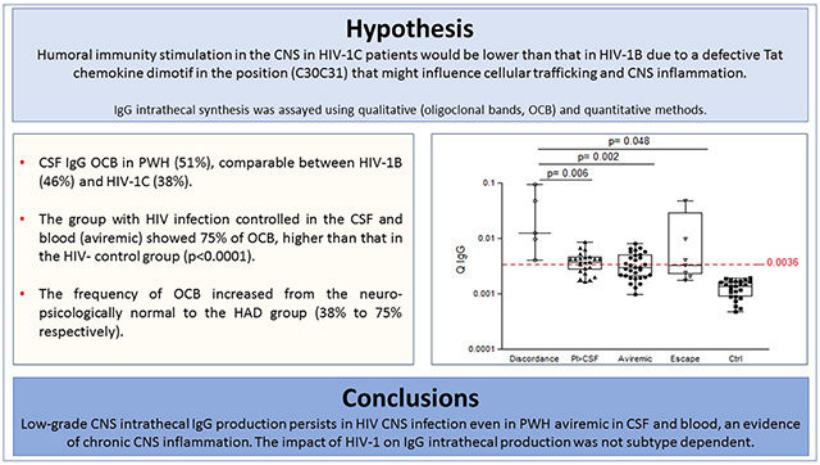
OCB. There was a poor positive correlation between the IgG quotient and GDS. The impact of HIV-1 on IgG intrathecal production was not subtype dependent. Low-grade CNS intrathecal IgG production persists in HIV CNS infection even in PWH with CSF and blood HIV RNA controlled.

Graphical Abstract



Keywords

IgG synthesis; HIV-1; subtypes; HAND; cerebrospinal fluid (CSF); discordance

1. INTRODUCTION

Intrathecal IgG synthesis occurs early in HIV-1 infection in more than 75% of patients and persists throughout the course of the disease (Bonnan et al., 2015) [1]. Many studies on IgG intrathecal synthesis on HIV-1 infection have been carried out in settings where HIV-1B predominates (Chiodi et al., 1988a; Kaiser et al., 1989; McArthur et al., 1989; Singer et al., 1994; Gisslen et al., 1999; Lackner et al., 2010; Elovaara et al., 1993; Abdulle et al., 2005). Little is known about IgG intrathecal synthesis in non-B HIV subtypes.

The HIV trans-activator of transcription (Tat) protein plays a pivotal role in chemotaxis, which is mediated by direct and indirect processes. Chemotaxis has been imputed to the C30C31 dicysteine motif (Albini et al., 1998; Beall et al., 1996). Tat has also been shown to induce chemokine secretion, mostly CC chemokines (β-chemokines), amplifying the recruitment of additional mononuclear phagocytes and lymphocytes across the blood–brain barrier (BBB) into the brain (Conant et al., 1998; Weiss et al., 1999). Some β-chemokines such as CCL19 and CCL21 play a role in the recruitment and retention of IgG-producing B lymphocytes to the CNS compartment during neuroinflammation (Cabezas et al., 2003; Kowarik et al., 2012; Metcalf et al., 2013). These chemokine levels are enhanced in patients with advanced AIDS (Damås et al., 2011). Besides, there is a crosslink between humoral and cellular immunity. Thus, intrathecal IgG production is a marker of greater immune activation in the CNS, and this, in turn has been associated with brain disease and neurocognitive impairment. HIV-1 subtype C has been proposed to be less neuropathogenic than subtype B

(Satishchandra et al., 2000), based on an in vitro defective Tat chemokine dimotif in the C30C31 position that might influence cellular trafficking and CNS inflammation (Ranga et al., 2004). The impact of defective Tat on HIV-1C humoral immunity was not investigated. Based on this, we hypothesized that the stimulation of humoral immunity in the CNS in HIV-1C would be reduced compared with that in HIV-1B.

This cross-sectional study was performed to investigate the impact of the defective Tat chemokine dimotif on humoral immunity in the CNS among people with HIV (PWH) infected with HIV-1C or HIV-1B. The aims of this study were to compare the effects of HIV-1 subtypes B and C on IgG intrathecal synthesis. Additionally, we compared IgG intrathecal synthesis levels in PWH compared with HIV-negative controls and associated it with neurocognitive impairment in HIV groups. Moreover, because viral replication is likely one important driver of immune activation we analyzed intrathecal IgG according to the distribution of HIV RNA in cerebrospinal fluid (CSF) and blood compartments.

2. METHODS

2.1 CSF and blood samples

In this study, ninety-three CSF and paired serum samples were collected from PWH (n=68) and HIV-negative controls (n=25).

Sixty eight PWH were recruited at HC-UFPR, Brazil. Individuals with opportunistic CNS infections were excluded. All volunteers underwent serological testing to confirm HIV status before enrollment (Brasil, 2018). For participants with a clinically resistant infection, the infecting HIV subtype was genotyped with *pol* sequences, whereas *env* sequences were used for all other participants. Genotyping revealed that 27 individuals were infected with HIV-1B and 40 with non-B HIV-1 subtypes (C, $n = 26$; BF, 10; BC, 1; CF, 1; and F, 2), and the subtype could not be genotyped in one participant.

Twenty-five HIV-negative participants were recruited from the CSF lumbar puncture (LP) outpatient clinic of the CHC-UFPR, with normal neurologic physical examination, clinical indication for CSF LP, symptoms not suggestive of IgG intrathecal synthesis (10 with epilepsy, 13 headache, and 2 others), CSF cytology or biochemistry characteristics [white blood cells (WBC), glucose, and total protein levels, protein electrophoresis on cellulose acetates] in normal ranges, negative immunologic reactions for cysticercosis (ELISA, IFI), and VDRL-RPR test in CSF and serum.

2.2 Laboratory methods

IgG and albumin levels in the CSF and serum were quantified using nephelometry (Dade Behring BNII, Deerfield, IL) with antiserum N human IgG (Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany) and N human albumin (Dade Behring BNII, Deerfield, IL).

2.2.1 Assay of IgG intrathecal synthesis using quantitative and qualitative methods—

Quantitative analysis was performed for the following, with reference values: absolute value of IgG (mg/dL; reference value, rv, ≤4 mg/dL); IgG/total protein (%; rv

≤18%, Thompson 1988); IgG CSF/Alb CSF (rv ≤0.28%, Caroscio et al., 1983); IgG CSF/IgG serum (Q IgG; rv ≤0.0036, Tibbling et al., 1977); IgG index (rv<0.8, Swanson, 1989); IgG extended index [(CSF IgG/serum IgG)/(CSF albumin/serum albumin)$^{1.12}$, rv 1.24, Ohman et al., 1989]; log IgG index (rv <0.84, McLean et al., 1990); daily intrathecal IgG synthesis (Tourtellotte formula; rv ≤10 mg/dia, Caroscio et al., 1983; Tourtellotte, 1970); Schuller formula (rv ≤2.25 mg/dL, Schuller e Sagar, 1983); IgG hyperbolic function (IgG HF; rv ≤0, Gallo et al., 1988a); IgG production (rv 0.4±5.6 mg/L, Blennnow et al., 1993); Auer formula (Auer et al., 2016); and Reibergram plot (CSF Research software, Reiber 1995).

For the qualitative analysis of IgG intrathecal synthesis, the CSF and serum samples were assayed to detect oligoclonal bands (OCBs) by agarose gel isoelectric focusing (IEF) followed by IgG immunofixation (Hydragel CSF Isofocusing, Sebia, Norcross, GA). The OCB pattern was classified according to the international consensus (Andersson et al., 1994), that is, band pattern type (2) CSF-restricted OCB or (3) CSF-restricted OCB with additional identical bands in CSF and serum representing local IgG synthesis within the CNS.

2.2.2 Quantification of plasma and CSF HIV RNA levels—

HIV RNA levels in plasma and CSF were measured by a branched DNA assay (VERSANT® HIV-1 RNA 3.0 bDNA Kit, Siemens Healthcare Diagnostics, Tarrytown, NY, USA) using 1 mL of CSF or plasma. The assays were performed immediately after sample collection. Samples with HIV RNA levels <50 copies/mL were considered under the detection limit.

The HIV group was categorized based on the quantification of HIV RNA in paired CSF and plasma samples: (1) Discordance between the CSF and plasma HIV RNA levels was defined as CSF HIV RNA level greater than 1 $\log_{10}$ of the plasma viral load (Canestri et al., 2010), and (2) CSF viral escape (CVE) was defined as any HIV RNA in the CSF above the detection limit of the assay used, despite undetectable plasma levels by the same assay. CVE reflects the loss of control of brain HIV infection in a patient on effective antiretroviral therapy (ART) (Peluso et al., 2012).

2.2.3 Clinical laboratory measures—

CD4 counts were quantified by flow cytometry (FACSCalibur-Multitest). Nadir CD4 levels were extracted from medical records.

CSF samples were collected by LP performed using an atraumatic spinal needle and aseptic technique. CSF total protein, glucose, and WBC counts were quantified by standard laboratory methods.

2.3 Neurobehavioral assessments, HAND diagnosis, and categorization

All PWH participants underwent neuropsychological (NP) assessment as described in detail previously (de Almeida et al., 2013). The NP test battery assessed seven domains and comprised 18 individual NP measures widely used to study HIV infection in multinational studies. Subjective neurocognitive difficulty was assessed using the Patient's Assessment of Own Functioning Inventory (PAOFI) (Chelune et al., 1986). HIV-associated neurocognitive disorder (HAND) diagnoses were assigned according to the Frascati criteria (Antinori et al.,

2007). Additionally, the global deficit score (GDS) method was used to classify the overall NP impairment status, as previously described. The GDS summarizes the number and severity of neurobehavioral deficits across the entire test battery. A GDS cutoff <0.50 was used to classify overall NP impairment (Carey et al., 2004; Heaton et al., 2004).

2.4 Data analyses

The results are presented as median and interquartile range (IQR) or number and percentage (%), as appropriate. Demographic data, HIV disease characteristics, and CSF biochemical, cytological, and virological measures were compared between individuals with subtypes B and C infections using the independent samples *t*-test for continuous variables and Fisher's exact test for binary and categorical variables (sex, AIDS diagnosis, ART, HIV RNA in plasma and CSF). The demographic data and CSF biochemical and cytological measures were compared between PWH (including subtypes B, C, BF, BC, CF, and F) and HIV-negative controls using similar methods. The CSF and serum biomarker values were $\log_{10}$ transformed to improve the normality of data distribution. The biomarkers were then compared between subtype B and C groups using linear regression (adjusted analysis), controlling for plasma HIV viral load suppression and nadir CD4 count. The comparison of PWH and HIV-negative controls was adjusted by age.

Comparisons of IgG intrathecal synthesis in the HIV-1 groups categorized by the distribution of HIV RNA in CSF and blood compartments, as well as those categorized by the HAND classification, were analyzed with the Kruskal–Wallis or Mann–Whitney nonparametric tests (unadjusted analysis). Comparisons of the frequency of impaired formulas were performed using the Chi-square or Fisher's exact test for binary and categorical variables.

Results were considered statistically significant at the 5% alpha level. Cohen's d effect sizes (and 95% confidence intervals [CIs]) were reported for between-group differences. For the correlation analysis, correlation coefficients (r_S) were estimated using Spearman's rank-order method.

2.5 Standard protocol approvals, registrations, and patient consent

This study, a cross-sectional survey using stored blood and CSF samples, was approved by UCSD (San Diego, CA, USA) IRB, Hospital de Clínicas–Universidade Federal do Paraná (HC-UFPR, Curitiba, Paraná, Brazil) IRB, and the National Commission of Ethics in Research (CONEP, Brazil). All participants signed informed consent forms approved by the IRBs in the United States and Brazil. CSF samples were collected using a NIMH-funded protocol (R21 MH076651–01).

3. RESULTS

The demographic, clinical, and laboratory characteristics were compared between PWH and HIV-negative controls, and in individuals infecteced with subtypes B and C (Table 1). Subtype B- and C-infected individuals were similar in age, sex, and education. Subtype B-infected participants had lower nadir CD4 counts (median, 82 versus 159 cell/mm^3, p=0.29), and were more likely to be on ART (89% versus 69%; p=0.099). Participants taking ART were more likely to be virologically suppressed than those not taking ART (plasma HIV

RNA <50 copies/mL, 81% versus 40%, p=0.01). ART included non-nucleoside reverse transcriptase inhibitors (NNRTIs) in 9/21 (43%) of those with subtype B infections vs. 4/15 (27%) of those with subtype C infections (p=0.48) (Table 1). The characteristics of the groups classified in accordance with the CSF and blood HIV RNA were previously described (de Almeida et al. 2020a).

3.1 PWH versus HIV-negative controls and subtype B and C HIV

OCBs were searched in 39 PWH samples, of which 13 were HIV-1B, 16 HIV-1C, 3 HIV-1F, and 7 circulating recombinant forms (CRFs). IgG OCB in CSF was observed in 20/39 of PWH (51.28%); 6/13 (46.15%) in HIV-1B, 6/16 (37.50%) in HIV-1C (p = 0.716), 5/6 (83.30%) in BF CRF, and 3/4 (75%) other subtypes or CRF (Table 2). Among PWH the median (IQR) of number of OCB was 7.0 (5.5; 13), range 1.0 to 15; concerning HIV-1B it was 6 (4; 13) and for HIV-1C it was 6 (3; 14) (p=0.937). The median of IgG intrathecal synthesis was higher among PWH than that in the HIV-negative controls (Table 2; p<0.05). Although all medians were under the reference value, less absolute IgG and Schuller formula, which were increased (Table 2). The frequency of cases higher than the reference was higher in PWH than that in the controls (Table 2).

IgG intrathecal synthesis metric values were comparable for HIV-1B and HIV-1C patients with all formulas applied (Table 2; p>0.05).

The HF (Reibergram plot) of the blood–CSF barrier function and intrathecal IgG synthesis in participants with HIV-1B or HIV-1C, according to GDS, compared with the HIV-negative controls, is shown in Figure 1A and B.

3.2 Classification by HIV RNA in CSF and plasma

All the HIV groups categorized according to HIV RNA in CSF and plasma showed OCB; the higher frequency was in the group with CSF and plasma discordance (100%), although the number of cases was low. The group with HIV infection controlled in CSF and blood (aviremic) showed that OCB in 9/12 (75%) was significantly higher than that in the control group (p<0.0001), although comparable with the other HIV groups (all p>0.05, Table 3).

The median of IgG intrathecal synthesis in PWH categorized according to the distribution of HIV RNA in the CSF and blood compartments was shown on Table 3 and Figure 2. Compared to the HIV- negative control group, values of IgG, Q IgG, IgG/TP, and IgG CSF/Alb CSF were higher in all groups categorized according to the HIV RNA in CSF and plasma (p<0.05). Almost all the formulas studied, except for the Tourtellotte, Schuller, and Blennow formulas, had higher medians in the aviremic group than the control group (Table 3). All HIV groups categorized according to the distribution of HIV RNA in CSF and blood compartments presented samples with values increased than the reference values for each formula studied, indicating intrathecal IgG synthesis, including the CSF and plasma aviremic group and the group with HIV RNA in plasma higher than that in the CSF. The frequency of cases with increased values for each formula is shown in Table 3.

The HF (Reibergram plot) of the blood–CSF barrier function and intrathecal IgG synthesis in PWH were categorized according to the distribution of HIV RNA in CSF and blood compartments (Figure 1C).

3.4 HAND

In the group with GDS ≥0.5, there was IgG OCB in 15/22 (68.18%) samples, and in the group with GDS <0.5, there was an OCB in 4/12 (33.33%) (p=0.075). In the HAND-categorized groups according to the Frascati classification, the frequency of OCB increased from the NP normal to the neurocognitivelly symptomatic groups, as HIV-associated dementia (HAD) [5/13 (38.46%) and 3/4 (75%) respectively, p= 0.295], (Suppl.Table 1).

The medians of all formulas for the IgG intrathecal synthesis studied were comparable for the GDS ≥0.5 and normal GDS groups (GDS <0.5); moreover, the frequencies of increased IgG intrathecal synthesis cases for each formula were comparable for groups with GDS >0.5 and normal (data not shown, all p>0.05). The median (IQR) IgG intrathecal synthesis across HAND groups categorized according to the Frascati criteria was comparable for all formulas applied (pairwise coparisons, all p>0.05). However, the medians were numerically higher in the HAD and MND groups than that in the NP-NML and ANI groups, although the difference was not significant, which was same for the frequency of increased cases for each formula studied (pairwise comparisons, all p>0.05, Suppl. Table 1, Figure 2C).

The HF (Reibergram plot) of the blood–CSF barrier function and intrathecal IgG synthesis, for PWH categorized according to the HAND classification is shown in Figure 1D.

3.5 Correlations

Higher IgG intrathecal synthesis correlated moderately to strongly with higher CSF HIV RNA and CSF WBC, and with less robust CD4 recovery (Suppl. Table 2). The only formula that correlated with GDS was Q IgG (Sr 0.271 [95% CI, 0.011–0.497], p=0.036). Shorter duration of infection correlated with higher IgG HF (mg/dL) and the Auer formula (mg/dL) (−0.281 [−0.492; −0.038], p=0.021, and −0.288 [−0.498; −0.046], p= 0.017, respectively). There was no correlation between any of the formulas for IgG intrathecal synthesis with nadir and current CD4 or CPE.

The number of OCBs was higher in those with shorter duration of treatment with the current ARV regimen (Sr −0.663, p=0.0219). Each band indicate a different clone of B lymphocyte stimulated. There was no correlation of number of OCBs with GDS; nadir, current CD4 or CD4 recovery; duration of the infection, current age or age at the beginning of the infection, plasma or CSF HIV RNA, CSF WBC or NFL, or CPE.

4. DISCUSSION

The present study showed intrathecal synthesis of IgG in PWH with chronic infection, identified by quantitative methods and the presence of OCB. The majority of PWH included in this study were on ART (81%); all were free of CNS opportunistic infections. IgG intrathecal synthesis in PWH was subtype independent; however, it was associated with CSF and plasma HIV RNA discordance. Intrathecal IgG synthesis was higher in PWH with CSF

plasma discordance, compared with that in other groups, suggesting that intrathecal viral replication drives immune activation. In the CSF and blood HIV infection controlled (aviremic) group, the IgG intrathecal synthesis was still higher than that in the HIV-negative control group. The correlation of higher IgG intrathecal synthesis with lower CD4 recovery may indicate that immune dysregulation also contributes to intrathecal immune activation. In contrast, IgG synthesis did not correlate with nadir or current CD4. In the present study, higher IgG quotient correlated with worse neurocognitive performance, while the other formulas did not correlate with neurocognition. In contrast to the findings of other studies, higher daily intrathecal IgG synthesis (Tourtellotte formula) correlated with worse cognitive impairment (Singer et al., 1994), perhaps because participants in the older study were not on effective antiretroviral therapies.

Increased IgG synthesis was observed in 22% to 93% of patients in all clinical stages of HIV infection (Andersson et al., 1988; Marshall et al., 1991; Resnick et al., 1998). This does not necessarily imply neurological damage and may simply indicate HIV persistence in the CNS (Sönnerborg et al., 1989; Goswami et al., 1991). Highly active antiretroviral therapy (HAART) does not significantly reduce immune activation, as a significant proportion of patients continue to have macrophage activation signals and an elevated IgG index after treatment (Abdulle et al., 2005; Edén et al., 2007).

In therapy-naïve patients, intrathecal IgG produced correlates with HIV load in the CSF (Cepok et al., 2007). In patients followed longitudinally for up to 2 years after antiretroviral initiation, the proportion of elevated IgG index slightly decreased from 56% to 41% ^{9} or less (Yilmaz et al., 2006), while CD4 counts were normalized, and blood HIV RNA was lowered.

In the present study, IgG OCB in CSF was observed in 51% of PWH, with a frequency comparable between HIV-1B and HIV-1C. The group with undetectable viral load in CSF and blood showed 75% with OCB, comparable with the other HIV groups categorized according to the distribution of HIV RNA in CSF and plasma. This indicates the persistence of the immunological stimulus in CNS despite of the viral control. The frequency of OCB was numerically higher in the group with cognitive impairment (GDS ≥0.5) than in the cognitively normal group (68% vs. 33%, respectively), although this difference was not statistically significant. In this study, for the groups with HAND categorized by the Frascati classification, the frequency of OCB increased from the NP normal to the HAD group. OCB has been detected in all stages of HIV infection, regardless of the presence of HIV neurological disease (Andersson et al., 1988; Appleman et al., 1988; Gallo et al., 1988b). IgG OCB by IEF immunofixation is present in 18 to 70% of HIV-positive patients (McArthur et al., 1989; Singer et al., 1994; Gisslen et al., 1999; Chiodi et al., 1988b; Bukasa et al., 1988; Goudsmit et al., 1986; McArthur et al., 1988; Fainardi et al., 2001). These studies included only HIV-1 subtype B infections.

Our group previously described OCB by isoelectric focusing in serial CSF samples (de Almeida et al., 2017). The pattern of OCB in the CSF persisted similarly in the three sequential samples over 18 months (Suppl. Figure S1) (de Almeida et al., 2017), indicating persistence of the immunological stimulus in HIV CNS infection. However, other authors

have described that the number of OCBs increases with the duration of HIV infection. Serial examinations over years revealed a one-year latency from infection to the appearance of OCB (Andersson et al., 1988; Goudsmit et al., 1986).

Here, we observed that the median IgG intrathecal synthesis was numerically higher in the HAD and MND groups than that in the NP-NML and ANI groups, although the difference was not statistically significant. Previous studies have shown that patients in the advanced stages of the disease and HAND had higher intrathecal IgG synthesis, suggesting CNS activity of the infection (Resnick et al., 1985).

Moreover, we showed high IgG intrathecal synthesis, by quantitative and qualitative methods, even in the group without demonstrable viral replication in CSF and plasma (aviremic group). Our group previously described 55% of NP impairment based on a GDS ≥0.5 in aviremic PWH. The odds of cognitive impairment for participants aviremic in CSF and plasma were five times higher than those for a person without HIV infection (p=0.003) (de Almeida et al., 2020a). The persistence of cognitive impairment in this group can be attributed to the persistence of the intrathecal immune activation, as the virus remains latent in microglia and astrocytes, and even uninfected glia remain activated.

Cognitive impairment in PWH with effective ARV treatment could also be explained by previous irreversible brain injury and chronic HIV infection in the CNS; ART does not eradicate infection in the CNS and systemically; thus, inflammation continues to interfere with function.

The findings of the present study will provide additional support for previously published studies that investigated differences between HIV-1B and HIV-1C, which found no difference in the frequency of HAND or major depression (de Almeida et al., 2013; de Almeida et al. 2016a). Moreover, CSF inflammatory stimulation, investigated by the increase in CSF WBC, β-chemokine, and interleukin levels as well as CSF discordance or CVE, occurred at comparable frequencies between HIV-1 subtypes C and B (de Almeida et al., 2016b; 2016c; 2020a). However, we found subtype-dependent differences in amyloid pathway impairment (de Almeida et al., 2018a; 2018b; 2020b).

The main strength of this study was the fact that it was the first to examine intrathecal IgG synthesis in HIV-1C patients. All previous studies of IgG intrathecal synthesis concern HIV-1 subtype B. IgG intrathecal synthesis was analyzed according to the association of quantitative and qualitative methods. Participants with HIV-1 subtypes B and C were from the same geographical region in Brazil and were similar in age and sex. This study will also add to the contributions of previous reports by further investigating the impact of the pattern of HIV RNA in CSF and blood on IgG intrathecal synthesis.

Additionally a recently described formula for quantification of intrathecal immunoglobulin synthesis as the Auer formula (Auer et al., 2016) was applied for the first time in samples from PWH.

However, the present study has some limitations. It presented PWH on antiretroviral treatment and untreated, with most of the cases on ART; we tried to overcome this problem

by taking into consideration the plasma HIV viral load in the multivariate analysis. However, HIV-1B and HIV-1C were comparable in the CSF to plasma HIV RNA ratio. The cross-sectional design limited the study. A longitudinal study might be able to predict the development of HAND in patients without apparent symptoms. The sample size was sufficient for power analysis because absolute values of Cohen's d effect sizes were medium to large; however, when the PWH group was categorized by HAND diagnosis, the number of cases was small, especially with symptomatic HAND subgroups (HAD and MND), limiting the conclusion on the association of CSF IgG intrathecal synthesis with neurocognitive impairment.

5. CONCLUSION

This study contributes to the understanding of the pathophysiology of HIV infection in the CNS and the impact of HIV-1 genetic diversity on intrathecal IgG production, which was not subtype dependent. Low-grade CNS humoral immunology stimulation persists in HIV CNS infection even in PWH with controlled CSF and blood HIV RNA. The number of OCBs was higher in those with shorter duration of treatment. Moreover this study will introduce new possibilities of diagnostic investigations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Declarations

Ethics committee approval: This study, a cross-sectional survey using stored blood and CSF samples, was approved by UCSD (San Diego, CA, USA) IRB, Hospital de Clínicas–Universidade Federal do Paraná (HC-UFPR, Curitiba, Paraná, Brazil) IRB, and the National Commission of Ethics in Research (CONEP, Brazil). All participants signed informed consent forms approved by the IRBs in the United States and Brazil.

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Highlights

- Low-grade CNS intrathecal IgG production persists in HIV infection even in people with HIV (PWH) with viral suppression in CSF and blood.
- This indicates the presence of chronic CNS inflammation that may be amenable to anti-inflammatory therapies.
- The impact of HIV-1 on IgG intrathecal production was not subtype dependent.
- IgG oligoclonal bands (OCB) in CSF were observed 51% of PWH.
- The group with HIV suppression in CSF and blood showed OCB in 75% of cases.
- The number of OCB was higher in those with shorter duration of treatment with ARV.
- The increase of Q IgG correlated with increase of cognitive impairment.

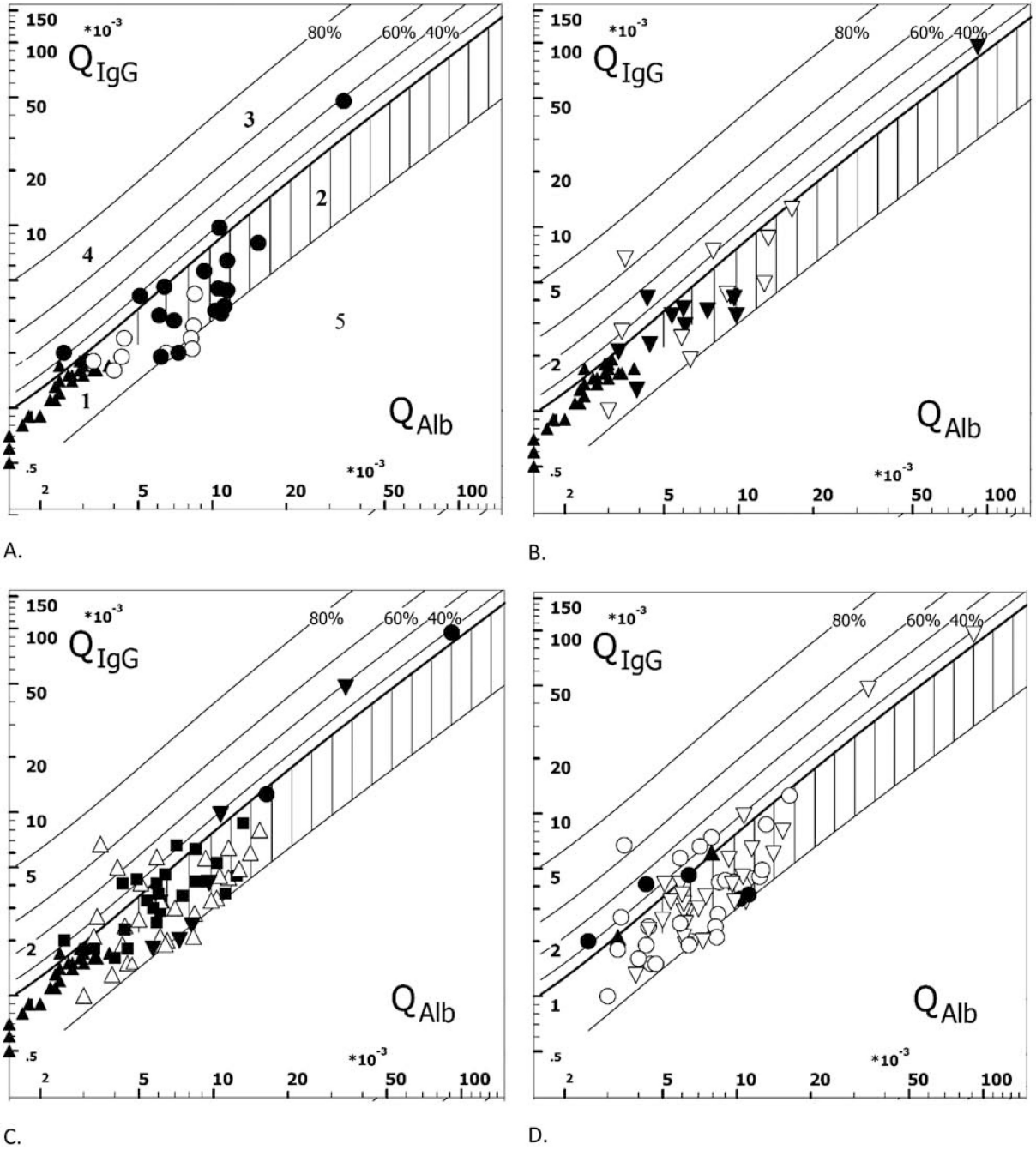

Figure 1. Hyperbolic function (Reibergram plot) of the blood–CSF barrier and intrathecal IgG synthesis

The graph shows the dynamics of intrathecal IgG synthesis and blood–CSF barrier (BCSFB) dysfunction reconstitution. Hyperbolic functions are a consequence of nonlinear interactions of molecular flux with the CSF flow rate as derived from the laws of diffusion (Reiber, 1995). The diagrams depict five ranges: 1 = normal, 2 = pure blood–CSF barrier dysfunction (i.e., reduced CSF turnover), 3 = intrathecal IgG synthesis with reduced CSF turnover, and 4 = intrathecal IgG synthesis without change in CSF turnover. Values below the lower hyperbolic line, in range 5, indicate a methodological fault. The plot was generated using

CSF Research software.A. Participants with HIV-1B: GDS≥0.5, filled circle; GDS <0.5, open circle; HIV-negative controls, filled triangle B. Participants with HIV-1C: GDS ≥0.5, filled side down triangle; GDS <0.5, open side down triangle; HIV-negative controls, filled triangle C. Participants with HIV-1 categorized by the distribution of HIV RNA in CSF and blood compartments: discordance, filled circle; escape, side down filled triangle; plasma >CSF, filled square; aviremic in CSF and plasma, open triangle; HIV-negative controls, filled triangle. D. Participants with HIV-1 categorized by HAND classification (Antinori et al., 2007), HIV-associated dementia (HAD), filled circle; mild neurocognitive disorder (MND), filled triangle; asymptomatic neurocognitive impairment (ANI), open side down triangle; cognitive normal, open circle.

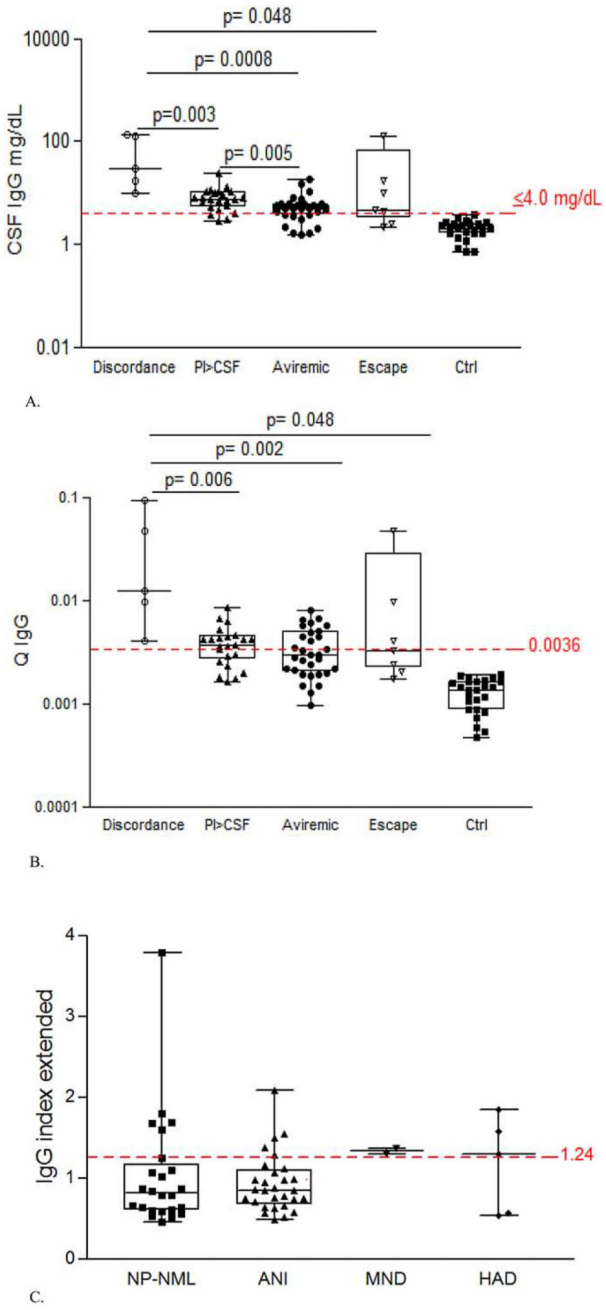

Figure 2

Figure 2. IgG intrathecal synthesis in the HIV-1 groups categorized by the distribution of HIV RNA in CSF and blood compartments and HAND classification

A. Absolute IgG in CSF (mg/dL). Red dotted line indicates reference value of absolute CSF IgG (4 mg/dL) B. Q IgG. Red dotted line indicates reference value of Q IgG (0.0036) C. IgG index extended. Red dotted line indicates reference value of IgG index extended (1.24). Ctrl= HIV-negative controls; NP-NML vs. ANI vs. MND+HAD (p=0.425); NP-NML vs. ANI vs. HAD (p=0.826)

Table 1.

Demographics and AIDS clinical characteristics in PWH, HIV-negative controls, and individual with HIV-1 subtypes B and C

	HIV− (n=25)	HIV+ (n= 68)	p	HIV1-B (n=27)	HIV1-C (n=26)	p
Demographics						
Age, years	31 (25; 34)	43 (35; 48)	<0.0001	44 (36.5; 50)	43 (34.5; 47.5)	0.450
Education, years	-	8 (5; 11)	-	8 (5; 12)	7 (5; 11.5)	0.550
Sex - male, n (%)	13 (52.0)	33 (49.0)	0.818	14 (51.9)	11 (42.3)	0.590
Disease and Treatment						
Duration of infection (months)	-	89 (31; 135)	-	91.03 (61.63; 144)	81.37 (27.82; 132)	0.450
AIDS, n (%)	0	55 (80.9)		22 (81.5)	19 (73.1)	0.526
GDS	-	0.65(0.30;105)	-	0.95 (0.275; 1.725)	0.50 (0.225; 0.875)	0.126
B/C, n	-	27/26	-	27	26	-
Current CD4 cells/mm^3	-	369 (201; 534)	-	457 (255; 614)	359.5 (176.5; 472.5)	0.200
Nadir CD4 cells/mm^3	-	90 (33; 266)	-	82 (26; 253.5)	159 (16.5; 359.5)	0.290
CART, n (%)	-	55 (80.9)	-	24 (88.9)	18 (69.2)	0.099
CPE	-	8 (6; 9)	-	8 (6; 9)	6(5.5; 9)	0.339
Adherence, n (%)	-	51/54 (94.4)	-	21/23 (91.3)	18/18 (100)	0.495
Plasma HIV RNA (Log_{10})	-	1.7 (1.7; 3.5)	-	1.7 (1.7; 1.97)	2.8 (1.7; 3.8)	0.012
Pl HIV RNA<50copies/mL, n (%)		38 (55.9)		20 (74.1)	9 (34.6)	0.006
CSF						
HIV RNA (Log_{10})	-	1.7 (1.7; 2.8)	-	1.7 (1.7; 2.2)	2.2 (1.7; 2.9)	0.084
HIV RNA <50 copies/mL, n (%)	-	35 (51.5)	-	16 (59.3)	10 (38.5)	0.173
HIV RNA CSF >plasma, n (%) [1]	-	12 (17.7)	-	5 (18.5)	5 (19.2)	1.00
CSF escape [2]	-	7 (10.3)	-	5 (14.8)	1 (3.9)	0.351
Discordance [3]	-	5 (7.4)	-	2 (7.4)	3 (11.5)	0.670
WBC cells/mm^3	0.60 (0.60; 2.00)	2.10 (0.60; 7.20)	0.015	1.60 (0.30; 4.85)	2.65 (0.60; 11.00)	0.160
RBC cells/mm^3	0.00	0.50 (0.00; 7.50)	0.007	1.00 (0.00; 24.00)	0.80 (0.00; 36.50)	0.900
Glucose mg/dL	56.00 (53.00; 62.50)	57.00 (53.00; 62.00)	0.649	63.00 (54.00; 66.00)	56.00 (51.50; 59.00)	0.007
Total protein mg/dL	23.00 (16.00; 27.00)	40.00 (32.00; 46.00)	<0.0001	42.00 (35.00; 47.50)	40.00 (28.50;47.00)	0.551
Total protein >45 mg/dL, n (%)	0.00	20 (29.4)	-	10 (37.0)	8 (30.8)	0.773
Albumin mg/L	159.50 (118.30; 184.70)	223.50 (164.00; 288.50)	<0.0001	248.00 (189.00; 309.00)	218.00 (138.50; 300.00)	0.328
Q. Albumin	0.002 (0.002; 0.003)	0.006 (0.005; 0.010)	<0.0001	0.008 (0.006; 0.011)	0.006 (0.004; 0.010)	0.52 [4]
Neopterin nmol/L	-	8.70(5.72; 15.81)	-	6.25(5.01; 16.53)	11.25 (5.89; 16.29)	0.910 [4]

	HIV− (n=25)	HIV+ (n= 68)	p	HIV1-B (n=27)	HIV1-C (n=26)	p
B_2M mg/L	-	1.73(1.32; 2.33)	-	1.45(1.26; 2.15)	1.93 (1.36; 2.39)	0.800 [4]

Data are median (IQR) or number of cases (%)

Comparisons were performed using independent samples t-test for continuous variables and Fisher's exact test for binary and categorical variables

[1]Any value of CSF or blood HIV RNA

[2]CSF escape is defined as any HIV RNA level in the CSF above the limit of detection of the assay used (usually 50 copies/mL) when the plasma HIV RNA levels are undetectable by the same assay

[3]CSF discordance is defined as a CSF viral load (VL) greater than $1\log_{10}$ of the plasma HIV RNA levels (independent of the number)

[4]Adjusted for plasma HIV VL suppression and nadir CD4

CART, combination antiretroviral therapy

CPE, CNS Penetration-Effectiveness rank

B/C, HIV-1 subtypes B / C; pl, plasma; GDS, global deficit score; RBC, red blood cells; WBC, white blood cells

Table 2.

IgG intrathecal synthesis in PWH, HIV-negative controls, and individuals with HIV subtypes B and C

a. Median (IQR)	**HIV+ (n= 68)**	**HIV− (n=25)**	**Cohen'sd (95%CI)**	**p**	**HIV1-B (n=27)**	**HIV1-C (n=26)**	**Cohen'sd (95%CI)**	**p**
IgG mg/dL	5.77(4.23;9.90)[1]	2.06(1.59;2.48)	−1.71(−2.24, −1.19)	**<0.001**	5.12(4.10;8.70)[1]	7.08(5.09;10.65)[1]	−0.29(−0.86;0.27)	0.710
IgG CSF/PT CSF (%)	16.38(11.17;23.27)	8.97(6.90;11.14)	−1.27(−1.77, −0.77)	**<0.001**	13.95(10.61;20.82)	19.97(12.37;28.54)[1]	−0.51(−1.09;0.06)	0.420
IgG CSF/Alb CSF	0.28(0.19;0.45)	0.13(0.11;0.14)	−1.59(−2.11, −1.07)	**<0.001**	0.23(0.18;0.39)	0.37(0.23;0.52)[1]	−0.6(−1.17;−0.02)	0.280
Q IgG	0.01(0.002;0.01)	0.001(0.001;0.002)	−1.70(−2.23, −1.17)	**<0.001**	0.003(0.002;0.005)	0.004(0.002;0.005)	−0.13(−0.69;0.44)	0.920
IgG Index	0.49(0.35;0.70)	0.49(0.48;0.57)	−0.03(−0.5, 0.43)	0.850	0.45(0.33;0.58)	0.52(0.42;0.79)	−0.45(−1.02;0.12)	0.280
IgG index extended	0.87(0.64;1.29)	1.02(0.95;1.17)	0.32(−0.15, 0.78)	0.130	0.86(0.57;1.07)	0.94(0.76;1.34)	−0.47(−1.03;0.09)	0.240
IgG HF (mg/dL)	1.77(−3.63;0.37)	−0.99(−1.23;−0.78)	0.23(−0.23, 0.70)	0.290	−2.88(−4.76;−0.87)	−1.70(−3.98;1.27)	−0.13(−0.69;0.43)	0.640
Tourtellotte (mg/24h)	−4.31(−9.33;6.28)	−5.11(−6.07;−4.06)	−0.12(−0.58;0.35)	0.880	−4.65(−12.91;4.35)	−3.94(−8.45;11.50)	−0.22(−0.78;0.35)	0.560
Schuller (mg/dL)	3.84(1.89;7.60)[1]	1.04(0.90;1.58)	−0.81(−1.29, −0.33)	**0.003**	2.49(1.14;5.85)[1]	5.81(2.92;8.23)[1]	−0.2(−0.76;0.36)	0.730
Auer (mg/dL)	−2.32(−5.15;−0.48)	−0.69(−0.90;−0.53)	0.42(−0.04, 0.89)	0.086	−3.67(−5.85;−1.26)	−2.43(−5.20;0.30)	−0.12(−0.69;0.44)	0.620
Auer, IgG (%)	20.79 (9.29;32.35)	0			12.22(7.42; 43.79)[1]	21.36(13.20;43.27)		0.352
Blennow (mg/L)	−2.82(−15.93;20.35)	−0.79(−1.57;2.44)	0.04(−0.42, 0.51)	0.600	−3.51(−21.97;9.04)	0.80(−10.36;30.87)	−0.34(−0.9;0.23)	0.330
Log IgG Index	0.28(−0.04;0.65)	0.29 (0.26;0.44)	0.09(−0.38, 0.55)	0.470	0.21(−0.13;0.46)	0.34 (0.13;0.76)	−0.17(−0.81;0.47)	0.600
b. Frequency of cases higher than the reference range, n (%)								
IgG mg/dL	49 (70.59)	0		**<0.0001**	19 (70.37)	22 (84.62)		0.215
IgG CSF/PT CSF (%)	27 (39.71)	0		**0.0002**	7 (25.93)	15 (57.69)		**0.019**
IgG CSF/Alb CSF	33 (48.53)	0		**<0.0001**	9 (33.33)	17 (65.38)		**0.020**
Q IgG	30 (44.12)	0		**<0.0001**	10 (37.04)	12 (46.15)		0.501
IgG index	15 (22.06)	0		**0.010**	4 (14.81)	7 (26.92)		0.277
IgG index extended	19 (27.94)	2 (8.00)		**0.041**	5 (18.52)	8 (30.77)		0.300
IgG HF (mg/dL)	18 (26.47)	0		**0.004**	5 (18.52)	7 (26.92)		0.465
Tourtellotte (mg/24h)	14 (20.59)	0		**0.014**	3 (11.11)	6 (23.08)		0.246
Schuller (mg/dL)	46 (67.65)	2 (8.00)		**<0.0001**	14 (51.85)	22 (84.62)		**0.011**
Auer (mg/dL)	14 (20.59)	0		**0.012**	4 (14.81)	6 (23.08)		0.442
Blennow (mg/L)	23 (33.82)	0		**0.001**	6 (22.22)	11 (42.31)		0.117
Log IgG Index	10 (14.71)	0		**0.042**	2 (7.41)	5 (19.23)		0.204
OCB, N (%)	20/39 (51.28)	0		**<0.0001**	6/13 (46.15)	6/16 (37.50)		0.716

a. Median (IQR)	HIV+ (n= 68)	HIV− (n=25)	Cohen'sd (95%CI)	p	HIV1-B (n=27)	HIV1-C (n=26)	Cohen'sd (95%CI)	p
N OCB (median;IQR)	7.00 (5.50;13.00)	0		-	6.00 (4.00;13.00)	6.00 (3.00;14.00)		0.937

OCB, oligoclonal bands;N OCB, number of oligoclonal bands

a. Values are presented as median (IQR)

[1] value of median higher than the reference value. HIV+ vs. HIV−, p value adjusted for age;HIV-1B vs. HIV-1C, p-value adjusted for plasma HIV viral load suppression and nadir CD4 count Auer, IgG (%) indicates the percentage of IgG intrathecal production in the cases with Auer formula higher the reference value.

b. Frequency of cases higher than the reference range;values are presented in number of cases (%);comparisons were performed using Fisher's exact test.

Table 3.

IgG intrathecal synthesis in the HIV groups categorized according to the distribution of HIV RNA in CSF and blood compartments

b. Frequency of cases higher than the reference range, n (%)									
IgG mg/dL	4 (57.14)	5 (100)	19 (82.61)	20 (64.51)	1.00	0.306	0.295	0.568	0.220
IgG CSF/PT CSF (%)	3 (42.86)	5 (100)	14 (60.87)	6 (19.35)	0.323	0.666	**0.001**	0.144	**0.004**
IgG CSF/Alb CSF	3 (42.86)	5 (100)	16 (69.57)	8 (25.81)	0.390	0.372	**0.003**	0.290	**0.002**
Q IgG	3 (42.86)	5 (100)	11 (47.83)	11 (35.48)	1.00	1.000	**0.012**	**0.053**	0.411
IgG index	2 (28.57)	4 (80)	4 (21.05)	5 (16.13)	0.592	0.603	**0.009**	**0.015**	1.000
IgG index extended	2 (28.57)	4 (80)	7 (43.73)	6 (19.35)	0.624	1.000	**0.015**	0.062	0.521
IgG HF (mg/dL)	2 (28.57)	4 (80)	7 (43.73)	5 (16.13)	0.592	1.000	**0.009**	0.062	0.322
Tourtellotte (mg/24h)	2 (28.57)	4 (80)	5 (27.77)	3 (9.68)	0.223	1.000	**0.003**	**0.026**	0.264
Schuller (mg/dL)	3 (42.86)	5 (100)	22 (95.65)	14 (45.16)	1.00	0.006	**0.047**	1.000	**<0.0001**
Auer (mg/dL)	2 (28.57)	3 (60)	4 (17.39)	5 (16.13)	0.592	0.603	0.062	0.083	1.000
Blennow (mg/L)	2 (28.57)	4 (80)	10 (43.48)	7 (22,58)	1.00	0.669	**0.023**	0.326	0.141
LN IgG Index	2 (28.57)	3 (60)	3 (13.04)	3 (9.68)	0.223	0.565	**0.024**	**0.050**	1.000
OCB, N (%)	1/3 (33.00)	3/3 (100)	7/17 (41.12)	9/12 (75.00)	0.242	1.00	1.00	0.214	0.130
N OCB (median; IQR)	3 (3; 3)[3]	6 (3; 3)[3]	6.0(5.0; 11.50)	11(6.5; 13)	-	-	0.481	1.00	0.114

a. Median (IQR)	**A-Escape n=7** [1]	**B-Discordance n= 5** [2, 3]	**C-HIV RNA Pl > CSF, n=23**	**D-CSF/Pl Aviremic n=31**	**Avs.D p**	**Avs.C p**	**Bvs.D p**	**Bvs.C p**	**Cvs.D p**
IgG mg/dL	4.57 (3.34; 69.55) [4]	30.20 (9.69; 138.0) [4]	7.52 (5.40; 10.55)[4]	5.05 (3.67; 6.11) [4]	0.651	0.624	**0.001**	**0.003**	**0.005**
IgG CSF/PT (%)	11.91 (9.80; 46.84)	33.19 (23.07; 68.54) [4]	19.55 (16.18; 26.33)[4]	11.75 (9.39; 17.86)	0.547	0.281	**0.003**	**0.008**	**0.0003**
IgG CSF/Alb CSF	0.21 (0.17; 0.94)	0.60 (0.31; 1.39) [4]	0.36 (0.28; 0.49)[4]	0.20 (0.14; 0.33)	0.679	0.155	**0.005**	**0.023**	**0.0003**
Q IgG	0.003 (0.002; 0.03)	0.01 (0.004; 0.10)	0.004 (0.003; 0.005)[4]	0.003 (0.002; 0.005)	0.572	0.883	**0.002**	**0.006**	0.310
IgG index	0.43 (0.30; 1.15)	0.90 (0.43; 1.39) [4]	0.53 (0.46; 0.73)	0.43 (0.33; 0.58)	0.821	0.303	**0.017**	0.055	**0.033**
IgG index extended	0.75 (0.55; 1.82)	1.38 (0.75; 2.09) [4]	0.99 (0.82; 1.31)	0.74 (0.62; 1.05)	0.763	0.220	**0.040**	0.208	**0.024**
IgG HF (mg/dL)	−2.82 (−4.29; 30.16)	4.16 (−5.55; 56.16) [4]	−1.52 (−2.45; 0.71)	−2.00 (−4.20; −0.87)	0.851	0.806	**0.040**	**0.036**	0.294
Tourtellotte (mg/24h)	−10.02 (−10.84; 224.2)	55.64 (−10.02; 411.10) [4]	−3.17 (−7.09; 10.37)	−5.18 (−10.46; 3.85)	1.00	0.492	**0.014**	**0.031**	0.133

Schuller (mg/dL)	2.12 (1.38; 51.43)	16.67 (3.90; 98.03)[4]	5.72 (3.65; 7.67)[4]	2.21 (0.22; 4.27)	0.474	0.170	**0.002**	**0.019**	**0.0003**
Auer (mg/dL)	−3.51 (−5.36; 28.13)	2.84 (−7.24; 53.43)[4]	−2.32 (−3.69; −0.24)	−2.53 (−5.181; −1.36)	0.792	1.00	**0.049**	0.055	0.518
Auer, IgG (%)	30.19 (16.58; 43.79)	21.83 (16.58; 43.79)	18.19 (7.424; 24.15)	24.30 (7.86; 62.39)	-	-	**1.000**	0.400	0.413
Blennow (mg/L)	−16.61 (−18.41; 424.0)	99.93 (−18.28; 773.80)[4]	2.03 (−7.26; 26.73)	−8.49 (−17.38; 8.14)	0.940	0.462	**0.020**	**0.031**	**0.054**
Log IgG Index	0.15 (−0.20; 1.12)	0.90 (0.15; 1.33) [4]	0.37 (0.22; 0.68)	0.15 (−0.10; 0.46)	0.821	0.303	**0.017**	0.055	**0.033**

OCB, oligoclonal bands; N OCB, number of oligoclonal bands

a. Values were presented as median (IQR); comparisons performed by Mann–Whitney nonparametric tests (unadjusted analysis).

[1] CSF escape is defined as any VL in the CSF above the limit of detection of the assay used (usually 50 copies/mL) when the VL in the plasma is undetectable by the same assay. All cases were on CART.

[2] CSF discordance is defined as a CSF viral load (VL) greater than 1$\log_{10}$ of the plasma VL (independent of the number).

[3] Data are median (min, max).

[4] values of median higher than the reference value

The comparison of escape and discordance was not done because of the superimposition of three participants

Auer, IgG (%) indicates the percentage of IgG intrathecal production in the cases with Auer formula higher the reference value.

The pairwise comparison of the groups categorized according to HIV RNA in CSF and plasma with the control group, were higher than the control group for absolute value of IgG, Q IgG, IgG/TP (%), IgG CSF/Alb CSF ($p<0.05$). Concerning Tourtellotte formula was significant only for discordance vs. control ($p=0.040$); IgG index only for discordance and aviremic group vs. control (0.025; 0,05 respectivelly); IgG index extended was only significant for aviremic vs control (0.003); Schuller formula was significant for cases with HIV RNA in plasma >CSF or discordance vs. control (<0.001; 0.0004 respectively); IgG HF for discordance or aviremic (0.0360; 0.0038 respectively); Concerning Auer formula all groups were significant when compared with HIV− control, less escape vs. control ($p=0.099$); Blennow only for discordance vs. control ($p= 0.032$); LN IgG index discordance or aviremic vs. control (0.0252; 0.051 respectively)

b. Frequency of cases higher than the reference range; values are presented in “n” (%); comparisons were performed using Fisher’s exact test.

D vs. Ctl: $p<0.0001$, 0.028, 0.006, 0.0006, 0.058, 0.277, 0.058, 0.245, 0.003, 0.058, 0.013, 0.245 (IgG, IgG CSF/PT, IgG CSF/Alb CSF, Q IgG, IgG index, IgG index extended, IgG HF, Tourtellotte, Schuller, Auer, and Blennow formulas, and Log IgG Index respectively).