

plasma viral load below detectable limits and age, race, or HIV transmission category.

One hundred and three women had been on HAART for over 5 months (mean, 1.0 years; range, 0.4–2 years), including 47 women who had been on HAART for more than 12 months (mean, 1.4 years). Women who were adherent to HAART were more likely to have plasma viral load below detectable limits than women who were not adherent after 5 months (OR, 17.6; 95% CI, 5.9–54.8;  $P < 0.01$ ) and 12 months (OR, 8.6; 95% CI, 1.9–41.5;  $P < 0.01$ ; Fig. 1) This study was a retrospective chart review and did not utilize pharmacy records, pill counts, or other measures of adherence.

HAART, third party health insurance, and an absence of active substance abuse are significantly associated with achieving undetectable plasma viral load. Among women on HAART, those who were adherent were significantly more likely to have plasma viral load below detectable limits than those who were non-adherent. Future research should examine long-term effects and adherence of different HAART regimens in the clinical setting.

### False positives for HIV using commercial viral load quantification assays

A 5-month-old boy was diagnosed with severe thrombopenia. His parents had been engaged in injecting drug use practices (sharing needles) for long time before they married. They had declined testing for HIV antibodies in the past, and both were currently in good health. The paediatrician decided to examine the child's HIV serological status, but subsequently it was suggested that he should perform a viral load test, because the result could be available the next day and the presence of HIV sequences rather than the detection of antibodies, which can reflect passive transfer from the mother, is more accurate for the diagnosis of HIV infection in newborns. The child's plasma viral load was 3044 HIV RNA copies/ml, and he began to receive zidovudine plus didanosine in combination once this information was available. Unexpectedly, days later it was discovered that HIV antibodies were not detected in sera collected from the child or from his parents. Moreover, additional serological screening assays, including Western blot analysis, yielded negative results for HIV antibodies. The plasma viral load was examined in a new specimen using the same technique (HIV Quantiplex, Chiron, Madrid, Spain) and again it gave a positive result of 5120 HIV RNA copies/ml.

Since sporadic cases of HIV-1 infection in the absence of specific antibodies have been reported in the literature [1–4], the presence of several HIV genomic

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regions (*gag*, *env*) in the child's plasma was examined using the PCR technique. Negative results were obtained in all instances. Furthermore, p24 antigenaemia was also negative, and CD4+ lymphocyte counts of the child and his parents were in the normal range. A suspicious false-positive viral load result becomes the sole explanation for this controversy.

Since viral load tests have been approved for the quantification of viraemia in already known HIV-seropositive individuals, we were interested to know their specificity. For this purpose, we selected 20 healthy volunteers, all of whom yielded negative results for HIV antibodies using different screening tests. Plasma from all of them were analysed by three different currently available HIV viral load tests: branched DNA (bDNA) signal amplification assay (Chiron), nucleic acid sequence-based amplification (NASBA) Nuclisens (Organon Teknika, Barcelona, Spain), and Ultradirect reverse transcriptase (RT)-PCR Monitor (Roche, Madrid, Spain). The detection limits of these assays are 500, 40 and 20 HIV RNA copies/ml, respectively. Moreover, we used two different HIV-1 Monitor kits (Roche, Madrid, Spain), one using primers exclusively designed for recognizing HIV-1 subtype B and another with non-B primers [4].

In summary, two samples yielded positive results by the bDNA assay, with values of 2020 and 10 620 HIV

RNA copies/ml. Another two specimens yielded false-positive results by the NASBA Nuclisens, with values of 150 and 480 HIV RNA copies/ml. Finally, one of the 20 samples was interpreted as positive by the Ultradirect RT-PCR Monitor assay, with a value of 73 HIV RNA copies/ml. Moreover, using the Monitor test with non-B primers, up to four of the 20 samples yielded positive values, ranging from 48 to 253 HIV RNA copies/ml. Results were reproduced in more than half of tested specimens for which plasma volumes were enough for repeat testing. The experiments were all performed by a single well-trained laboratory technician. Furthermore, controls run during the study excluded contamination as a source of false-positive results.

The performance and sensitivity of the three commercially available viral load assays has been analysed in several comparative studies, concluding that relatively good agreement exists when comparing the results provided [1–3]. However, non-specific hybridization between the probes or the primers used in the current tests has been noted. Recently, bDNA has incorporated new molecules that lead to a substantial reduction in unspecific hybridizations [4]. The coamplification of internal standard nucleic acid sequences, either DNA or RNA, has been applied successfully for RT-PCR and NASBA [5,6]. Although false positives have been recognized in some studies, retesting does not reproduce the error in most instances.

Short fragments of cellular RNA can be misleading, being recognized or interfering with the amplification systems used by the different quantification methods. Targeting other nucleic acid sequences could lead to the amplification of background, providing false-positive results, usually with low values, as it was seen in our cases.

Our data support the notion that viral load quantification methods must be used for monitoring plasma HIV-1 RNA levels in patients already known to be HIV-infected. Since their specificity is not well known, these tests must not be used for diagnostic purposes (as

has been suggested by others) [7], substituting the well-probed serological methods. Finally, since unspecific results may occur, the extent to which this phenomenon can affect the values provided by testing HIV-positive specimens, leading to an underestimation of the real number of undetectable samples, should be examined. In light of this, recent reports [8,9] have pointed out that some individuals show a dramatic and sustained improvement in their CD4+ lymphocyte counts despite remaining with detectable viraemia. It is clear that false-positive viral load results should be excluded in these circumstances.

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## Protease inhibitor-associated hyperinsulinaemia

Protease inhibitors have proved to be powerful drugs capable of changing the natural history of HIV-1 infection. However, knowledge of the long-term safety profile of these drugs is limited because they were approved under an accelerated mechanism. Adverse effects such as hyperglycaemia and abnormal body-fat distribution in association with protease inhibitors have

been recently reported [1–7], although their pathogenesis remains unknown.

To determine whether protease inhibitor therapy is associated with metabolic effects and to avoid confounding factors biasing study results, we studied two groups of otherwise clinically stable (CD4 cells