Correspondence

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Concerns and clarifications regarding the article 'Virological, weight, and drug resistance outcomes among patients initiating a dolutegravir-based first line ART regimen in Zimbabwe'

I hope this letter finds you well. I recently read with great interest the article by Kouamou *et al.*, published in your journal, discussing the real-life data of patients using dolutegravir [1]. However, I would like to bring attention to some points in the study that require clarification.

The study initially indicated the inclusion of 172 participants, yet it was stated that 137 participants completed the crucial 24-week follow-up period, implying that the research was actually conducted with 137 participants. While the average age of the participants was reported to be 39, it was noted in the article that 15 individuals, constituting 9%, had died. The mortality rate in a series of individuals living with AIDS and undergoing treatment is a significant cause for concern, and it is necessary to provide information on whether these deaths are associated with AIDS. I believe it is imperative to provide a more detailed explanation of the causes of death in these patients, as it is essential for the readers, and also because the deaths mentioned in the article may be related to opportunistic infections, treatment failures or AIDS-related cancers.

Another critical concern is that the statistical analysis seems to have been conducted based on 137 cases rather than the initially recruited 172 cases. This situation can affect both the power and statistical evaluation of the study, potentially impacting its internal and external validity. From an internal validity perspective, the oversight of missing data and loss to follow-up could lead to results that do not accurately represent reality. Externally, replicating similar results from this study may become challenging for other researchers or institutions.

To address these concerns and improve the study's reliability, I suggest adding a detailed explanation of how missing data and loss to follow-up were handled in the methodology section of the research report. In addition, clarifying the reasons for the reported deaths and reassessing the statistical analysis based on the correct number of cases will contribute to the overall quality of the study. I think that correcting these concerns and believing that addressing them will enhance the scientific rigor and credibility of the research.

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Conflicts of interest

There are no conflicts of interest.

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Further considerations on the use of cerebrospinal fluid C-X-C motif chemokine ligand 13 in the diagnosis of neurosyphilis among people with HIV

We commend the work by Carvalho *et al.* [1] on the role of cerebrospinal fluid (CSF) C-X-C motif chemokine ligand 13 (CXCL13) in the diagnosis and monitoring of neurosyphilis in people with HIV (PWH). We agree with the authors that the central nervous system (CNS) involvement by *Treponema pallidum* represents a clinical challenge in PWH because of several HIV-related factors that weaken the performance of current diagnostic tools. We also agree on the fact that the measurement of CSF CXCL13 is a promising biomarker that could help in the detection of neurosyphilis. However, further considerations should be made before drawing strong conclusions on the diagnostic accuracy of CSF CXCL13 for neurosyphilis in PWH.

The authors stated that CSF CXCL13 is a highly specific marker of neurosyphilis and having observed no correlation between CSF CXCL13 and plasma HIV RNA, they concluded that the levels of this biomarker are not affected by HIV activity. However, there is robust evidence that HIV RNA directly induces CXCL13 production [2,3]. We recently described the role of CXCL13 in intrathecal immune activation and cognition among 175 PWH with no confounding condition: increased CSF CXCL13 levels were observed in 22.2% of the study participants [4]. Of note, six participants had CSF levels significantly higher than the cut-off of 60 pg/ml proposed by Carvalho *et al.* to detect neurosyphilis [median 550, interquartile range (IQR) 119–600 pg/ml). The fact that we also observed no correlation between the CSF chemokine and plasma HIV RNA depends on the compartmentalized production of the chemokine, as previously described in multiple sclerosis [5,6]. In fact, when we regress CSF CXCL13 values against CSF HIV RNA in the participants with detectable HIV ($n = 86$), we observe a strong linear relationship: CSF CXCL13 increases by 36.8 pg/ml (β 95% confidence interval (CI) 15.6–58.0; $P < 0.001$] for every unit increase in $\log_{10}$ copies/ml of CSF HIV RNA. Moreover, compared with PWH with plasma greater than CSF HIV RNA ($n = 61$), those with CSF greater than plasma HIV RNA ($n = 25$) had higher CSF CXCL13 ($P = 0.003$) but no difference in CSF HIV RNA ($P = 0.566$), suggesting that the relationship between HIV and CXCL13 is not only quantitative but also that the chemokine levels may reflect immune activity to cope with viruses that have higher sequence diversity (as it can occur in CSF escape [7,8]). Therefore, the accuracy of CSF CXCL13 in diagnosing neurosyphilis may need to be adapted to the individual context, as distinct clinical settings (e.g. off ART, CSF escape) likely benefit from different cut-offs.

The authors described a better diagnostic accuracy of CSF CXCL13 compared with CSF TPHA; however, only seven index cases were included, and among the two neurosyphilis cases that were excluded by referencing to CSF VDRL as the gold standard, one would have been missed by the proposed cut-off. Although we recognize a potential benefit in integrating CXCL13 in the clinical workout, we struggle to foresee the possibility of relying only on CSF CXCL13 to diagnose neurosyphilis for several reasons.

Up to 35% of PWH have some degree of blood–brain barrier (BBB) impairment [9]. The molecular weight of CXCL13 is about 10 kDa [10], rendering this chemokine able to cross permeable BBB; despite one study disputed this possibility [6], the contribution of plasma CXCL13 (e.g. produced in response to syphilis outside the CNS) to the CSF levels of this chemokine should be further investigated in case of moderate–severe BBB injury (e.g. by CSF-to-plasma ratio) and diagnostic cut-offs should be adjusted accordingly.

The stringent eligibility criteria used by Carvalho *et al.* [1] limit the generalizability of the findings. Among the PWH tested for CXCL13 but excluded from our previous study because of CNS confounding diseases, CSF CXCL13 levels ranged widely (Fig. 1a): 28.6% of people with

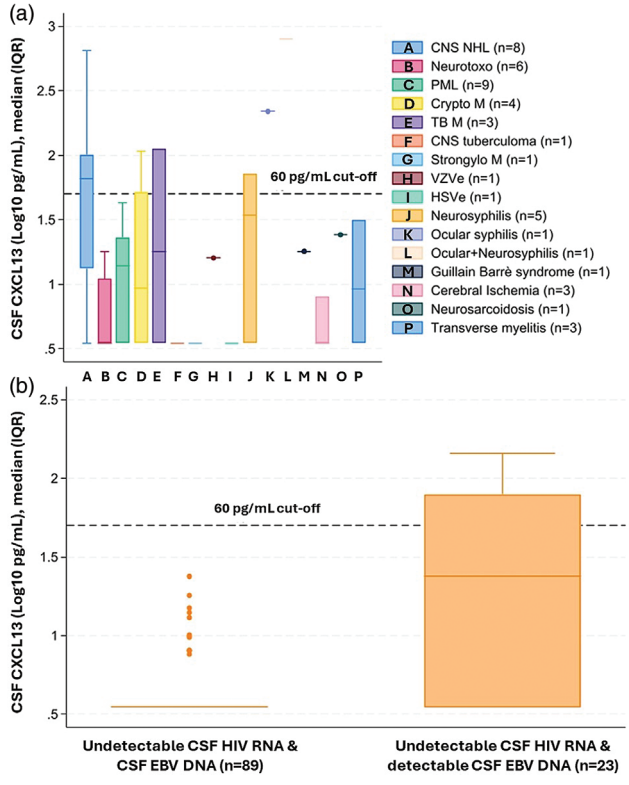

Fig. 1. Cerebrospinal fluid levels of CXCL13 by central nervous system disorders and by cerebrospinal fluid Epstein–Barr virus DNA in people with HIV. Panel a shows the levels of CSF CXCL13 (median, interquartile range) across different infectious and noninfectious CNS disorders in 49 PWH, regardless of CSF HIV RNA. Half of the people with neurosyphilis (all CSF VDRL+) had values below the cut-off of 60 pg/ml; it is also notable that the large variability of the conditions that can trigger the production of CSF CXCL13, including PML, cerebral ischemia, and neurosarcoidosis, which have been rarely acknowledged in this regard before. Panel b compares CSF CXCL13 values (median, interquartile range) in PWH with ($n = 23$) vs. without CSF EBV DNA ($n = 89$; $P = 0.014$; both the groups had CSF HIV RNA<20 copies/ml). Seven cases out of 23 have CSF CXCL13 levels above the 60 pg/ml cut-off (30.4% of false positive). All the original data presented in this correspondence have been collected and analyzed following the approval of the Ethics Committee of San Luigi Hospital, Orbassano (p.n.103/2015). All participants provided written informed consent. CSF, cerebrospinal fluid; CXCL13, C-X-C motif chemokine ligand 13; CNS, central nervous system; NHL, non-Hodgkin lymphomas; Neurotoxo, neurotoxoplasmosis; PML progressive multifocal leukoencephalopathy; Crypto M, cryptococcal meningitis; TB M, tuberculous meningitis; Strongylo M, *Strongyloides stercoralis* meningitis; VZVe, varicella zoster virus encephalitis; HSVe, herpes simplex virus encephalitis; EBV, Epstein–Barr virus.

neurosyphilis had concentrations below the proposed cut-off, people with other disorders had levels above it (CNS tuberculosis, CNS lymphomas, cryptococcal meningitis), and others increased CSF CXCL13 (e.g. PML and neurotoxoplasmosis). It is true that the co-occurrence of neurosyphilis or of neuroborreliosis [11] with all the above is infrequent; however, other confounding conditions can be more prevalent. For example, EBV DNA is detectable in the CSF of up to 20% of asymptomatic PWH [12]. As shown in Fig. 1b, among virally suppressed PWH, even low-level replication of EBV in the CSF (min–max 31–893 DNA copies/ml) was associated with significantly higher levels of CSF CXCL13. Interestingly, both CSF CXCL13 and CSF anti-EBV IgG have been associated with the cognitive performance of PWH [4,12].

In conclusion, the results of Carvalho *et al*. are a valuable proof-of-concept of the link between CSF CXCL13 and neurosyphilis but cannot endorse the clinical application of CXCL13. Stronger ecological validity is required, and further essential factors should be detailed to define the normal range and tailored cut-offs of CSF CXCL13 in PWH.

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Towards a refined understanding of cerebrospinal fluid CXCL13 in neurosyphilis diagnosis among people with HIV

We value the insights from Trunfio *et al*. [1] on our investigation of cerebrospinal fluid (CSF) CXCL13 as a biomarker for neurosyphilis in people with HIV (PWH) [2], acknowledging its promise yet urging caution before making definitive conclusions. In contrast to our results, they provide robust evidence that HIV-RNA boosts CXCL13 production, demonstrating a significant correlation between CSF CXCL13 and CSF HIV-RNA levels in their cohort of 175 PWH, with 22.2% exhibiting increased CSF CXCL13 levels [1]. Their study reveals a direct moderate correlation between CSF HIV RNA and CSF CXCL13, with each $\log_{10}$ increment in CSF HIV-RNA leading to a 36.8 pg/ml rise in CSF CXCL13 [3]. Furthermore, they observed that PWH under virological suppression with low CSF EBV-DNA levels had notably higher CSF CXCL13 [4].