Clinical Features and Virologic Characteristics of Primary and Early HIV-1 Infection in Slovenian Patients

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ABSTRACT

Analysis of time trends in newly diagnosed HIV-1 infected patients in Slovenia over a 10-year period (1996-2005) showed an increase in the number of newly diagnosed HIV-1 infected patients in 2004 and 2005 as well as increase in the number of newly diagnosed patients with primary/early HIV-1 infection. A retrospective analysis was performed in order to evaluate the clinical, epidemiological, laboratory and virological parameters of primary/early HIV-1 infection presenting with or without acute retroviral syndrome (ARS). Primary/early HIV-1 infection was diagnosed in 33 (19.5%) out of 169 newly diagnosed HIV-1 infected patients during the 10-year period. Most patients experienced ARS, the most commonly reported symptoms being fever, malaise and pharyngitis, followed by rash and lymphadenopathy. Median CD4 cell count was 415 cells/mm³, median CD8 cell count was 865 cells/mm³ and median HIV-1 viral load at the time of diagnosis was 5.1 log₁₀ copies/mL. The increase in the number of newly diagnosed HIV-1 infected patients of symptoms of ARS among persons at high risk of infection.

Key words: acute retroviral syndrome, primary HIV-1 infection, seroconversion

Introduction

Primary or acute HIV-1 infection (PHI) is an early dynamic part of HIV-1 infection, defined as the time from initial exposure to and infection with HIV-1 to the completion of seroconversion. This stage of HIV-1 infection is characterized by an extremely high level of viremia, followed by a vigorous immune response. During the acute phase of the infection, an estimated 50 to 90% of patients experience symptoms of acute retroviral syndrome (ARS). The following so-called early stage of HIV-1 infection is less well defined, and it usually refers to an interval between seroconversion and the establishment of the viral load set-point¹. Namely, during early HIV-1 infection, a balance between viral replication and immune response is achieved and the viral load set-point is established, usually 6-12 months after infection. The level of the viral load set-point is prognostic for disease progression². The events associated with PHI are likely critical determinants of the subsequent course of HIV/AIDS¹.

According to data from the Institute of Public Health of Republic of Slovenia, 276 individuals were diagnosed with HIV/AIDS infection from 1986 to the end of 2005 in Slovenia³. By the end of 2005, at least 192 people were living with HIV-1, including 48 AIDS patients. The number of newly diagnosed HIV-1 infected patients has been steadily increasing over recent years, peaking in 2005 with 35 new cases of HIV-1 infection³. Apart from the overall increase in newly diagnosed HIV-1 infected patients, a dramatic increase in the number of primary/early HIV infections was noted in 2004 and 2005³.

The aim of the present study was to evaluate the clinical features, epidemiological, laboratory and virologic parameters of primary/early HIV-1 infection in Slovenian patients diagnosed between 1996 and 2005 and to establish changes of time trends in newly diagnosed HIV-1 infections.

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Material and Methods

Definitions

Data from all newly diagnosed HIV-1 infected patients between 1996 and 2005 in Slovenia were retrospectively analyzed to identify patients who were diagnosed during primary or early phase of HIV-1 infection. The patients were included in the study if they fulfilled one of the following criteria at the time of diagnosis: (i) negative or undetermined HIV-1 serology (negative or positive anti-HIV screening tests with negative or undetermined anti-HIV confirmatory tests) associated with the detection of HIV-1 RNA; (ii) evidence of seroconversion during the last 12 months; (iii) documented ARS with or without a negative HIV-1 serology during the last 12 months. PHI was defined as negative or undetermined HIV-1 serology (negative or positive anti-HIV screening tests with negative or undetermined anti-HIV confirmatory tests) which became positive on follow-up, or positive HIV-1 RNA tests and negative results of HIV-1 antibody tests. Early HIV-1 infection was defined as positive results of anti-HIV confirmatory tests and evidence of seroconversion within the previous 12 months, or documented ARS within the last 12 months.

Virological and immunological tests

Anti-HIV status was determined in all samples by two screening assays, Vitros Anti-HIV 1+2 (Ortho Clinical Diagnostics, Amersham, UK) and VIDAS HIV DUO Ultra (bioMerieux, Marcy-l'Etoile, France). In case of a reactive result of one or both screening tests, the results were confirmed by two confirmatory assays, Western blot-based (WB) test HIV BLOT 2.2 (Genelabs Diagnostics, Singapore) and immunoblot-based assay (IB) INNO-LIA HIV I/II Score (Innogenetics, Ghent, Belgium). The results of WB were interpreted using the American Red Cross criteria – a test was considered HIV-1 positive if anti-HIV-1 antibodies against at least one HIV-1 protein derived from each of the env, gag and pol regions were detected. If no reactivity against HIV-1 proteins was detected, the test was considered negative. If some but not all criteria for a positive result were found, the result was considered indeterminate. The results of IB were interpreted according to the manufacturer's criteria⁴.

HIV-1 viral load was determined using the Cobas Amplicor HIV-1 Monitor[™] Test version 1.5 (Roche Diagnostics Systems, Branchburg, NJ). Genotypic resistance testing was performed for 31 patients, whose plasma samples were obtained at the time when primary/early HIV-1 infection was diagnosed using the ViroSeq HIV-1[™] Genotyping System version 2 (Celera Diagnostics, Alameda, California) or the TRUGENE[®] HIV-1 Genotyping Kit (Visible Genetics, Toronto, Canada) and the results were reported according to the consensus document of the International AIDS Society-USA (March/April 2005)⁵. HIV-1 subtype was determined using the REGA HIV-1 subtyping tool as described previously⁶. CD4 and CD8 cell counts were assessed by standard flow cytometry methods on a FACScan flow cytometer (Becton-Dickinson, Heidelberg, Germany).

In all cases, epidemiological information, the presence and type of symptoms, and laboratory tests carried out during the first visit, were reviewed by analyzing the patients' records. Data from newly diagnosed HIV-1 infected patients with chronic HIV-1 infection between 1996 and 2005 were also analyzed in relation to CD4 cell count and HIV-1 viral load, and compared with patients with newly diagnosed primary/early HIV-1 infection in the same time period.

Statistical analysis

Statistical analysis was performed using SPSS version 13.0 (SPSS Inc., Chicago, IL). Variables were expressed as medians and range. Between-group differences were analyzed with the Mann-Whitney U test. p-values were 2-sided and considered significant at a level of <0.05.

Results

Time trends in newly diagnosed HIV-1 infected patients in Slovenia from 1996-2005

According to data from the Institute of Public Health of Republic of Slovenia, 169 new cases of HIV-1 infection were diagnosed between 1996 and 2005. While the overall incidence of HIV-1 infection remains relatively low (the estimated overall incidence is less than 1 per 1,000 persons), in the last three years an increase in the number of newly diagnosed HIV-1 infected patients, and especially in the number of patients diagnosed with primary/early HIV-1 infections, was noted (Figure 1).

As shown in Figure 1, in 2003 there were 16 newly diagnosed HIV-1 infected patients, 4 (25%) of them diagnosed during primary/early infection, with 3 confirmed



Fig. 1. Number of newly diagnosed HIV-1 infected patients in Slovenia over a 10-year period (1996–2005).

cases of ARS and one case of asymptomatic seroconversion. In 2004, among 25 newly diagnosed HIV-1 infected patients, primary/early infection was confirmed in 10 (40%); all of the patients developed ARS with 4 cases of confirmed and 6 cases of probable ARS. In 2005, there were 35 newly diagnosed HIV-1 infected patients, 13 (37%) of them in the primary/early phase of infection. Most of them experienced ARS, with 9 cases of confirmed and 3 cases of probable ARS, and in one case seroconversion was asymptomatic. Analysis of the time trends showed a similar annual number of newly diagnosed HIV-1 infected patients with advanced HIV-1 infection with a CD4 cell count below 200 cells/mm³ (average 6.4 cases per year; range 2-8), with a slight increase in the number of patients with chronic HIV-1 infection with higher CD4 cell counts.

Primary/early HIV-1 infection in Slovenia between the years 1996-2005

In total, primary/early HIV-1 infection was diagnosed in 33 out of 169 (19%) newly diagnosed HIV-1 infected patients between 1996 and 2005. As shown in Table 1, 32 (97%) were males and one (3%) female. All but one of the men included in the study reported a homosexual or bisexual mode of transmission. The only woman in the study group reported a heterosexual mode of transmission. The median age at the time of diagnosis was 34.9 years (range 20.7 - 65.5). Seroconversion was virologically confirmed in 23 out of 33 (70%) patients with primary/early HIV-1 infection. In the other 10 (30%) patients the diagnosis of early HIV-1 infection was based on a clinical diagnosis of ARS without seroconversion and an estimated date of infection within 12 months prior to diagnosis based on epidemiological data. In accordance with above described criteria, 19 cases of PHI and 14 cases of early HIV-1 infection were diagnosed (Table 1).

Out of 33 patients with primary/early HIV-1 infection, 15 (45%) were first clinically examined by their general

 TABLE 1

 CHARACTERISTICS OF PRIMARY/EARLY HIV-1 INFECTION IN

 SLOVENIA BETWEEN 1996 AND 2005

Characteristics	N (%)	
Number of patients	33	
Gender		
Male	32 (97%)	
Female	1 (3%)	
Mode of transmission		
Homo/bisexual	31 (94%)	
Heterosexual	2(6%)	
Primary HIV-1 infection	19 (58%)	
Early HIV-1 infection	14 (42%)	
Seroconversion	23 (70%)	
Acute retroviral syndrome	31 (94%)	
Confirmed	21	
Probable	10	

practitioner and then referred to various departments for infectious diseases, where HIV testing was performed, 3 (9%) patients were diagnosed in a sexually transmitted diseases outpatient clinic, 9 (27%) patients through voluntary confidential HIV testing and the remaining 6 (18%) patients were blood donors. Seven out of 31 (23%) symptomatic patients were hospitalized.

Among 33 patients with primary/early HIV-1 infection, 31 (94%) patients experienced ARS and two patients had asymptomatic seroconversion. ARS was confirmed in 21 patients, while 10 patients described symptoms of probable ARS. As shown in Figure 2, the most commonly reported symptoms were fever, malaise and pharyngitis, followed by rash and lymphadenopathy.



Fig. 2. Symptoms and signs of acute retroviral syndrome in Slovenian patients.

In four out of 33 (12%) patients with primary/early HIV-1 infection, viral meningitis was diagnosed, one of them requiring treatment in an intensive care unit because of the severity of encephalitis.

Laboratory tests were available for 31 out of 33 patients, most frequently elevated liver function tests and relative lymphocytopenia were present (35% and 29% of patients, respectively), while leukopenia and thrombocytopenia were found in less than 10% of patients.

The virological and immunological characteristics of patients with primary/early HIV-1 infection at the time of diagnosis were analyzed with respect to their CD4 and CD8 cell counts and HIV-1 viral load. Out of 33 patients with primary/early HIV-1 infection, 14 (42%) had a CD4 cell count above 500 cells/mm³, 15 (46%) had a CD4 cell count between 200 and 500 cells/mm³ and 4 (12%) patients had a CD4 cell count below 200 cells/mm³ (range 38 –1002 cells/mm³) at the time of first diagnosis. As shown in Table 2, the median CD4 cell count was 865 cells/mm³ and the median HIV-1 viral load at the time of diagnosis was 5.1 log₁₀ copies/mL.

HIV-1 subtypes and the presence of mutations associated with resistance to antiviral drugs were determined in 31 out of 33 (94%) patients with primary/early HIV-1 infection, whose plasma samples had been obtained at the time of diagnosis. All analyzed patients were infected with HIV-1 subtype B and no major drug resistance mutations were identified on the protease or reverse transcriptase gene. However, almost all of the patients carried one or more secondary mutations/polymorphisms on the protease gene. The most frequent secondary mutations/polymorphisms were seen at positions 63 (81%), 93 (55%) and 77 (42%).

Antiretroviral treatment was initiated shortly following diagnosis in 20 out of 33 patients, 15 of them received highly active antiretroviral treatment (HAART) within the first month following diagnosis (mean time to treatment 12.4 days); the other 5 patients received treatment within the first three months following diagnosis due to a persistently high viral load. Out of the 20 treated patients, 16 patients with good adherence to treatment became aviremic after a median time of 16.7 weeks (1.8–41.2 weeks) following the initiation of HAART. The time to virological suppression was estimated as the calendar midpoint between the last positive and first undetectable HIV-1 viral load test⁷. Nine out of 16 (56%) patients with good adherence had achieved an undetectable HIV-1 viral load at week 16, and 15 (93%) at week 24.

Primary/early HIV-1 infection versus chronic HIV-1 infection in Slovenia

Data from patients diagnosed with chronic HIV-1 infection at the time of first presentation, diagnosed during the last decade, were analyzed in relation to their CD4 cell count and HIV-1 viral load at the time of diagnosis. During the 10-year period, 136 out of 169 (80%) patients were diagnosed with chronic HIV-1 infection, but data was only available for 132 patients. The median age of patients with primary/early HIV-1 infection at the time of diagnosis was 34.9 years, while the median age of 132 patients with chronic HIV-1 infection at the time of diagnosis was 36.6 (Table 2). Among 132 patients with chronic HIV-1 infection, 26 (20%) were females, 106 (80%) males. The proportion of women infected with HIV-1 has decreased from a maximum of over one third of all newly diagnosed cases in the late nineties to less than 6% in 2005.

As shown in Table 2, there were no statistically significant differences in the age at the time of diagnosis between patients with primary/early and those with chronic HIV-1 infection (p=0.274). As expected, there was a statistically significant difference in the CD4 cell count between primary/early and chronically HIV-1 infected patients (p=0.001). Among patients newly diagnosed with chronic HIV-1 infection, 64 (48%) had CD4 cell counts below 200 cells/mm³, 61 (36%) had CD4 cells counts between 200 and 500 cells/mm³, while 21 (16%) patients had CD4 cells counts over 500 cells/mm³. The difference in the HIV-1 viral load between the group of newly diagnosed HIV-1 infected patients with primary/early and the group with chronic HIV-1 infections at the time of first presentation, was not statistically significant (p=0.288).

Discussion

Primary HIV-1 infection is frequently symptomatic, however as the symptoms are usually non-specific, the diagnosis may be delayed or missed. Early recognition of PHI can provide benefits for the infected person, since HAART can be initiated early, especially in severe cases of ARS. However, studies have not so far confirmed a long-term immunological benefit of early treatment. Perhaps even more important are the benefits on the public health level, since the risk of transmission in this more contagious stage of HIV-1 is reduced^{1,8,9}.

Analysis of the time trends in newly diagnosed HIV-1 infected patients in Slovenia during the 10-year period (1996-2005) showed an increase in the number of newly diagnosed HIV-1 infected patients in 2004 and 2005, as well as an increase in the number of newly diagnosed HIV-1 infected patients with primary/early HIV-1 infections, with more patients presenting with ARS with its classic non-specific symptoms. Our analysis showed that clinical manifestations and laboratory parameters of ARS in Slovenia are similar to previously published reports^{1,10-13}. The most commonly reported symptoms were

TABLE 2

COMPARISON OF DEMOGRAPHIC AND VIROLOGICAL PARAMETERS BETWEEN NEWLY DIAGNOSED HIV-1 INFECTED PATIENTS WITH PRIMARY/EARLY AND THOSE WITH CHRONIC HIV-1 INFECTION AT THE TIME OF DIAGNOSIS IN SLOVENIA BETWEEN 1996 AND 2005

Characteristics	Primary/early HIV-1	Chronic HIV-1	р
Number of patients	33	136	
Age at the time of diagnosis			
Median years, range	34.9 (20.7-65.5)	36.6 (18.8–68.3)	0.274
Gender, n (%)			
Male	32 (97%)	106 (80%)	
Female	1 (3%)	26 (20%)	
CD4 cell count			
Median (range)	415.5 (38-1002)	200.0 (2-1362)	0.001
(% of study group) <200 cells/mm ³	12%	48%	
$>500 \text{ cells/mm}^3$	42%	23%	
HIV-1 RNA			
Median log ₁₀ copies/mL (range)	5.2 (1.6-6.8)	5.1 (1.3-6.8)	0.288

fever, malaise and pharyngitis, followed by rash and lymphadenopathy. Among laboratory tests elevated liver transaminases and relative lymphocytopenia were the most common finding. Seven out of 31 (23%) symptomatic patients were hospitalized, which is similar to previously published reports¹². Two thirds of patients with primary/early HIV-1 infection were given HAART; after 16 weeks of treatment the proportion of patients that had achieved undetectable viral load was lower than in previously reported study (53% versus 78%), but a comparable proportion of patients had achieved an undetectable viral load after 24 weeks of HAART (93% versus 90%)⁷. The overall response and tolerance of treatment among patients during PHI and early course of HIV-1 infection was satisfactory.

Comparison of Slovenian patients diagnosed with primary/early HIV-1 infection and patients diagnosed with chronic HIV-1 infection in the same time period somewhat surprisingly showed that there was no significant difference in the age at presentation. We had expected that patients presenting with acute illness would be younger, but this was not the case. In contrast, the median CD4 cell count was statistically significantly lower in patients with chronic HIV-1 infection, while their median HIV-1 viral load was not significantly different. Our study showed that the increase in the number of newly diagnosed primary/early HIV-1 infections is mainly due to infections among men who have sex with men. At the same time, the proportion of women among patients with newly diagnosed HIV-1 infection has been steadily decreasing.

The increase in the number of newly diagnosed HIV-1 infections may be due in part to increased awareness among clinicians of the possibility of ARS, since the majority of patients presenting with primary/early HIV-1 infection were diagnosed in the last two years. Another factor which may contribute to the rising number of diagnosed primary/early HIV-1 infections is the possibility of increased awareness of ARS symptoms among persons at high risk of infection. There was, however, practically no increase in the overall number of HIV-1 screening tests performed annually in Slovenia; the rate of annual HIV-1 testing between 1996 and 2000 was only 9.9 per 1,000 population with only a minor increase in 2004, with 11.1 tests

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In contrast to several countries in Western and Central Europe and North America, where a shift towards heterosexual transmission has been noted recently, our epidemiological data shows that the predominant mode of HIV-1 transmission in Slovenia is still through unprotected homosexual intercourse²⁵. The data also shows that the intravenous drug user population in Slovenia has so far been largely spared HIV-1 infection²⁶.

Primary HIV-1 infection should be considered in the differential diagnosis for patients presenting with symptoms and signs of acute viral disease. One study has shown that the majority of patients experiencing ARS seek medical attention, 48% in general practice and 52% in various urgent care facilities. Only one quarter of patients were correctly diagnosed at the time¹¹. In a similar study performed in an urgent care center in the United States, all patients who had symptoms of viral infection and at least one risk factor for HIV-1 infection (defined as unprotected sex in the last two months or intravenous drug abuse) were tested for HIV-1 infection. They found that 1% of subjects had PHI and 1.2% had chronic HIV-1 infection¹².

Since a significant increase in primary/early HIV-1 infections has been recognized recently in Slovenia, PHI should be considered in the differential diagnosis for patients presenting with the symptoms and signs of acute viral disease, and an appropriate epidemiological history taken. At the same time, further effort should be directed towards more awareness campaigns to highlight the possible symptoms of PHI in groups with a high risk of infection. Persons at high risk should be encouraged first towards primary prevention of HIV-1 infection, through safe sex practices and second to seek medical attention in case of possible PHI to enable timely diagnosis, management and prevention of the further spread of infection.

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KLINIČKA OBILJEŽJA I VIROLOŠKE ZNAČAJKE PRIMARNE I RANE HIV-1-INFEKCIJE U BOLESNIKA IZ SLOVENIJE

SAŽETAK

Analiza učestalosti novootkrivene HIV-1 infekcije u Sloveniji kroz desetogodišnje razdoblje (1996.-2005.) pokazala je porast broja novodijagnosticiranih HIV-om tipa 1 u 2004. i 2005. g. kao i porast u broju novodijagnosticiranih bolesnika s primarnom/ranom HIV-1-infekcijom. Provedena je retrospektivna analiza da bi se procijenili klinički, epidemiološki, laboratorijski i virološki parametri primarne/rane HIV-1 infekcije s ili bez kliničke manifestacije akutnog retrovirusnog sindroma (ARS). Primarna/rana HIV-1 infekcija je dijagnosticirana u 33 (19,5%) od 169 novodijagnosticiranih pacijenata kroz navedeno desetogodišnje razdoblje. Većina bolesnika je imala simptome ARS, češći simptomi su bili vrućica, umor i faringitis potom osip i limfadenopatija. Medijan vrijednost limfocita T CD4+ bio je 415 stanica/mm³, medijan limfocita T CD8+ bio je 865 stanica/mm³, a broj virusnih kopija po mililitru u vrijeme dijagnoze bio je 5.1 log kopija/mL. Porast u broju novodijagnosticiranih HIV-1 bolesnika može biti dijelom uzrokovan povećanom osviještenošću liječnika o pojavi ARS-a i mogućoj povećanoj osviještenosti o simptomima ARS-a među visokorizičnom populacijom.