

# HIV disease among immigrants coming to Italy from outside of the European Union: a case-control study of epidemiological and clinical features

R. MANFREDI\*, L. CALZA AND F. CHIODO

*Department of Clinical and Experimental Medicine, Division of Infectious Diseases, University of Bologna, S. Orsola Hospital, Bologna, Italy*

(Accepted 24 June 2001)

## SUMMARY

The epidemiological, clinical and therapeutic features of HIV disease diagnosed in 41 immigrants from outside of the European Union (EU), were compared with those of 123 Italian and EU patients, in a cross-sectional case-control study, with individuals matched according to age and gender. In total 4·15% of our patients came from outside of the EU (51·2% of them from subsaharan Africa), with a proportional predominance of females, and heterosexual and perinatal transmission of HIV disease ( $P < 0\cdot0001$  and  $P < 0\cdot02$ , respectively). Compared with Italian and EU subjects, patients coming from abroad had a shorter duration of known HIV infection ( $P < 0\cdot001$ ), but only some subjects were aware of their HIV disease prior to immigration, or acquired HIV infection only after coming to Italy (14·6% and 12·2%, respectively). No cases of HIV-2 infection or co-infection were detected in either study group. Compared with controls, patients coming from outside of the EU had a comparable clinical and immunological status, and had similar antiretroviral therapy, which was administered earlier ( $P < 0\cdot0001$ ), and proved better tolerated ( $P < 0\cdot04$ ), than in Italian and EU subjects. The apparently more limited virological response (as expressed by a higher mean plasma viral load, and a lower rate of viral suppression at the last visit;  $P < 0\cdot001$  and  $P < 0\cdot05$ , respectively), was probably a bias due to the shorter mean overall follow-up time ( $P < 0\cdot0001$ ), and the shorter mean duration of antiretroviral treatment ( $11\cdot1 \pm 2\cdot2$  months of immigrants *vs.*  $16\cdot2 \pm 6\cdot7$  months of controls;  $P < 0\cdot0001$ ).

## INTRODUCTION

The epidemiology, natural history, clinical features and outcome of HIV infection have undergone considerable changes in recent years, because of the proportional increase of heterosexual transmission, the progressive rise of patients' age, and especially the virological monitoring and the introduction of highly

active antiretroviral therapy (HAART) [1]. This has changed HIV disease from an acute-relapsing life-threatening disease to a chronic infection, after the sharp drop of related morbidity and mortality [1–4], although drug resistance, reduced patient compliance with complicated therapeutic regimens, drug–drug interactions, and long-term toxicity of anti-HIV therapy have become apparent as significant problems [1, 2, 5, 6].

This ever-changing epidemiological and clinical scenario has been influenced by the concurrent, massive immigration of people from developing

\* Author for correspondence: Department of Clinical and Experimental Medicine, Division of Infectious Diseases, University of Bologna, S. Orsola Hospital, Via Massarenti 11, I-40138 Bologna, Italy.

countries to the European Union (EU), and especially Italy during the last few years [7–15]. A large number of persons seeking jobs and refugee status in Italy have come from central Africa, Eastern Europe, Mediterranean countries and Asia. This population has an increased prevalence of HIV disease, as well as other infectious illnesses (especially tuberculosis and sexually transmitted diseases) [1, 7, 10, 13–22], either because of their prior residence in highly endemic regions (i.e. sub-Saharan Africa), or because of high-risk behaviour after their arrival in Italy or other industrialized countries. Poor socio-economic conditions, unemployment and low income, poor hygiene and feeding, limited health care, and concurrent drug abuse, sexual promiscuity, prostitution and recurrent visits to country of origin, are frequent among recently immigrated people, looking for a permanent or a temporary stay in Italy [7, 10, 15, 17–21].

The aim of our study was to assess the epidemiological, clinical and therapeutic features of HIV infection in patients coming from outside of the EU, compared with those in Italian and EU HIV-infected subjects, in a cross-sectional case-control study.

## METHODS

Among the 988 HIV-infected patients referred to our tertiary care outpatient centre located in Bologna (a large city of Northern Italy) in December 2000, and regularly followed-up for at least 9 months, 41 subjects (4.15%) came from outside of the EU. These were retrospectively compared with 123 Italian or EU individuals randomly selected from the 947 remaining HIV-infected patients (after matching for age and gender), in a 1:3 case-control study. Ten Italian citizens born outside the EU were not considered among immigrants, while eight patients coming from Switzerland or San Marino Republic, were excluded from the study.

Several epidemiological, clinical, laboratory and therapeutic features of HIV disease were evaluated and compared in the two study groups, using Mantel–Haenszel  $\chi^2$  test, Fisher's exact test, or Student's *t* test where applicable, for statistical analysis (with significance levels set at  $P < 0.05$ ).

HIV-1 and HIV-2 antibody testing was performed by different commercial EIA and immunoblotting assays, while determination of plasma HIV viral load was carried out by the branched-DNA technique (Quantiplex® HIV-RNA 2.0, Chiron, Emeryville, CA,

USA), with a sensitivity threshold of 50 HIV-RNA copies/ml.

Administered antiretroviral combinations included all compounds which were available in Italy in December 2000: zidovudine, didanosine, zalcitabine, lamivudine, stavudine and abacavir (nucleoside analogues); nevirapine, delavirdine and efavirenz (non-nucleoside reverse transcriptase inhibitors); and indinavir, ritonavir, nelfinavir and saquinavir in both hard-gel and soft-gel formulations (protease inhibitors). These were used as recommended by the international guidelines of antiretroviral therapy [2]. In particular, in our study highly active antiretroviral therapy (HAART) was defined as an association of at least three different anti-HIV compounds, including at least one protease inhibitor and/or one non-nucleoside reverse transcriptase inhibitor.

The adherence to clinical and laboratory monitoring and the correct compliance to recommended drug regimens during the last 9 months were checked by monthly outpatient visits carried out at our centre, which matched self-reported declarations of patients, and accountability of all administered drugs (directly distributed by physicians in charge of our unit).

The efficacy of antiretroviral therapy was evaluated according to the updated international recommendations [2], while tolerability of administered regimens was controlled through monthly clinical examination, and laboratory tests carried out at least quarterly, and including haematology, serum biochemistry, liver and kidney function, blood clotting assays, plasma lipid levels and urine analysis.

## RESULTS

The 41 patients coming from outside of the EU were comparable with the 123 Italian or EU controls according to mean age:  $32.1 \pm 11.4$  (range 3–53) years, and gender distribution: 46.3% of foreign subjects were females compared with 47.2% of controls (Table 1). However, when compared with the whole population of Italian or EU HIV-positive patients treated at our centre, a significantly greater proportion of females were found among immigrants ( $P < 0.0001$ ) (data not shown).

Compared with the control group, immigrants had a significantly greater prevalence of presumed heterosexual transmission of HIV (75.6% *vs.* 20.3% of cases;  $P < 0.0001$ ), and congenital infection (9.8% *vs.* 0.8%;  $P < 0.02$ ), as opposed to i.v. drug addiction

Table 1. *Epidemiological, clinical, laboratory, and therapeutic features of HIV disease in the 41 patients from outside of the European Union, and the 123 control subjects from Italy or countries belonging to the European Union*

Patients' features	Patients coming from outside of the European Union ( <i>n</i> = 41)	Patients from Italy or European Union ( <i>n</i> = 123)
Mean age (years $\pm$ s.d.)	32.1 $\pm$ 11.4	33.2 $\pm$ 12.2
Gender (males/females)	22/19	65/58
Risk for HIV infection: i.v. drug users/men who had sex with men/heterosexuals/children with congenital disease	3/3/31/4	74/23/25/1
Mean duration of known HIV infection (months $\pm$ s.d.)	14.1 $\pm$ 3.9	22.2 $\pm$ 6.9
Percentage of patients with a diagnosis of full-blown AIDS	14.6	17.1
Mean CD4+ lymphocyte count at the last available control (cells/ $\mu$ l $\pm$ s.d.)	278.3 $\pm$ 72.6	296.1 $\pm$ 68.1
Mean plasma viremia at the last available control (Log <sub>10</sub> HIV-RNA copies/ml)	3.9 $\pm$ 0.5	3.1 $\pm$ 0.3
Percentage of patients with viral suppression at the last available control	53.7	70.7
Percentage of patients receiving antiretroviral therapy (treated with HAART)	95.1 (87.8)	92.7 (86.2)
Mean duration of antiretroviral therapy (time to start of the first anti-HIV regimen) (months $\pm$ s.d.)	11.1 $\pm$ 2.2 (3.0 $\pm$ 2.6)	16.2 $\pm$ 6.7 (6.0 $\pm$ 5.4)
Percentage of patients with prior modification of antiretroviral therapy (due to failure/poor tolerability)	39.0 (31.7/7.3)	48.0 (26.0/22.0)

(7.3% of immigrants *vs.* 60.2% of controls:  $P < 0.0001$ ), while a tendency towards a lower incidence of homo-bisexual transmission was also observed (7.3% *vs.* 18.7%:  $P = 0.08$ ) (Table 1). One African child with perinatal HIV disease had been adopted by Italian parents. The majority of immigrated patients originated from Africa (61%): 21 patients from subsaharan Africa (with Eritrea, Nigeria and Zambia as the most frequent nations), and four subjects from Northern Africa (with Tunisia as the predominating country of origin). The remaining patients were from non-EU European countries (21.9%) (all from Eastern Europe, but none from Albania), South America (Argentina and Brazil) in 9.8% of cases, and Asia (Pakistan and Iran), in the remaining 7.3% of cases (Table 2).

Patients from outside the EU had a shorter duration of known HIV infection, compared with controls ( $P < 0.0001$ ) (Table 1). Among the 41 foreign patients, only 6 (14.6%) were aware of their disease before their immigration and referral to our clinic, while 5 patients (12.2%) acquired HIV infection only after their arrival in Italy (as confirmed by a negative HIV serology

preceding immigration, or by a verified seroconversion). On these grounds, in the majority of immigrant patients (73.2%), it could not be determined whether HIV infection had been acquired in their country of birth, other regions, or only after their immigration to Italy. No cases of HIV-2 infection were found in either study group.

A similar clinical picture of HIV disease was found in both study groups, as expressed by a comparable frequency of prior or concurrent diagnosis of full-blown AIDS (14.6% *vs.* 17.1%) (Table 1), a similar spectrum of AIDS-defining illnesses (data not shown), and a comparable mean CD4+ lymphocyte count at last laboratory examination (278.3  $\pm$  72.6 in immigrants *vs.* 296.1  $\pm$  68.1 cells/ $\mu$ l in controls) (Table 1). However, the most recent available virological assay showed a greater mean plasma viraemia ( $P < 0.001$ ), and a significantly lower rate of complete viral suppression among immigrants (53.7%), as opposed to Italian or EU controls (70.7%:  $P < 0.05$ ) (Table 1). In spite of this, at the time of our cross-sectional survey no significant difference was found between immigrants and controls, as to frequency of adminis-

Table 2. Distribution of the 41 patients coming from countries other than the European Union, according to the different nations of origin

Country of origin	No. patients
Africa (Subsaharan)	21
Eritrea	4
Nigeria	3
Zambia	3
Ethiopia	2
Zaire	2
Senegal	1
Burkina Faso	1
Côte d'Ivoire	1
Somalia	1
Angola	1
Mozambique	1
Kenya	1
Africa (Northern)	4
Tunisia	3
Morocco	1
Europe	9
Ukraine	3
Czech Republic	2
Poland	2
Croazia	1
Yugoslavia	1
South America	4
Argentina	2
Brasil	2
Asia	3
Pakistan	2
Iran	1

tration of antiretroviral therapy, with foreign patients showing even a slightly greater anti-HIV treatment (and HAART) use (Table 1). Also the selection of the different drug associations did not show differences between immigrants and controls (data not shown). Moreover, compliance with both clinical and laboratory examinations, and to prescribed drugs was 85–90% in both study groups during the 9 months of retrospective monitoring. Patients from outside the EU had a significantly shorter time to initiation of antiretroviral therapy ( $3.0 \pm 2.6$  months), compared with controls ( $6.0 \pm 5.4$  months;  $P < 0.0001$ ) (Table 1).

As a consequence, the apparently less evident virological response to antiretroviral therapy encountered in immigrant subjects, was probably dependent only on their shorter mean duration of anti-HIV therapy ( $P < 0.0001$ ) (Table 1), due to the significantly shorter mean follow-up of these patients (as expressed

by 'duration of known HIV infection') ( $P < 0.0001$ ), compared with those of Italian and EU citizens.

Finally, patients who came from outside of the EU experienced a lower overall frequency of modification of antiretroviral therapy due to both treatment failure or lack of tolerability (39% *vs.* 48% of control group); it depended on adverse events or toxicity in a significantly lower number of cases among immigrants compared with EU controls ( $P < 0.04$ ), while the failure rate did not differ significantly between study groups (Table 1). Again, all these variables could also have been notably affected by the significantly different mean duration of anti-HIV therapy between the two patient groups. When performing a multivariate analysis which considered the different demographic, epidemiological, clinical, laboratory, and therapeutic parameters available for immigrant patients, no significant relationship was demonstrated according to the different country or continent of origin.

## DISCUSSION

Many immigrants who recently moved to Italy are from highly endemic regions for HIV infection (i.e. subsaharan Africa, Western, or Eastern Central Africa) [7–15], where the majority of worldwide cases of HIV disease (which often remains undiagnosed), is presently concentrated. In these countries, the available health resources cannot cover more than 1% of needs, in terms of HIV treatment and prophylaxis [1, 2, 22, 23]. The immigration of individuals coming from developing areas of the world to industrialized regions is responsible for a direct increase of imported infectious diseases (which are endemic in countries of origin), and an indirect rise of infectious illnesses related to poverty, poor socio-economic conditions, substance abuse, sex working, reduced levels of personal and community hygiene, and limited health care delivery [1, 7, 10, 13, 14, 17–22, 24].

Tuberculosis (which has at least a seven-fold increase in incidence in HIV-infected individuals [19, 25], has a major epidemiological and social impact [8, 11, 12, 25, 26], but also sexually-transmitted diseases [7, 13, 14, 17, 20], infectious or parasitic skin illnesses [9, 16], and HIV infection itself [7, 10, 13, 14, 17–21], are increasingly being reported as imported diseases in immigrants to the EU.

Some countries (such as the United States) impose formal restrictions to immigration of subjects suffering from transmissible diseases, including HIV [27], and

there are limitations on medical assistance for illegal immigrants [17]. Unlike the situation of the United States [17, 27], no substantial limitations of access to infectious diseases or sexually-transmitted diseases centres occurs in Italy, and all clinical, laboratory, and needed pharmacological therapies (including antiretrovirals), are free for HIV-infected immigrants, even during the period preceding their regularization as authorized residents in our country [7, 10, 13, 14].

In a multicentre surveillance study carried out in Italy in the period 1991–5, over 4000 novel cases of sexually-transmitted disease were reported in immigrants, coming from Northern Africa in over one third of cases. The overall prevalence of HIV infection in this population was around 5%, but remarkable differences were found according to the country of origin, and co-existing risk behaviours (i.v. drug addiction and homosexual contacts). When considering tuberculosis, a recent epidemiological survey performed in the Milan area identified 596 episodes diagnosed in immigrants, and stressed that 22.8% of all episodes reported to a reference centre occurred in foreign people (who had a eightfold greater prevalence, compared with that of the general Italian population) [11]. This last study, and similar Italian surveys of tuberculosis in immigrants [8, 12], confirmed the increased frequency of acquired or re-activated disease in this population, the possible occurrence of epidemic in closed communities, and the reduced sensitivity of tuberculin skin testing, when screening programme are implemented [8, 11, 12].

Almost all metropolitan areas of Western Europe have registered a steady increase in the number of people recently immigrated because of socio-economic or political reasons. As a consequence, appropriate strategies of health care and prevention are needed [15, 20, 21, 24, 26, 28]. Further concerns stem from the recent report of HIV-1 serotypes other than B (usually imported from outside Europe), in Iceland, Spain, and other European countries, as a potential consequence of contacts with persons from developing countries, through travel or immigration [19, 29, 30].

Information and prevention campaigns ‘targeted’ to recently immigrated people with prior or present risk of exposure to HIV infection and other transmissible diseases, have been scheduled by the majority of developed countries [7, 10, 14, 19–21, 24, 28, 31], but the specific cultural and social background of immigrants has to be taken into special consideration in order to deliver a successful educational message [28].

When considering HIV infection, the large-scale introduction of measures to prevent viral transmission [7, 19, 23, 31], the virological and immunological monitoring, and especially the combination antiretroviral regimens (HAART), has had a largely favourable cost–benefit ratio [1, 22]. This is despite the emergence of a number of long-term drawbacks related to HAART administration [1, 2, 5, 6], and the elevated costs of both treatment and monitoring of HIV disease [1]. However, at the present time no prospective controlled studies are available regarding the epidemiology, clinical features, treatment and evolution of HIV infection in the HAART era, specifically addressed to patients immigrated to industrialized countries, including Italy.

Our cross-sectional observational survey, although based on a limited patient sample, has revealed an appreciable percentage (slightly over 4%) of the HIV positive 988 patients followed at our reference centre for at least 9 months, come from outside of the EU: over 50% of cases from central Africa, followed by Eastern Europe (but surprisingly not from Albania) [7, 10, 13–15], Northern Africa, and South America, with a large prevalence of HIV-1 infection acquired through heterosexual or perinatal transmission. A significantly higher prevalence of females (46.3%) was found among immigrants, compared with that observed overall in our cohort of HIV-infected individuals. The shorter duration of known HIV infection among foreigners is clearly related to the recent immigration and the frequent, late recognition of HIV disease: however, the arrival in our country allowed the diagnosis of a probable prior infection acquired in the country of origin in the majority of cases (73.2%). A lower proportion of patients’ HIV infection was already known, or was acquired only after immigration (at least 12.2% of cases). These data underline the elevated risk of HIV disease for patients coming from endemic areas, but also the increased exposure to HIV acquired because of poor socio-economic and health care conditions [7, 13, 14]. The persisting absence of HIV-2 infection is noteworthy [32], especially when the majority of our HIV-infected immigrants come from regions where HIV-2 is endemic. The immigrants experienced a more limited virological response, as shown by a significantly greater mean viral load, and a lower viral suppression rate at the last available laboratory examination. This is probably a bias induced by the shorter mean duration of follow-up period and antiretroviral therapy administration in immigrants,

compared with Italian and EU citizens, at the time of our cross-sectional study (Table 1). No significant demographic, epidemiological, clinical or therapeutic difference was shown according to the country (or continent) or origin of immigrated HIV-infected patients.

Besides the increased commitment of public health structures in terms of early screening, information, counselling and prevention campaigns [7, 10, 15, 19], frequently poor socio-economic and hygienic conditions, and a different cultural approach to illness [28], patients immigrated to Italy and diagnosed with HIV disease seem to show limited epidemiological and clinical differences, while compared with Italian or EU controls, and do not have a significantly different disease evolution, and trend of acceptance, efficacy and tolerability of therapeutic regimens. In our experience, the less evident virological response of immigrants is likely to be due to their shorter laboratory and therapeutic follow-up, which could be also responsible for the apparently reduced toxicity and need of therapy modification. Further prospective studies are warranted to evaluate the frequency and epidemiological trends of HIV infection among immigrants to developed countries, and to assess their immunological, virological, and clinical outcome during antiretroviral therapy, their level of adherence to recommended drugs, and their short- and long-term tolerability of anti-HIV regimens.

## REFERENCES

- Nicoll A, Gill ON. The global impact of HIV infection and disease. *Commun Dis Publ Hlth* 1999; **2**: 85–95.
- Carpenter CCJ, Cooper DA, Fischl MA et al. Antiretroviral therapy in adults. Updated recommendations of the International AIDS Society-USA Panel. *JAMA* 2000; **283**: 381–90.
- Palella FJ, Delaney KM, Moorman AC et al. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. *N Engl J Med* 1998; **338**: 853–60.
- Manfredi R, Chiodo F. Features of AIDS and AIDS-defining diseases during the highly active antiretroviral therapy (HAART) era, compared with the pre-HAART period: a case-control study. *Sex Transm Infect* 2000; **76**: 145–6.
- Andrews L, Friedland G. Progress in HIV therapeutics and the challenge of adherence to antiretroviral therapy. *Infect Dis Clin North Am* 2000; **14**: 901–28.
- Mauss S. HIV-associated lipodystrophy syndrome. *AIDS* 2000; **14** (Suppl. 3): S197–207.
- Suligoi B, Giuliani M. Sexually transmitted diseases among foreigners in Italy. *Migration Medicine Study Group. Epidemiol Infect* 1997; **118**: 235–41.
- Rapiti E, Fano V, Forastiere F et al. Determinants of tuberculosis in an immigrant population in Rome: a case-control study. *Int J Tuberc Lung Dis* 1998; **2**: 479–83.
- Bottoni U, Dianzani C, Rossi ME, Carlesimo M, Calvieri S. Skin diseases in immigrants seen as outpatients in the Institute of Dermatology of the University of Rome 'La Sapienza' from 1989 to 1994. *Eur J Epidemiol* 1998; **14**: 201–4.
- Colucci A, Covre P, D'Agostini A et al. Immigration and health: the Italian situation. Italian National Focal Point (NFP) of the AIDS & Mobility Project. *Ann Ist Super Sanità* 1998; **34**: 473–87.
- Codecasa LR, Porretta AD, Gori A et al. Tuberculosis among immigrants from developing countries in the province of Milan 1993–1996. *Int J Tuberc Lung Dis* 1999; **3**: 589–95.
- Scolari C, El-Hamad I, Matteelli A et al. Incidence of tuberculosis in a community of Senegalese immigrants in Northern Italy. *Int J Tuberc Lung Dis* 1999; **3**: 18–22.
- D'Antuono A, Cocci C, Carla E, De Tommaso S. Prevalence of STDs and HIV infection among immigrant sex workers attending an STD centre in Bologna, Italy. *Sex Transm Infect* 1999; **75**: 273–4.
- Smacchia C, Di Perri G, Boschini A, Parolin A, Concia E. Immigration, HIV infection, and sexually transmitted diseases in Europe. *AIDS Patient Care STDs* 2000; **14**: 233–7.
- Matteelli A, Signorini L. Population movements and STD/HIV hazard. *Health Co-operation Papers* 2000; **16**: 199–204.
- Manfredi R, Mazzoni A, Nanetti A, Chiodo F. Histoplasmosis capsulati and duboisii in Europe: the impact of the HIV pandemic, travel and immigration. *Eur J Epidemiol* 1994; **10**: 675–81.
- Asch S, Rulnick S, Todoroff C, Richwald G. Potential impact of restricting STD/HIV care for immigrants in Los Angeles County. *Int J STD AIDS* 1996; **7**: 532–5.
- McMunn AM, Mwanje R, Pozniak AL. Issues facing Africans in London with HIV infection. *Genitourin Med* 1997; **73**: 157–8.
- Decosas J, Adrien A. Migration and HIV. *AIDS* 1997; **11** (Suppl. A): S77–84.
- Gras MJ, Weide JF, Langendam MW, Coutinho RA, Van den Hoek A. HIV prevalence, sexual risk behaviour and sexual mixing patterns among migrants in Amsterdam, The Netherlands. *AIDS* 1999; **13**: 1953–62.
- Freeman RC, Williams ML, Saunders LA. Drug use, knowledge, and HIV risk behaviors of Cuban-, Mexican- and Puerto-Rican-born drug injectors who are recent entrants into the United States. *Subst Use Misuse* 1999; **34**: 1765–93.
- Kumaranayake L, Watts C. Economic costs of HIV/AIDS prevention activities in sub-Saharan Africa. *AIDS* 2000; **14** (Suppl. 3): S239–52.
- Fessel J, Follansbee S, Hurley L, Klein D. Does use of

- potent antiretroviral therapy differ by health care setting?. *JAMA* 1999; **281**: 1696–7.
24. Bardsley M, Storkey M. Estimating the numbers of refugees in London. *J Public Health Med* 2000; **22**: 406–12.
  25. Glynn JR. Resurgence of tuberculosis and the impact of HIV infection. *Br Med Bull* 1998; **54**: 579–93.
  26. Schwartzman K, Menzies D. Tuberculosis screening of immigrants to low-prevalence countries. A cost-effectiveness analysis. *Am J Respir Crit Care Med* 2000; **161**: 780–9.
  27. Shoop LG. Health based exclusion grounds in United States immigration policy: homosexuals, HIV infection and the medical examination of aliens. *J Contemp Health Law Policy* 1993; **9**: 521–44.
  28. Lechky O. Multiculturalism and AIDS: different communities mean different educational messages required. *CMAJ* 1997; **156**: 1446–8.
  29. Holguin A, Rodes B, Dietrich U, Soriano V. Human immunodeficiency viruses type 1 subtypes circulating in Spain. *J Med Virol* 1999; **59**: 189–93.
  30. Love A, Chen M, Sallberg M. Changing profile of HIV-1 serotypes in Iceland during 1989–96. *Scand J Infect Dis* 2000; **32**: 445–6.
  31. Shriver MD, Everett C, Morin SF. Structural interventions to encourage primary HIV prevention among people living with HIV. *AIDS* 2000; **14** (Suppl. 1): S57–62.
  32. Costigliola P, Ricchi E, Manfredi R. et al. No evidence of HIV-2 infection amongst HIV-1 Ab positive people in the largest cities of north-eastern Italy. *Eur J Epidemiol* 1992; **8**: 140–1.