Cerebrospinal fluid viral load and neopterin in HIV-positive patients with undetectable viraemia

Plasma Non-detectable HIV RNA and Cerebrospinal Fluid Viral Load and Neopterin in HIV-positive HAART-treated patients

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Running Head: CSF undetectable HIV RNA

Type of article: Short communication

Figures: 2

Tables: 1

Words count: 1249

Abstract words count: 191

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Abbreviations:

TND Target not detected, CSF cerebrospinal fluid, LLV low level viremia

Abstract

Background

Cerebrospinal fluid (CSF) HIV RNA is commonly used as a marker of compartmental antiviral activity in HIV-positive patients. Undetectable CSF HIV RNA levels have been associated with low CSF neopterin levels and better neurocognitive performances. Aim of this study was to analyse the prevalence and predictors of non-detectable CSF HIV RNA using a commercial assay.

Methods

In adult HIV-positive HAART-treated patients with confirmed plasma HIV RNA <50 copies/mL, CSF HIV RNA (with Roche Amplicor Assay) and neopterin were measured.

Results

112 adult patients were included. Plasma and CSF HIV-RNA was non-detectable (TND) in 29 (25.9%) and 36 (32.1%) patients, respectively. CSF TND was observed more frequently in patients with plasma TND (p=0.005, OR=3.87). CSF neopterin levels were associated with age (rho=0.333, p=0.002) and current (rho= -0.272, p=0.015) and nadir (rho=-0.240, p=0.038) CD4+ T lymphocytes; the lowest CSF neopterin concentration was observed in patients with CSF TND versus other viral load strata (0.62 mg/dl vs. 0.78 mg/dL, p=0.048).

Conclusions

Efficaciously treated HIV-positive patients with detectable plasma HIV RNA might imperfectly control CSF viral replication. Prospective studies addressing the management and neurocognitive consequences of CSF low level viremia are warranted.

Key words: low level viral load, CSF, central nervous system, neuroefficacy, neopterin.

Background

After penetration in central nervous system HIV-infected cells may express neurotoxins leading to permeable blood brain barrier and to neuronal injury. (1,2) Cerebrospinal fluid (CSF) HIV RNA is the most commonly used marker of compartmental viral replication in HIV-positive patients although its relationship with brain tissue viral load is not uniform. (3,4)

Neopterin is a well-established early marker of intrathecal immune response, and particularly of macrophage activation. The CSF neopterin levels are elevated in most HIV-1-infected individuals and decrease significantly after initiation of antiretroviral therapy achieving CSF concentrations observed in HIV-negative subjects. (5) Two exceptions have been reported: low CSF neopterin levels in patients treated during primary HIV infection (6) and in those with undetectable CSF HIV RNA by ultra-sensitive methods. (7-10)

Following such evidence, maintaining an undetectable CSF HIV RNA would be one of the long-term objectives of antiretroviral therapy. Unfortunately proven strategies to reach such target are lacking.

Objectives

Aim of this study was to analyse the prevalence and predictors of non-detectable CSF HIV RNA.

Study design

The study was conducted at the Unit of Infectious Diseases, Department of Medical Sciences, University of Torino, Italy between January 2013 and February 2015. Ethics Committee approval was obtained (Comitato Etico Interaziendale, Ospedale San Luigi Gonzaga, Orbassano Torino) and all patients provided a written informed consent.

Adult HIV-positive HAART-treated patients enrolled in a prospective ongoing cohort with confirmed plasma HIV RNA <50 copies/mL (at least two determinations, the latter in the previous

30 days) undergoing lumbar punctures for clinical reasons were included. Exclusion criteria included the presence of central nervous system infectious, inflammatory or neoplastic lesions.

Routine cerebrospinal fluid examinations (including HIV RNA, resistance associated mutations and neopterin) were performed. Neopterin (normal value <1.5 ng/ml) was analysed with an enzyme-linked immunosorbent assay. HIV-1 RNA quantification was performed with the Roche Amplicor assay (version 2.0; Hoffman-La Roche, Basel, Switzerland) with a lower limit of quantification of 20 copies/mL. CNS concentration penetration effectiveness score (CPE) was derived from Hammond et al. (11)

Data are expressed as medians (interquartile range). Non-parametric tests were used for all analysis using SPSS software for Macintosh (version 22.0, IBM Corp.).

Results

One hundred-twelve patients were included; subjects' characteristics are shown in **Table 1**. Previous syphilis was recorded in 18 (16.1%) subjects. Plasma HIV-RNA was TND, <20 cp/ml and 20-50 cp/ml in 29 (25.9%), 65 (58%) and 18 (16.1%) patients. Antiretroviral regimens were mostly based on the association of 2 NRTIs (95 patients, 84.8%) plus either a PI (81 patients, 72.3%) followed by a NNRTI (16 patients, 14.3%) or a integrase inhibitor (14 patients, 12.5%). Median CPE was 7 (IQR 6-8).

CSF HIV RNA was TND, <20, 20-50 and >50 cp/ml in 36 (32.1%), 35 (31.3%), 11 (9.8%) and 30 (26.8%) patients, respectively; in those subjects with CSF HIV RNA >50 copies/mL its median value was 122 copies/mL (73-185). Median CSF neopterin was 0.6 (0.48-1.07) ng/ml; it was >1.5 ng/ml in 9 patients (8%).

	n or median	% or IQR
Gender: male	74	66.1%
Ethnicity: Caucasian	81	72.3%
Age: years	47.9	40.9-56.2
HBsAg positive	12	10.7%
HCV Ab positive	30	26.8%
Liver cirrhosis	15	13.4%
Nadir CD4 cell count: n/uL	86	11-215
Current CD4 cell count: n/uL	387	194-601
Estimated duration of HIV infection: years	11.9	2.8-18.9
Reason for lumbar puncture		
Longitudinal cohort	46	41.1%
Neurocognitive disorders	24	21.4%
White matter abnormalities	13	11.6%
Differential diagnosis	13	11.6%
Long-term follow up of opportunistic infections	11	9.8%
Persistent headache	7	6.2%

Table 1. Characteristics of included patients.

HBsAg, hepatitis B surface antigen. **HCV Ab**, hepatitis C virus antibody. **IQR**, interquartile range.

CSF TND was more common in patients with plasma TND [16 out of 29 patients (55.2%) vs. 20 out of 83 patients (24.1%), p=0.005, OR=3.87] (**Fig.1**). No other variable was significantly associated with non-detectable HIV RNA in the CSF.

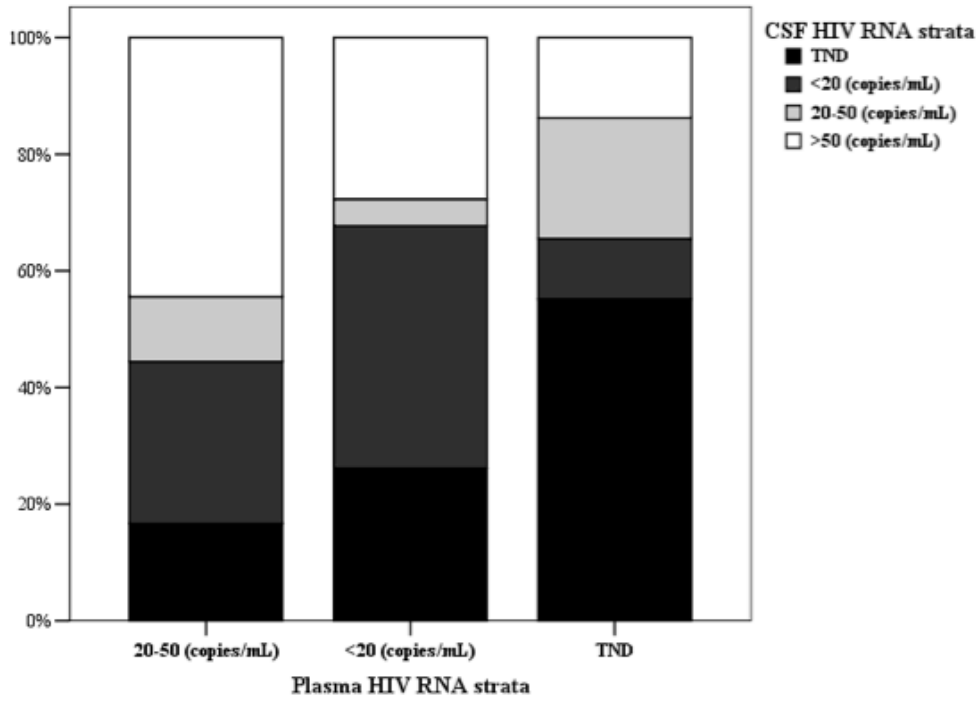
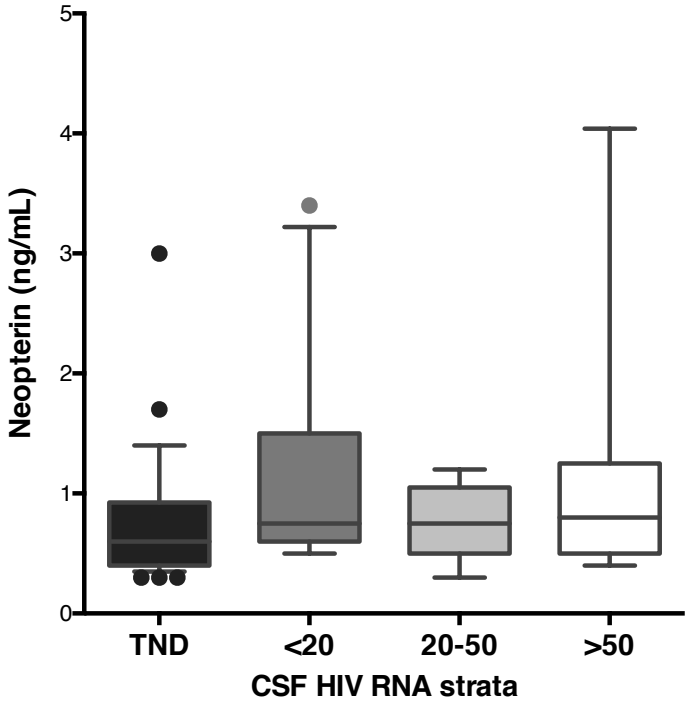


Figure 1. Prevalence of cerebrospinal fluid viral load strata according to plasma viral load levels. TND, target not detectable.

CSF neopterin levels correlated with CSF HIV RNA (rho=-0.22, p=0.048) with lower values in patients with TND as compared to those in other CSF viral load strata [0.62 ng/mL (0.41-0.923) vs. others 0.78 ng/mL (0.54-1.21), p=0.048] (**Fig. 2**) but not in patients with plasma TND (0.73 vs. 0.63 ng/mL, p=0.512). Furthermore CSF neopterin levels showed a weak inverse correlation with current (rho= -0.272, p=0.015) and nadir (rho=-0.240, p=0.038) CD4+ T lymphocytes and a direct correlation with age (rho=0.333, p=0.002).



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142 **Fig 2. Cerebrospinal fluid neopterin concentrations according to CSF viral load strata.**

143 Horizontal lines represent median values; boxes represent interquartile ranges while whiskers are

144 10-90 percentiles. TND, target not detectable.

145

146 **Conclusions**

147 The main results of this analysis are that the non-detectable CSF HIV RNA was observed more

148 frequently in patients with plasma non-detectable plasma HIV RNA (suggesting this as a possible

149 screening marker for optimal viral control) and that it was associated with the lowest CSF neopterin

150 concentrations (suggesting that it may be a good target for CNS-oriented antiretroviral therapy).

151 Our aim was to analyse the prevalence and predictors of non-detectable CSF HIV viral load by

152 using the Roche Amplicor Assay: this test is able to discriminate non-detectable RNA (associated

153 with better virological control and less risk of future treatment failures) to non-quantifiable but

154 detectable RNA (less than 20 copies/mL). Sensitive CSF HIV RNA analyses have been performed

155 so far using single copy assay thus requiring a large quantity of cerebrospinal fluid collected (above

156 7 mL for this determination) and expensive laboratory work. (5,8) Using a commercial assay might
157 provide useful and clinically applicable information.

158 Some limitations should be acknowledged such as the cross-sectional design and the heterogeneous
159 reasons for performing lumbar punctures.

160 Several determinants of CSF viral control have been identified so far including plasma HIV RNA
161 <50 copies/mL, depression, duration of HAART, HAART adherence and nadir CD4 cell count.
162 (11-14) The role of different drugs is debated: higher CPE scores have been associated in most
163 studies with lower CSF HIV RNA (13,15,16) even if treatment intensification was reported to have
164 no effect in controlling residual CSF replication. (17) The association of CPE with better
165 neurocognitive outcomes is even more controversial. (18,19) In patients with less drug pressure and
166 specifically on protease inhibitors monotherapy a low nadir CD4 cell count was associated with
167 CSF escape and neurological symptoms. (20) Furthermore individual CSF concentrations, rather
168 than estimated penetration, might inform on the compartmental efficacy of antiretroviral regimens
169 (12).

170 We were here able to identify optimal plasma control as the only predictor of CSF undetectable
171 HIV RNA. In several studies LLV was associated with virological failure, with viral reservoirs size
172 and, recently, with CSF escape. (21-27)

173 We also confirmed what Yilmaz and Dahl (5,8) observed in previous studies: only patients with the
174 lowest residual CSF HIV RNA showed the lowest levels of immune activation. Impact of residual
175 HIV RNA has been linked to higher levels of immune activation and pro-inflammatory cytokines in
176 periphery and we suggest this observation could be assumed as possible explanation of our result.

177 Early treatment for all HIV-positive patients might be beneficial in preventing advanced immune
178 suppression, even if an overall neurocognitive advantage (or disadvantage) was not found for
179 immediate ART initiation in asymptomatic treatment-naive individuals with high CD4 counts.

(27,28). Data have been published showing neurocognitive improvement in HAART patients treated regardless of CD4 cell count and those treated during primary HIV infection where neopterin level normalized after early initiation of HAART. (6, 30, 31)

Occasional CSF HIV-RNA may be clinically benign and represent low fluctuations in release of virus into the CSF rather than lack of treatment efficacy. (32,33) Dahl et al. found that patients who had detectable viral replication at one point did not have elevated CSF neopterin levels at other time points. (8) This observation suggests that such patients may not have "chronic" immune activation in the CSF but rather the release of very low levels of HIV-RNA that may lead to macrophage activation and neopterin production even during suppressive therapy.

Is HAART not enough to control viral induced immune response? Reducing HIV replication in the brain may not have effects on other processes involved in neuronal injury, including ongoing immune activation. Several data suggested that maraviroc might have additional positive effect in the central nervous system possibly by interfering with immune activation processes. (34,35)

Prospective studies addressing the management and neurocognitive consequences of very low level CSF HIV RNA and immune-activation are warranted. Possible adjunctive therapies, beside efficacious antiretroviral treatments, are needed.

Acknowledgements

GDP and SB has received grants, travel grants, and consultancy fees from Abbvie, Boehringer-Inghelheim, BMS, MSD, Gilead, Janssen-Cilag and Viiv. AC has received grants from Gilead and Bristol-Myers Squibb (BMS), travel grants and speaker's honoraria from Abbvie, BMS, Merck Sharp & Dohme (MSD), Gilead, Janssen-Cilag and Viiv. Other authors declare no potential conflict of interest.

Authors' contribution

204 AC, SB, DI, VG designed the study. IM, MF and SE enrolled the patients and followed the study
205 procedures. AR, TA performed the plasma and CSF analysis. AC, IM and SB analysed the data and
206 wrote the draft of the manuscript. AC, SB, VG, DI and GDP reviewed the paper. All authors made
207 substantial contributions to this manuscript and agreed on its preparation in tis present form.
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