Letter

Inflammation and microbial translocation in treatment-controlled HIV patients

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Bastard et al. [1] reported that circulating levels of interleukin (IL)-6, a commonly used marker of inflammation, correlated with HIV viral load (VL) in antiretroviral-therapy-controlled patients with HIV viraemia <500 copies/ml, and they suggested that the VL threshold for significantly increased IL-6 was 31 copies/ml. Our group also showed that patients with low-level HIV VL (20–200 copies/ml) had higher levels of inflammatory markers than patients with HIV VL<20 copies/ml, but the relationship between HIV VL and inflammation did not seem to be direct, and rather it depended on the presence of microbial translocation (MT) [2].

In clinical practice, ultrasensitive VL assays (able to quantify a few HIV-1 RNA copies) are not available, but third-generation VL assays can detect HIV-1 RNA below the limit of quantification [3]. It is not known whether the relationship between HIV VL, inflammation and MT is maintained in patients with HIV-1 RNA that is detectable under the limit of quantification. To settle this point, we have performed a study to determine the prevalence of MT and inflammation according to the qualitative detection of HIV-1 RNA under the limit of quantification of the widely used third-generation VL assay of Roche.

Between November 2011 and December 2012, patients on antiretroviral therapy were invited to participate in the study if they had HIV VL<20 copies/ml for ≥6 months and self-reported good adherence to antiretroviral therapy. Patients with active infection or antibiotic treatment in the past month were excluded. After written informed consent, a blood sample was obtained to determine MT, inflammation markers, lymphocyte count and HIV VL. MT was determined by identification of plasma 16S ribosomal DNA (rDNA) using a broad-range PCR according to the methodology previously described [4]. Soluble CD14 (sCD14), IL-6 and tumour necrosis factor (TNF-α) were measured by ELISA according to manufacturer instructions (R&D Systems, Minneapolis, MN, USA) [5]. HIV VL was determined by real-time reverse transcriptase PCR using COBAS AmpliPrep/COBAS TaqMan HIV-1 test version 2.0 (Roche Diagnostics, Meylan, France), a test with a lower limit of quantification of 20 copies/ml but able to detect the presence of HIV-1 RNA under that limit [3]. The study was approved by the local ethics committee.

A total of 57 patients with HIV VL under the limit of quantification (<20 copies/ml) were studied. They were 39 (68%) men, median age 45 years (range 31–65), 24 (42%) acquired HIV through parenteral drug use, 16 (28%) had chronic hepatitis C, 24 (42%) were in stage C of the CDC, and the median CD4+ T-cell count was 475 cells/mm3 (25th–75th percentile 378–744, range 64–1,640). Their antiretroviral treatment was non-nucleoside-reverse-transcriptase-inhibitor-based in 37 (65%) and protease-inhibitor-based in 25 (44%) patients.

HIV-1 RNA was detectable in 17 (30%) patients and there was no significant difference between these patients (RNA-positive group) and patients with undetectable HIV-1 RNA (RNA-negative group) in the characteristics previously mentioned. rDNA was found in 6/11 (35%) patients from the RNA-positive group and 3/40 (8%) patients from the RNA-negative group (P<0.01). TNF-α levels were higher in patients from the RNA-positive group compared to the RNA-negative group: 50 pg/ml (25th–75th percentile 29–72) versus 35 pg/ml (25th–75th percentile 23–45; P<0.05). Although levels of sCD14 and IL-6 were also higher in the RNA-positive group, the difference was not significant. No other variables were associated with high levels of inflammation markers.

To study the independent effect of HIV VL and MT on inflammation markers, analysis of variance for two factors was used. Table 1 shows that levels of inflammation markers are related to the presence of
MT and are independent of the detection of low levels of HIV-1 RNA, a fact already reported for patients with HIV VL between 20–400 copies/ml [2].

Those data suggest that low-level viraemias might be dangerous not only through the increased risk of virological failures [6,7] but also because they are accompanied by MT, which is in fact the factor associated with chronic inflammation. We do not know if the results of our transversal study have clinical relevance but many reports link MT and inflammation to poor outcomes of HIV infection [8].

Disclosure statement

The authors declare no competing interests.

References


Authors’ reply

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We were very interested by the data presented by Reus et al. on the relationship between microbial translocation, inflammation markers and low-level viraemia (LLV) in antiretroviral therapy-controlled HIV-infected patients. Very recently [1], the authors reported that a viral load (VL)<20 copies/ml was associated with a lower level of interleukin (IL)-6 than a VL between 20–200 copies/ml. These results are in agreement with our results [2], which identified only IL-6, among the inflammatory and immune activation markers, for which the level was related to the level of VL in the low range.

Moreover the authors propose that the difference in the inflammatory markers was driven by microbial translocation, as evaluated by the plasma level of 16S ribosomal DNA, rather than by the level of VL. Of concern are the values recorded in the paper and in the present letter for the inflammatory/immune activation markers, since IL-6, tumour necrosis factor (TNF)-α and soluble CD14 (sCD14) values are markedly over the range reported in HIV-infected patients in the absence of acute inflammation. It is not known whether the units recorded were inadequate or whether those patients had severe inflammation.

Since the authors have not previously evaluated VL<20, they searched for a similar relationship in

Table 1. Biomarker levels according to HIV viral load and microbial translocation in patients with HIV viral load under the quantification limit of 20 copies/ml

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>RNA-positive (n=17)</th>
<th>RNA-negative (n=40)</th>
<th>P-value</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>rDNA-positive</td>
<td>rDNA-negative</td>
<td></td>
</tr>
<tr>
<td></td>
<td>rDNA-positive</td>
<td>rDNA-negative</td>
<td>P1</td>
</tr>
<tr>
<td></td>
<td>rDNA-negative</td>
<td></td>
<td>P2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P3</td>
</tr>
<tr>
<td>TNF-α, pg/ml</td>
<td>80 (65–91)</td>
<td>34 (23–55)</td>
<td></td>
</tr>
<tr>
<td>IL-6, pg/ml</td>
<td>133 (117–147)</td>
<td>69 (68–76)</td>
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</table>

Total n=57. Data are expressed as median (25th-75th percentile). IL-6, interleukin 6; P1, significance for detectable HIV-1 RNA; P2, significance for ribosomal DNA (rDNA); P3, significance for the interaction between detectable HIV-1 RNA and rDNA; rDNA-negative, absence of 16S rDNA; rDNA-positive, presence of 16S rDNA; RNA-negative, patients with undetectable HIV-1 RNA; RNA-positive, patients with detectable HIV-1 RNA; TNF-α, tumour necrosis factor-α.
patients with VL<20. They report here that, in patients identified as HIV-RNA-negative or -positive by the COBAS Taq-Man HIV-1 test version 2.0 (Roche Diagnostics, Meylan, France), the TNF-α level was lower in patients with negative as compared to positive HIV RNA. Again, this difference was explained by the levels of 16S ribosomal DNA rather than by VL. The authors conclude that LLV, even in the range 0–20 copies/ml, might be dangerous not only through the risk of virological failure but also because they are accompanied by microbial translocation, which is in fact the factor associated with chronic inflammation.

This study raises important points related to the management of HIV-infected patients: what is the level of undetectability which is clinically relevant for the routine care and whether clinicians have to routinely check low-grade inflammation? Given that inflammation and microbial translocation markers are not routinely used, the remaining evaluable factor is VL. Therefore, could VL be considered as a surrogate marker of inflammation and/or microbial translocation and what VL threshold has to be proposed in the routine care?

At present, the level of undetectability offered by diagnosis tests, as the routinely used Roche test, is in the range of < or >20 copies/ml. Their and our results [1,2] suggest that being under this threshold could mildly improve inflammation regarding IL-6 but not other markers and that microbial translocation was less common [1]. Therefore, maintaining VL<20 is probably beneficial, even if its clinical relevance has not been established.

Moreover, the authors propose to discriminate between undetectable versus detectable HIV RNA in patients with VL<20 copies/ml suggesting that a negative HIV RNA would be beneficial regarding inflammation.

The first concern is that, in this range of values, the variability of the COBAS Taq-Man HIV-1 test is very high and the reproducibility quite poor [3].

Otherwise, it was important to evaluate if, for VL<20, the level of inflammatory/immune activation markers was different, and whether this difference was due directly or indirectly through microbial translocation to VL. In the letter, lower levels of TNF-α were reported in patients with negative HIV RNA.

We further analysed the patients included in our study [2], selecting patients with VL<1 versus 1–20 copies/ml, as evaluated by the ultra-sensitive assay able to detect 1 copy/ml and to quantify LLV [4]. We recorded respectively 34 and 18 patients in each group. The levels of IL-6, high-sensitivity C-reactive protein (hsCRP), soluble TNF receptor 1, soluble TNF receptor 2 and sCD14 were not significantly different between the two groups (Table 1). This could be clinically relevant given that hsCRP, IL-6 and sCD14 have been related to an increased incidence of deaths or cardiovascular complications [5–7] in HIV-1-infected patients and to negative outcomes in HIV-2 infection [8].

Therefore, we suggest that there is no indication, when considering the inflammatory/immune activation markers, to decrease the VL threshold <20 copies/ml for the routine patients’ care. There is also no indication at present, in the absence of a situation of acute infection or inflammation, to check the level of the inflammation/immune activation and microbial translocation markers in the patients’ care.

Disclosure statement

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References


Table 1. Mean values of the inflammation/immune activation markers are similar in patients with viral load <1 copies/ml as compared to those with viral load 1–20 copies/ml

<table>
<thead>
<tr>
<th>Marker</th>
<th>Viral load &lt;1 copies/ml (n=34)</th>
<th>Viral load 1–20 copies/ml (n=18)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6, pg/ml</td>
<td>0.80 (0.18–2.79)</td>
<td>1.17 (0.15–5.40)</td>
<td>0.327</td>
</tr>
<tr>
<td>sTNFR2, pg/ml</td>
<td>2.709 (1.353–7.388)</td>
<td>2.676 (1.277–4.357)</td>
<td>1.000</td>
</tr>
<tr>
<td>sTNFR1, pg/ml</td>
<td>1.344 (726–5,834)</td>
<td>1.382 (583–3,826)</td>
<td>0.658</td>
</tr>
<tr>
<td>hsCRP, mg/ml</td>
<td>2.66 (0.42–7.44)</td>
<td>1.64 (0.29–6.65)</td>
<td>0.053</td>
</tr>
<tr>
<td>sCD14, ng/ml</td>
<td>1,238 (351–2,520)</td>
<td>1,125 (480–1,846)</td>
<td>0.472</td>
</tr>
</tbody>
</table>

Data are mean (range). hsCRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; sCD14, soluble CD14; sTNFR1, soluble tumour necrosis factor-α receptor 1; sTNFR2, soluble tumour necrosis factor-α receptor 2.


