International Journal of Infectious Diseases 14 (2010) e1008–e1012

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Incomplete IgG response to HIV-1 proteins and low avidity levels in recently converted HIV patients treated with early antiretroviral therapy

Maria Carla Re [a,*], Pasqua Schiavone [a], Isabella Bon [a], Francesca Vitone [a], Elisa De Crignis [a], Carlo Biagetti [b], Davide Gibellini [a]

[a] Microbiology Section of the Department of Hematology and Oncologic Sciences, University of Bologna, Via Massarenti 9, 40138 Bologna, Italy
[b] Infectious Diseases Section, Department of Internal Medicine, Geriatric Medicine and Nephrology, University of Bologna, Bologna, Italy

ARTICLE INFO

Article history:
Received 8 January 2010
Received in revised form 8 June 2010
Accepted 18 June 2010

Keywords:
Primary HIV-1 infection
Western blot
Antibody avidity
HAART

SUMMARY

Objectives: To evaluate the evolution of antibody avidity and Western blot reactivity in recently infected HIV-1 subjects and to study the impact of highly active antiretroviral therapy (HAART) on avidity maturation of HIV-1-specific immunoglobulin G (IgG) in patients with recent HIV-1 infection.
Methods: Thirty-six HIV-1 seroconverters were enrolled in this study and followed longitudinally over 24 months to evaluate if the administration of antiretroviral therapy during primary infection affects Western blot reactivity and the evolution of antibody avidity. The patients were divided into two groups; group A consisted of 19 HIV-1-untreated patients who did not receive any drug treatment during our follow-up period; group B consisted of 17 subjects who were treated early with an association of two nucleoside reverse transcriptase inhibitors (NRTI) and one non-nucleoside reverse transcriptase inhibitor (NNRTI) within 3 months after seroconversion.
Results: At diagnosis, Western blot analysis and avidity index (mean value) were exactly matched in untreated and treated patients; subsequently, however, a significantly lower reactivity to HIV-1 pol and gag proteins and a lower avidity index (mean values) were observed in HAART-treated patients up until the end of the follow-up period.
Conclusions: The impaired production and maturation of the humoral immunological response in antiretroviral-treated patients might be related to a rapid suppression of HIV replication, driven by HAART. These results could have important implications in understanding the complex mechanism of the immune response during HIV infection.
© 2010 International Society for Infectious Diseases. Published by Elsevier Ltd. All rights reserved.

1. Introduction

The diagnosis of HIV in adults is routinely performed using serological tests able to detect specific antibodies to the virus.[1] During recent years, serological tests have evolved rapidly, with combined antigen and antibody tests reaching high levels of sensitivity for the detection of primary infections.[2,3] Despite the introduction of new generation assays, it is difficult to differentiate recent and long-standing infections, even though this information may be crucial to establish the incubation period and for partner notification, epidemiological surveillance, and patient management.[4–6]

One approach to identifying recent infections is the investigation of the level of antibody avidity, as routinely used for several infectious diseases, such as rubella, cytomegalovirus and *Toxoplasma gondii* infections.[7–9]

To date, it is still not clear whether an early therapeutic approach affects antibody maturation or is related to seroreversion or decreased HIV-1 antibody titers,[10–19] although early antiretroviral treatment during primary HIV infection might block the formation of HIV-1-specific antibodies, leading to a rapid reappearance upon cessation of therapy.[19]

Even though several approaches identify acute seroconverters, including detuned assay, BED-CEIA assay, avidity assays, IDE-V3 assay, IgG3, anti-HIV and Inno-LIA HIV adaptation tests[20,21] up to now all these assays are not yet well standardized and performed on an in-house basis.

To evaluate the evolution of antibody avidity and Western blot reactivity over time, we undertook a retrospective study in two groups of recently seroconverted patients; the first group consisting of patients who were not treated during our observation period (untreated HIV) was compared with a second group of subjects who were treated within 3 months after seroconversion (treated with highly active antiretroviral therapy (HAART)).

Avidity tests were performed after guanidine serum pretreatment, as previously described[22] and were analyzed using a

* Corresponding author. Tel.: +39 0 51 4290900; fax: +39 0 51 307397.
E-mail address: mariacarla.re@unibo.it (M.C. Re).

1201-9712/$36.00 – see front matter © 2010 International Society for Infectious Diseases. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.ijid.2010.06.015

different serological platform (BioMérieux, Boxtel, the Netherlands). Avidity index results were correlated to immunological and virological parameters from time of diagnosis until the end of the follow-up period.

2. Materials and methods

2.1. Patients and sera

Serum samples from 36 HIV-1 seroconverters attending the infectious disease outpatient clinic between February 2005 and October 2007 were enrolled in this study; informed consent was obtained according to the Helsinki Declaration of 1975. Intensive medical evaluation established that transmission had occurred by sexual contact in all subjects, excluding any drug abuse.

All selected patients met the following criteria: (1) a first indeterminate or positive enzyme-linked immunosorbent assay followed by Western blot profile compatible with ongoing seroconversion; (2) a previous confirmed-negative test for HIV antibody carried out a maximum of 6 months before the first positive test; (3) detectable HIV-1 RNA plasma levels.

The patients were divided into two groups. The first group (group A) comprised 19 untreated HIV-1 patients who did not receive any drug treatment during our follow-up period, since they did not meet the criteria for a therapy regimen or had refused any drug treatment. The second group (group B) included 17 subjects treated early with an antiretroviral combination (two nucleoside reverse transcriptase inhibitors (NRTI) and one non-nucleoside reverse transcriptase inhibitor (NNRTI)) within 3 months after seroconversion, following HIV guidelines (http://aidsinfo.nih.gov/contentfiles/AdultandAdolescentGL.pdf). The two groups were matched for age and sex, sharing a similar gender composition (15 men and four women in group A; 13 men and four women in group B) and mean age (34 ± 9 years in group A; 36 ± 4 years in group B) (Table 1). All patients were followed longitudinally over 24 months; plasma and serum samples were collected at time 0 (first positive serum) and at 6, 12, 18, and 24 months thereafter. Longitudinal serum and plasma samples were stored at $-20\,^{\circ}C$ until use and tested by immunoblot assay and guanidine-based avidity assay, and for RNA viral load and CD4 count.

2.2. ELISA and immunoblot assay

Each sample was tested by ELISA (Vironostika; BioMérieux, Boxtel, the Netherlands) and immunoblot (INNO-LIA™ HIV-1/2 Score Assay; Innogenetics, Ghent, Belgium) according to the manufacturers' instructions. Immunoblot strips were interpreted following the criteria of the INNO LIA HIV-1/2 score test and read and interpreted by LiRAS™ software; the intensity of the antibody response to specific HIV-1/2 proteins spotted on the filter is analyzed by scanner and compared with an internal control. The software describes the intensity strength with a value ranging from 4+ to ±. We considered a line positive when the intensity of the antigen–antibody reaction was between 2+ and 4+.

Table 1
Baseline characteristics of HIV-1-positive patients (group A: untreated HIV subjects; group B: HIV subjects treated early) enrolled in the study.

	Group A	Group B
Number of patients	19	17
Gender	15 M and 4 F	13 M and 4 F
Age, years (mean ± SD)	34 ± 9	36 ± 4
CD4 + T-cell count, cell/μl (mean ± SD)	668 ± 10	391 ± 150
RNA viral load, copies/ml (mean)	1×10^4	2.7×10^4

M, male; F, female; SD, standard deviation.

2.3. HIV antibody avidity assay

For each serum sample, two aliquots were prepared, one by a 1/10 dilution with phosphate-buffered saline (PBS) and one with 1 M guanidine (G) as denaturing agent. Both aliquots were shaken and incubated for 10 min at room temperature and then tested as previously described[21,22] using the Vironostika ELISA platform (BioMérieux). Sample to cut-off ratios (S/CO) were calculated and the avidity index of HIV antibodies was computed as (S/CO of the G aliquot)/(S/CO of the PBS aliquot). A cut-off of 0.75 was selected according to previous observations.[19,21,22]

2.4. HIV-1 RNA quantification

The quantification of HIV-1 RNA viral load was carried out by Quantiplex HIV-RNA 3.0 assay (Siemens, Munich, Germany) according to the manufacturer's instructions.

2.5. Peripheral blood CD4 lymphocytes

Peripheral blood CD4 + T lymphocytes were counted by flow cytometry (FACScan; Becton and Dickinson, Mountain View, CA, USA) using commercially available monoclonal antibody (Becton-Dickinson).

2.6. Statistical analysis

Simple descriptive statistical measures for the avidity index were calculated (median and range). Fisher's test and the Wilcoxon rank sum test were used for immunoblot and avidity index results, respectively.

3. Results

3.1. Immunoblot assay

Antibody patterns were studied by immunoblot analysis in all serum samples from time 0 (first positive serum) onwards. As discussed in the Materials and methods, we considered the antibody response to specific HIV protein to be positive when the intensity of the antigen line reaction was between 2+ and 4+ positive rating, validated by software comparison with positive and negative control strips. The specific HIV-1 proteins used as targets in the INNO-LIA system are the gp120, gp41, p31, p24 and p17 viral proteins.

The percentage of serum reactivity (from 2+ to 4+ rating) to HIV-1 proteins was similar in the two groups of patients when the analysis was performed at time 0 (Fig. 1), showing a comparable percentage of sera that reacted to env glycoprotein gp120 (84% group A vs. 76% group B, $p = 0.7$), to env glycoprotein gp41 (100% in both groups of patients analyzed), to pol protein p31 (52% group A vs. 47% group B, $p = 1.0$), to gag protein p24 (68% group A vs. 70% group B, $p = 1.0$), and to gag protein p17 (63% group A vs. 58% group B, $p = 1.0$).

By contrast, subsequent analyses at later times (from 6 months onwards) showed a significantly lower reactivity to HIV-1 pol and gag proteins in HAART-treated patients (Fig. 1). In particular, among treated patients (group B) at 6 months after seroconversion, only seven reacted to p31 (41% vs. 94%; $p = 0.00$), 12 to p24 (70% vs. 94%; $p = 0.08$), and 10 to p17 (58% vs. 89%; $p = 0.05$). This lower reactivity persisted throughout the observation period, showing significantly low levels of antibody to p31 ($p < 0.01$ at 12, 18 and 24 months), to p24 ($p < 0.01$ at 12, 18 and 24 months), and to p17 ($p < 0.01$ at 12, 18 and 24 months).

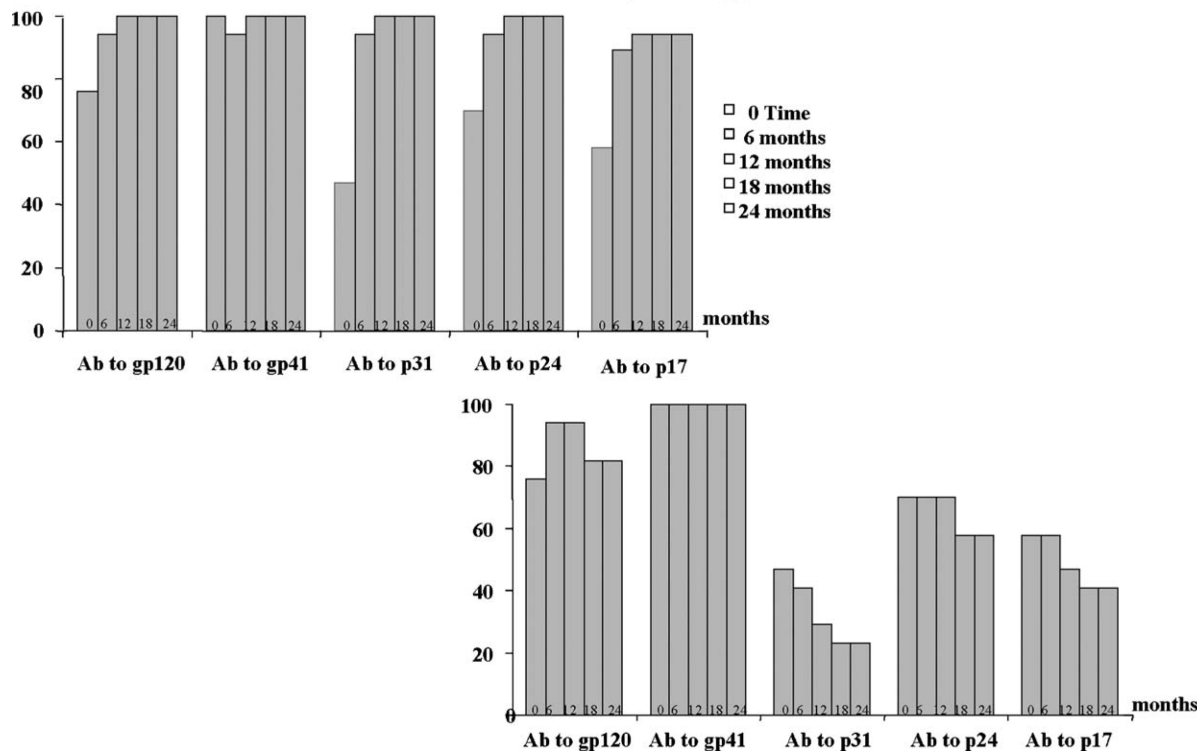

Fig. 1. Reactivity to env (gp120 and gp41), pol (p31), and gag (p24 and p17) proteins in untreated (top) and early treated (bottom) HIV-1 patients, from certified infection (0 months) onwards (6, 12, 18 and 24 months).

3.2. HIV antibody avidity assay

At diagnosis (time 0), the mean avidity index value was absolutely matched ($p = 0.83$) in untreated (OD values ranging from 0.2 to 0.72; avidity index mean 0.51 ± 0.15) and treated (OD from 0.3 to 0.7; avidity index mean 0.50 ± 0.13) patients. Among sera analyzed at later times, a clear difference in the mean avidity index values between treated and untreated patients was already evident at 6 months, with avidity index values in treated patients lower than those observed in untreated patients (0.66 vs. 0.8, $p = 0.03$). Similarly, the difference was significant up to the end of the observation period (0.74 vs. 0.92, $p = 0.001$ at 12 months; 0.79 vs. 0.98, $p = 0.000$ at 18 months; 0.90 vs.1.00, $p = 0.00$ at 24 months) (Fig. 2).

Even though individual variability in the rapidity of avidity maturation exists and different avidity index cut-offs (a conservative cut-off <0.75 (Chawla et al.[6]) or more conventional cut-off <0.8 (Suligoi et al.[5])) have been proposed for the antibody avidity assay to identify 'recent infection', our results show that most (82%) early treated patients had a low avidity index value (ranging from 0.5 to 0.7 OD) in comparison to untreated patients (26%).

3.3. HIV-1 RNA viral load and CD4 levels

The overall viral load at baseline showed a lower ($p = 0.07$) mean value in naïve individuals (mean value 1×10^4 copies/ml) in comparison with treated individuals (mean value 2.7×10^4 copies/ml). As expected, constant high levels of RNA viral load (3.7×10^4, 5.3×10^4, 3.2×10^4 and 6.8×10^4 HIV-1 RNA copies/ml at 6, 12, 18 and 24 months, respectively) were observed in untreated patients, whereas a significant decrease was observed from 6 months onwards following antiretroviral treatment (4.5×10^2, 6.4×10^1, 6.1×10^1 and less that 50 HIV-1 RNA copies/ml at 6, 12, 18 and 24 months, respectively).

CD4 cell counts (cell/μl) tended to be higher in untreated patients than treated patients at 0 and 6 months of observation

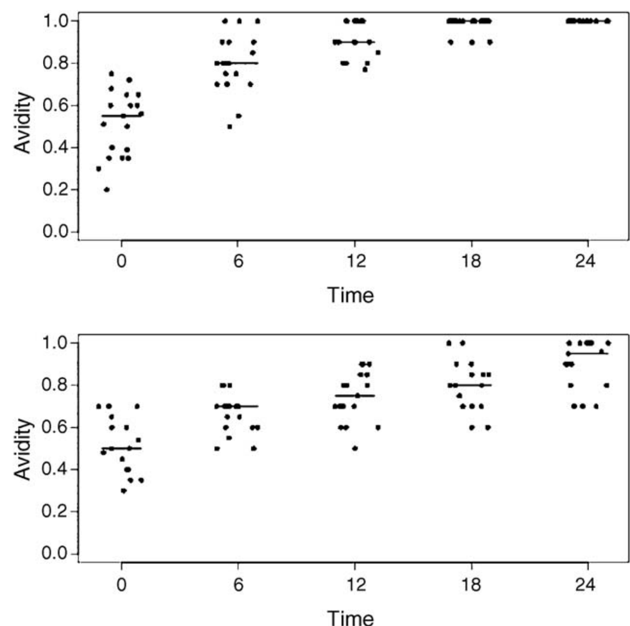

Fig. 2. Evolution of antibody avidity (expressed as avidity index) from time 0 (certified infection) onwards (6, 12, 18 and 24 months) in (A) untreated HIV-1 patients and (B) HIV-1 patients treated with HAART (within 3 months of seroconversion).

(668 ± 10 vs. 391 ± 150 at time 0 and 652 ± 110 vs. 437 ± 81 at 6 months, $p < 0.01$). No significant differences were found thereafter (665 ± 141 vs. 641 ± 435 at 12 months; 659 ± 184 vs. 689 ± 668 at 18 months, and 647 ± 182 vs. 825 ± 551 at the end of observation period; $p = 0.8$, 0.85 and 0.2, respectively). However, the CD4 follow-up confirmed a constant trend in untreated subjects ($p > 0.5$) and a significant recovery of CD4 following antiretroviral treatment ($p = 0.04$).

4. Discussion

This study reports a longitudinal retrospective analysis of immunological and virological parameters detected in 36 selected HIV-positive patients with recent seroconversion. The progression of HIV infection was followed in these patients for 2 years to determine the evolution of antibody avidity and Western blot reactivity in recently infected HIV-1 subjects and to study the impact of HAART on avidity maturation of HIV-1-specific immunoglobulin G (IgG) in patients with recent HIV-1 infection.

Our results show an impaired production and maturation of the humoral immunological response in patients treated early with HAART, as demonstrated by low levels of antibody to pol and gag proteins detected by immunoblot and low avidity levels confirmed by a lower index at 6 months (0.66 vs. 0.8, $p = 0.03$, treated vs. untreated patients). In particular, most early treated subjects (82%) showed a low avidity index value (ranging from 0.5 to 0.7 OD) in comparison to untreated patients (26%).

Several reports on this topic[15,17–19,24] have given rise to lively debate on the impact of antiretroviral treatment on antibody maturation. HAART appears to prevent the emergence of IgG antibodies, since HIV replication elicits the production of anti-HIV antibodies directly, through the persistent production of stimulating antigens, or indirectly, through the release of cytokines stimulating B-lymphocytes such as interleukin-6 (IL-6) and interferons (IFN).[15,23]

We found that treated patients developed a complete immunoblot response only to envelope proteins (HIV-1 gp120 and gp41) within 6 months and only a limited number of patients showed a high reactivity to pol and gag proteins. At 6 months only 41% of HAART-treated patients showed a detectable presence of antibody to p31, whereas most untreated patients (94%) reacted to p31. In addition, a significant difference in the immunologic response to p31 and to gag viral proteins p24 and p17 was also observed at later times (12–24 months) in HAART-treated patients.

On the other hand, Selleri and coworkers[19] showed that antibody to HIV-1 reached a complete repertoire 6 months after diagnosis in most treated patients (seven out eight subjects enrolled in the study) and in only one later on (within 10 months). This relative discrepancy may be related to several factors, such as the length of follow-up period, elevated individual variability, the specific HAART combination used, and the serological techniques employed.

Besides the low or slow IgG maturation process, we also found that early institution of HAART prevents the gradual evolution of the avidity index of HIV-1-specific antibodies. Our results are in agreement with the data of several authors[17,19,24,25] who have demonstrated not only the relationship between viral suppression established by HAART and low antibody avidity, but also the increase in avidity index values after discontinuation of HAART therapy.[19]

In addition, studies by other authors[15,19] on a limited but suggestive number of HAART-treated patients have failed to show any difference in IgG maturation (as demonstrated by Western blot and avidity index) in NNRTI or protease inhibitor (PI)-treated patients.

Moreover, not all data concerning the low response to some HIV proteins and the low avidity index levels can be explained in terms of CD4 counts: although the CD4 cell count tended to be higher in untreated patients at 0 and 6 months of observation, no significant differences were found thereafter. Moreover, the significant increase in CD4 levels in patients seems to be exclusively related to antiretroviral therapy leading to a CD4 recovery. However a careful evaluation of serological and immunological markers is necessary to fully characterize the course of HIV-1 infection and to provide a more complete laboratory-based assessment of disease progression.

Even though the role of the avidity index as a marker to evaluate the use of HAART in acute or early HIV infection or to predict treatment outcome remains unknown, our results have at least two major implications. First, the altered kinetics of the avidity index, often used in seroepidemiological studies to determine the approximate time of infection, is probably related to early control of viremia during HAART and could lead to inconsistent estimates in a substantial proportion of patients. Second, our data add new information on the complexity of the initial host–virus interaction during primary HIV infection.

The mechanisms underlying the low antibody response (both in terms of antibody maturation to single viral proteins and levels of avidity index) remain undetermined. However, some studies have demonstrated an IFN type I production defect during the course of primary HIV infection, suggesting a severe impairment in anti-HIV antibody production in recently converted patients.[23]

In particular, since HIV replication stimulates HIV antibody directly by a persistent production of antigens and indirectly by the release IL-6 and IFN able to stimulate B lymphocytes, the impairment of both types of cytokine due to antiretroviral therapy might also explain why HAART could be responsible for a reduced amount of HIV antibody.[15]

In addition, the different pharmacological combinations used to switch off viral replication could play a role in the humoral response, determining a different impairment in antibody affinity maturation. In fact, affinity maturation of the humoral immune response is regulated by antigen somatic hypermutation (SHM) of the genes encoding immunoglobulin variable regions, with the subsequent antigenic selection of mutant B clones. DNA hypermutagenesis at dC/dG and dA/dT pairs is linked to DNA polymerase-θ and DNA polymerase-η, which are partially inhibited by nucleoside analysis.[26] Hence, affinity maturation and isotype switching may be more or less damaged by different therapeutic associations.

In vitro studies to verify the B-lymphocyte response to single drugs (both reverse transcriptase and protease inhibitors) are in progress, to study the effect of single or associated drugs on B cell maturation and their capacity for antibody production. However, in a complex scenario also characterized by the so-called ‘late presenters’, the importance of diagnosing HIV infection at an early stage is unquestionable. Besides all the public health implications, the distinction between recent and chronic infection might serve to establish the time of infection and therefore allow any potential partners who could have been infected during a specific period of time to be reached.

Although results based on avidity index in patients who started therapy early may represent a limitation to this objective, the routine application of methods able to distinguish recent from long-lasting infection might have great impact on public health in monitoring disease incidence, in identifying high-risk groups, and in enhancing epidemiological conclusions. Lastly, as standardization of the test used is one of the most important points for test results to be clinically significant, all the data available on this topic suggest indications on the method and its applicability, even if its utility will depend on operating characteristics, HIV-1 subtype limitations, and selection of appropriate assay cut-off values.

Conflict of interest

All the authors declare that they have no relationship (commercial or otherwise) that may constitute a dual or conflicting interest.

Acknowledgements

This work was funded by Fondazione Cassa di Risparmio Bologna, Italy (grant number 2006.0035, June 2006), the AIDS projects (30G.27) of the Italian Ministry of Health, the SIVIM study group for test standardization, funds for selected research topics of the University of Bologna, and MURST (60%).

References

1. Kassutto S, Rosenberg ES. Primary HIV type 1 infection. *Clin Infect Dis* 2004;**38**: 1447–53.
2. Iweala OI. HIV diagnostic tests: an overview. *Contraception* 2004;**70**:141–7.
3. Hecht FM, Busch MP, Rawal B, Webb M, Rosenberg E, Swanson M, et al. Use of laboratory tests and clinical symptoms for identification of primary HIV infection. *AIDS* 2002;**16**:1119–29.
4. McFarland W, Busch MP, Kellogg TA, Rawal BD, Satten GA, Katz MH, et al. Detection of early HIV infection and estimation of incidence using a sensitive/less-sensitive enzyme immunoassay testing strategy at anonymous counseling and testing sites in San Francisco. *J Acquir Immune Defic Syndr* 1999;**22**: 484–9.
5. Suligoi B, Massi M, Galli C, Sciandra M, Di Sora F, Pezzotti P, et al. Identifying recent HIV infections using the avidity index and an automated enzyme immunoassay. *J Acquir Immune Defic Syndr* 2003;**32**:424–8.
6. Chawla A, Murphy G, Donnelly C, Booth CL, Johnson M, Parry JV, et al. Human immunodeficiency virus (HIV) antibody avidity testing to identify recent infection in newly diagnosed HIV type 1 (HIV-1)-seropositive persons infected with diverse HIV-1 subtypes. *J Clin Microbiol* 2007;**45**:415–20.
7. Lazzarotto T, Galli C, Pulvirenti R, Rescaldani R, Vezzo R, La Gioia A, et al. Evaluation of the Abbott AxSYM cytomegalovirus (CMV) immunoglobulin M (IgM) assay in conjunction with other CMV IgM tests and a CMV IgG avidity assay. *Clin Diagn Lab Immunol* 2001;**8**:196–8.
8. Mubareka S, Richards H, Gray M, Tipples GA. Evaluation of commercial rubella immunoglobulin G avidity assays. *J Clin Microbiol* 2007;**45**:231–3.
9. Sickinger E, Braun HB, Praast G, Stieler M, Gundlach C, Birkenbach C, et al. Evaluation of the Abbott ARCHITECT Toxo IgM assay. *Diagn Microbiol Infect Dis* 2009;**64**:275–82.
10. Lacabaratz-Porret C, Urrutia A, Doisne JM, Goujard C, Deveau C, Dalod M, et al. Impact of antiretroviral therapy and changes in virus load on human immunodeficiency virus (HIV)-specific T cell responses in primary HIV infection. *J Infect Dis* 2003;**187**:748–57.
11. Altfeld M, Rosenberg ES, Shankarappa R, Mukherjee JS, Hecht FM, Eldridge RL, et al. Cellular immune responses and viral diversity in individuals treated during acute and early HIV-1 infection. *J Exp Med* 2001;**193**: 169–80.
12. Smith D, Berrey MM, Robertson M, Mehrotra D, Markowitz M, Perrin L, et al. Virological and immunological effects of combination antiretroviral therapy with zidovudine, lamivudine, and indinavir during primary human immunodeficiency virus type 1 infection. *J Infect Dis* 2000;**182**:950–4.
13. Roca B. Adverse drug reactions to antiretroviral medication. *Front Biosci* 2009;**14**:1785–92.
14. Gupta RK, Hill A, Sawyer AW, Cozzi-Lepri A, von Wyl V, Yerly S, et al. Virological monitoring and resistance to first-line highly active antiretroviral therapy in adults infected with HIV-1 treated under WHO guidelines: a systematic review and meta-analysis. *Lancet Infect Dis* 2009;**9**:409–17.
15. Adalid-Peralta L, Grangeot-Keros L, Rudent A, Ngo-Giang-Huong N, Krzysiek R, Goujard C, et al. Impact of highly active antiretroviral therapy on the maturation of anti-HIV-1 antibodies during primary HIV-1 infection. *HIV Med* 2006;**7**: 514–9.
16. Coyne KM, Parry JV, Atkins M, Pozniak A, McOwan A. Spontaneous HIV-1 seroreversion in an adult male. *Sex Transm Dis* 2007;**34**:627–30.
17. Kassutto S, Johnston MN, Rosenberg ES. Incomplete HIV type 1 antibody evolution and seroreversion in acutely infected individuals treated with early antiretroviral therapy. *Clin Infect Dis* 2005;**40**:868–73.
18. Amor A, Toro C, Jiménez V, Simón A, Ramos B, Soriano V. Seroreversion of HIV antibodies in patients with prolonged suppression of viraemia under HAART. *AIDS* 2006;**20**:1460–2.
19. Selleri M, Orchi N, Zaniratti MS, Bellagamba R, Corpolongo A, Angeletti C, et al. Effective highly active antiretroviral therapy in patients with primary HIV-1 infection prevents the evolution of the avidity of HIV-1-specific antibodies. *J Acquir Immune Defic Syndr* 2007;**46**:145–50.
20. Murphy G, Parry JV. Assays for the detection of recent infections with human immunodeficiency virus type 1. *Euro Surveill* 2008;**13**. pii:18966.
21. Suligoi B, Galli C, Massi M, Di Sora F, Sciandra M, Pezzotti P, et al. Precision and accuracy of a procedure for detecting recent human immunodeficiency virus infections by calculating the antibody avidity index by an automated immunoassay-based method. *J Clin Microbiol* 2002;**40**: 4015–20.
22. Re MC, Schiavone P, Vitone F, Bon I, De Crignis E, Biagetti C, et al. Low avidity antibody: a reliable method to diagnose a recent HIV-1 infection. *New Microbiol* 2008;**31**:19–26.
23. Adalid-Peralta L, Godot V, Colin C, Krzysiek R, Tran T, Poignard P, et al. Stimulation of the primary anti-HIV antibody response by IFN-alpha in patients with acute HIV-1 infection. *J Leukoc Biol* 2008;**83**:1060–7.
24. Hare CB, Pappalardo BL, Busch MP, Karlsson AC, Phelps BH, Alexander SS, et al. Seroreversion in subjects receiving antiretroviral therapy during acute/early HIV infection. *Clin Infect Dis* 2006;**42**:700–8.
25. Markowitz M, Vesanen M, Tenner-Racz K, Cao Y, Binley JM, Talal A, et al. The effect of commencing combination antiretroviral therapy soon after human immunodeficiency virus type 1 infection on viral replication and antiviral immune responses. *J Infect Dis* 1999;**179**:527–37.
26. Franklin A, Blanden RV. Potential inhibition of somatic hypermutation by nucleoside analogues. *Mol Immunol* 2007;**44**:666–9.