Evaluation of Elisa test for therapeutic monitoring of Nelfinavir in HIV-positive patients

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Therapeutic drug monitoring (TDM) is an important tool in the management of antiretroviral (ARV) therapy. The gold standard for measuring drugs plasma levels is High-Performance Liquid Chromatographic Assay (HPLC) however it is technically-demanding and time-consuming.
We evaluated a new immunoenzymatic test (TDM-ELISA®, Biostrands, Trieste, Italy) for nelfinavir and its active metabolite M8 in comparison with HPLC. A statistically significant difference in Ctrough between the two different tests was demonstrated but this difference was no longer significant when a value of 29% due to M8 aliquot was deleted. This faster TDM-ELISA® may have an important role for TDM in HIV patients taking ARVs.

KEY WORDS: Nelfinavir, Therapeutic drug monitoring, TDM-ELISA

The clinical usefulness of therapeutic drug monitoring (TDM) in HIV-positive, ARV-treated patients is still debated. This may be due to a number of different reasons, including:
1) no clear-cut reproducible demonstration of a correlation between the blood level of protease inhibitors (PIs) or non-nucleoside reverse transcriptase inhibitors (NNRTIs) and either the efficacy and/or the toxicity of the HAART combinations based on them;
2) wide intra-patient variability, as suggested by recently-available reports (Koo et al., 2006; Nettles et al., 2006);
3) limited access to reference pharmacology laboratories able to measuring PI and NNRTI plasma levels and the use of time-consuming, costly and technically-demanding technologies;
4) problems of reproducibility and interlaboratory variability when using in High Performance Liquid Cromatography (HPLC), that is the gold standard for assessing PI concentrations in plasma (Aarnouste et al., 2002; Droste JA et al., 2003).

A possible solution to these problems may be the development of simpler, cheaper and wide-available technologies that may allow most, if not all the centres currently treating HIV-positive patients to determine PI and NNRTI plasma levels. This basically means to assess trough (pre-dose) concentrations, that are those most commonly determined in pharmacokinetics studies. The recent availability of an immunoenzymatic test based on the use of polyclonal rabbit antibodies (TDM-ELISA®, Biostrands, Trieste, Italy) allows the determination of total plasma levels of lopinavir (LPV), amprenavir (APV), nelfinavir (NFV), and ritonavir (RTV). The NFV test measures both the parental drug and its active metabolite M8 as was also reported in published studies on this PI (Pellegrin et al., 2002). The corre-

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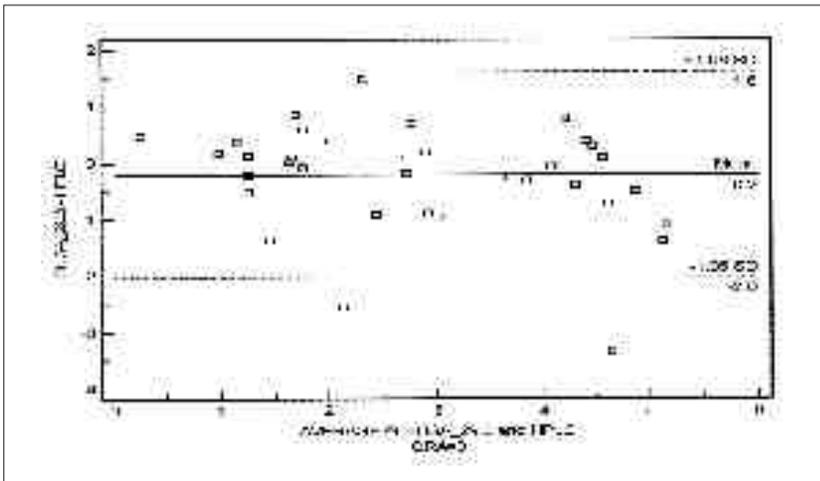E-mail: v.meroni@smatteo.pv.it

FIGURA 1 - *Bland&Altaman Plot: difference between mean of Ctrough values obtained with ELISA and HPLC test.*

lation between the PI level as measured with TDM-ELISA® and HPLC has been validated by a series of studies performed either by the TDM-ELISA® manufacturer or by independent investigators (Montagna *et al.*, 2003).

The aim of the study was to evaluate the correlation between HPLC and TDM-ELISA® results of nelfinavir and M8 plasma concentrations in

blood samples, taken from HIV-positive patients treated with nelfinavir-containing combination therapy. HIV+ patients were followed at HIV/AIDS Outpatient Clinic of Infectious Diseases, Foundation IRCCS San Matteo Hospital (Pavia). All blood samples were stored frozen at –80°C until tested, after inactivation to a 56°C for 30'. Concentrations of nelfinavir were
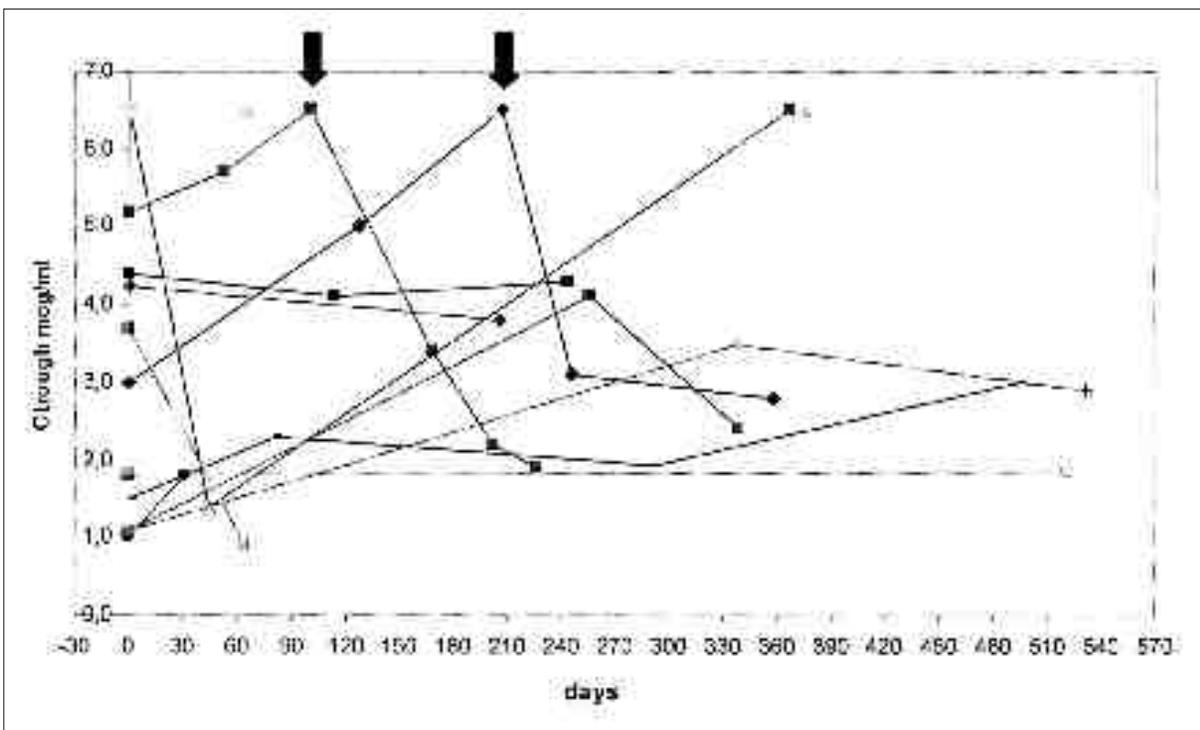

FIGURA 2 - *NFV+ M8 intrapatient variability in HIV-HCV subjects. All samples were drawn pre-dose (Ctrough) single line identify each patient. In two cases (arrows) a dose reduction was performed.*

determined by HPLC method at the department of Clinical Pharmacology, Foundation IRCCS San Matteo Hospital (Pavia). The TDM-ELISA® test is a competitive immunoenzymatic assay that measures nelfinavir and its pharmacologic active metabolite, M8 (hydroxy-t-butylamide) within a dynamic range (0.8-6.25 mcg/ml).

We analysed 186 blood samples with TDM-ELISA® and 70 with HPLC. Then, we considered ELISA and HPLC values obtained on 37 base-line samples (before drug intake, *Ctrough level*), taken from 7 patients in different days.

All ELISA values were higher than HPLC and this difference was statistically significant (Paired samples t-test, P=0.0075), but when M8 quote (29% Baede-van Dijk 2001 *et al., 2001*) was subtracted from each result this difference became not statistically significant (P=0.45). The difference between mean values of the two tests when Bland &Altman Plot (-0,2) also demonstrated that the results became comparable (Figure 1).

Further, to assess the intrapatient variability of nelfinavir concentration, 39 samples from 12 subjects, 10 males and 2 females (mean 3 samples per patient) were evaluated in TDM-ELISA® on different days and at the same time point (*Ctrough*).

The values obtained with TDM-ELISA® in these samples were always above therapeutic Cmin (1 mcg/ml), and these data were also confirmed by virological response. In most of our patients, the intrapatient variability was very low. In two coinfected HIV-HCV patients the therapeutic switch to a lower dose was the reason behind a drop between the third and the fourth samples (Figure 2).

Because the immunoenzymatic test is simple, rapid and does not require specific equipment so it could be performed in all laboratories. This assay also provides a wide array of information to the clinician such as patient compliance with treatment and makes clinical management of patients with associated comorbidity (viral hepatitis) easier.

TDM-ELISA® values are usually higher than HPLC concentrations due to the concomitant determination of nelfinavir active metabolite, M8 and this should be taken into account when we use these data. Further studies are underway to evaluate PK of nelfinavir in a larger cohort of HIV + patients.

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