MAJOR ARTICLE

Factors Associated with the Development of Opportunistic Infections in HIV-1–Infected Adults with High CD4$^+$ Cell Counts: A EuroSIDA Study

Daria Podlekareva,[1] Amanda Mocroft,[2] Ulrik B. Dragsted,[1] Bruno Ledergerber,[4] Marek Beniowski,[5] Adriano Lazzarin,[6] Jonathan Weber,[3] Nathan Clumeck,[7] Norbert Vetter,[8] Andrew Phillips,[2] and Jens D. Lundgren,[1] for the EuroSIDA study group[a]

[1]Hvidovre University Hospital, Hvidovre, Denmark; [2]Royal Free Hospital and University College London Medical School and [3]St. Mary's Hospital, London, United Kingdom; [4]University Hospital Zürich, Zürich, Switzerland; [5]Medical University of Silesia, Chorzow, Poland; [6]San Raffaele Scientific Institute, Milan, Italy; [7]Saint-Pierre Hospital, Brussels, Belgium; [8]Pulmonary Center of the City of Vienna, Vienna, Austria

Background. Limited data exist on factors predicting the development of opportunistic infections (OIs) at higher-than-expected CD4$^+$ cell counts in human immunodeficiency virus (HIV) type 1–infected adults.

Methods. Multivariate Poisson regression models were used to determine factors related to the development of groups of OIs above their respective traditional upper CD4$^+$ cell count thresholds: group 1 ($\geqslant$100 cells/μL), OIs caused by cytomegalovirus, *Mycobacterium avium* complex, and *Toxoplasma gondii;* group 2 ($\geqslant$200 cells/μL), *Pneumocystis* pneumonia and esophageal candidiasis; and group 3 ($\geqslant$300 cells/μL), pulmonary and extrapulmonary tuberculosis.

Results. In groups 1, 2, and 3, 71 of 9219, 125 of 7934, and 36 of 7838 patients, respectively, developed $\geqslant$1 intragroup OI. The strongest predictor of an OI in groups 1 and 2 was current CD4$^+$ cell count (for group 1, incidence rate ratio [IRR] per 50% lower CD4$^+$ cell count, 5.37 [95% confidence interval {CI}, 3.71–7.77]; for group 2, 4.28 [95% CI, 2.98–6.14]). Injection drug use but not current CD4$^+$ cell count predicted risk in group 3. Use of antiretroviral treatment was associated with a lower incidence of OIs in all groups, likely by reducing HIV-1 RNA levels (IRR per 1-$\log_{10}$ copies/mL higher HIV-1 RNA levels for group 1, 1.50 [95% CI, 1.15–1.95]; for group 2, 1.68 [95% CI, 1.40–2.02]; and for group 3, 1.89 [95% CI, 1.40–2.54]).

Conclusion. Although the absolute incidence is low, the current CD4$^+$ cell count and HIV-1 RNA level are strong predictors of most OIs in patients with high CD4$^+$ cell counts.

In the natural history of HIV-1 infection, opportunistic infections (OIs) occur throughout a range of immune function as measured by CD4$^+$ cell count [1]. The introduction of combination antiretroviral therapy (CART) in 1996–1997 resulted in a markedly reduced risk of OIs in HIV-1–infected patients at all levels of immunodeficiency [2]. The risk of OIs is known to decrease with decreasing immunodeficiency [1, 3]. As a consequence, CD4$^+$ cell count thresholds have been identified below which the risk of clinical progression and specific OIs is sufficiently high to warrant the initiation of CART and prophylaxis for opportunistic pathogens [4]. However, case reports and retrospective studies have suggested that a minority of HIV-1–infected patients develop OIs at higher-than-expected CD4$^+$ cell counts during CART, when the immune function would appear to be only moderately compromised, if at all [5, 6].

Some infections (e.g., tuberculosis [TB] [7]) can develop over a wide range of CD4$^+$ cell counts and might also develop in persons without recognized immunodeficiency. Analyses of HIV-1–infected cohorts prior to the introduction of CART suggested that patients with

Received 16 March 2006; accepted 13 April 2006; electronically published 24 July 2006.

Presented in part: 13th Conference on Retroviruses and Opportunistic Infections, Denver, Colorado, 5–8 February 2006 (poster O-125).

Potential conflicts of interest: none reported.

Financial support: European Commission BIOMED 1 (grant CT94-1637), BIOMED 2 (grant CT97-2713), and 5th framework (grant QLK2-2000-00773); Bristol-Myers Squibb; GlaxoSmithKline; Roche; Boehringer Ingelheim; Swiss Federal Office for Education and Science (grant for the participation of centers in Switzerland).

[a] Study group members are listed after the text.

Reprints or correspondence: Dr. Daria Podlekareva, Copenhagen HIV Programme, Dept. 044, Hvidovre University Hospital, Kettegaard alle 30, DK-2650 Hvidovre, Denmark (dpo@cphiv.dk).

The Journal of Infectious Diseases 2006; 194:633–41

0022-1899/2006/19405-0015$15.00

high $CD4^+$ cell counts with clinical signs of one OI were at excess risk of another [8]. Thus, several factors may influence the risk of developing OIs at relatively high $CD4^+$ cell counts. These factors have, however, not been thoroughly investigated, and, to our knowledge, they have not been previously reported from large international cohort studies.

PATIENTS AND METHODS

Cohort. The EuroSIDA study is a prospective observational study of HIV-1–infected patients from 82 centers in 28 European countries, Israel, and Argentina. Details of the study have been published elsewhere [2]. Six cohorts of patients have been recruited to date. Information was provided on a standardized data-collection form at recruitment and at 6-month intervals. Follow-up lasted through April 2005, and all $CD4^+$ cell counts, plasma HIV-1 RNA levels, dates of starting and stopping each antiretroviral drug, and information about disease-specific chemoprophylaxis and treatment were routinely collected. Both $CD4^+$ cell counts and HIV-1 RNA levels were measured at median intervals of 3 months (interquartile range [IQR], 2–4 months). Data on reasons for the initiation or discontinuation of disease-specific prophylaxis and adherence to antiretroviral drugs were not collected. AIDS-defining diagnoses were recorded, including distinct diagnoses made subsequent to the initial diagnosis, using the 1993 clinical definition of AIDS of the US Centers for Disease Control and Prevention [9]. An extensive quality-assurance program was established that included data quality control at the coordinating center and monitoring visits to each site.

Patients. Three groups of patients were defined a priori according to $CD4^+$ cell count thresholds. Baseline was defined as the date of the first $CD4^+$ cell count measurement above the predefined threshold. In group 1, patients were required to have at least 1 $CD4^+$ cell count of $\geqslant$100 cells/μL during prospective follow-up in the EuroSIDA study. A diagnosis of cytomegalovirus (CMV) retinitis, *Mycobacterium avium* complex (MAC) infection, or cerebral toxoplasmosis (TOXO) was defined as the end point. In group 2, the $CD4^+$ cell count threshold was $\geqslant$200 cells/μL, with an end point of *Pneumocystis jirovecii* pneumonia (PCP) or esophageal candidiasis (EC). In group 3, the $CD4^+$ cell count threshold was $\geqslant$300 cells/μL, with an end point of pulmonary or extrapulmonary TB. Patients were excluded from their individual groups if they had no $CD4^+$ cell counts recorded after baseline or had been given a diagnosis of any of the diseases that constituted the end point prior to baseline. We defined CART as any combination of $\geqslant$3 antiretroviral drugs that included at least 1 protease inhibitor, 1 nonnucleoside reverse-transcriptase inhibitor, or abacavir.

Statistical methods. Descriptive statistics were used to describe the baseline characteristics of the patients in each group and to describe patient characteristics at the date of diagnosis of the relevant OI. Characteristics of interest were $CD4^+$ cell count, nadir of the $CD4^+$ cell count, HIV-1 RNA (when available), previous and current antiretroviral therapy (ART), prior AIDS diagnosis, and use of disease-specific prophylaxis. Of note is the fact that patients could be included in >1 group.

At each $CD4^+$ cell count measurement, follow-up since the last $CD4^+$ cell count was calculated; person-years of follow-up (PYFU) accrued in 2 categories—above or below the $CD4^+$ cell count threshold—and OIs were categorized in the same way. The incidence of OIs in each of the 3 groups was calculated using the latest, or "current," $CD4^+$ cell count.

Multivariate Poisson regression was used to determine the factors related to the development of the relevant OI in the 3 groups at a $CD4^+$ cell count above the threshold level. Patients were removed from the analyses if their $CD4^+$ cell counts fell below the threshold, and they were reinstated at the date when the $CD4^+$ cell count returned to a level above the threshold. Therefore, events that occurred below the $CD4^+$ cell count threshold were not included. Only the first OI within each group was included in the analyses. Factors included in univariate models were demographic factors (date of birth, ethnic origin, sex, country of origin, risk group, and region of Europe), current $CD4^+$ cell count, nadir of the $CD4^+$ cell count (either as a continuous or categorical variable, both of which were included as $\log_2$-transformed variables when modeled as continuous variables), time with $CD4^+$ cell count below the threshold, starting CART, starting any ART, year of follow-up, and prior AIDS-defining diagnosis. Factors that were significant in univariate models ($P < .1$) were included in the multivariate model for the corresponding group. Factors that were not significant in univariate analyses were added in turn to the final model, to test whether they were confounding variables or were related to the development of the OI. All statistical analyses were performed using SAS software (version 9.1; SAS Institute).

RESULTS

As of spring 2005, the EuroSIDA database contained prospective follow-up data on 11,928 patients, of whom 9219 (77.3%) were eligible for inclusion in group 1, 7934 (66.5%) were eligible for inclusion in group 2, and 7838 (65.7%) were eligible for inclusion in group 3. There were 71 cases of CMV retinitis (19 cases), MAC infection (22 cases), and TOXO (31 cases) during 45,555 PYFU in group 1 (1 patient was diagnosed with both CMV retinitis and MAC infection on the same date); 125 cases of PCP (31 cases) and EC (94 cases) during 35,456 PYFU in group 2; and 36 cases of TB (27 pulmonary and 10 extrapulmonary; 1 patient was diagnosed with both pulmonary and extrapulmonary TB on the same date) during 29,828 PYFU in group 3. The median (IQR) date of diagnosis of OIs was 2/1997 (12/1995–1/2000) in group 1, 12/1998 (4/1996–5/2002) in group 2, and 4/1999 (8/1997–1/2002) in group 3.

Patient characteristics and the incidence of OIs. The baseline characteristics of patients are shown in table 1. We compared baseline characteristics of patients who did and did not develop OIs within each group (table 2). In group 1, patients who developed CMV retinitis, MAC, or TOXO at a $CD4^+$ cell count of >100 cells/μL had a significantly lower nadir of the $CD4^+$ cell count ($P = .0039$) and earlier baseline $CD4^+$ cell count ($P < .0001$) than did those who did not develop an OI. In group 2, injection drug users (IDUs) developed PCP or EC significantly more often at a $CD4^+$ cell count of >200 cells/μL than did persons in other exposure groups ($P = .010$). In group 3, patients with a diagnosis of TB had a considerably higher nadir of the $CD4^+$ cell count than did those who did not develop TB ($P = .0006$). In all groups, patients who developed an OI had significantly higher HIV-1 RNA levels at baseline and were less likely to be receiving CART than were patients who did not develop an OI.

In all 3 groups, the incidence of OIs was significantly lower in patients who had started CART, compared with those who had not (for group 1, incidence rate ratio [IRR], 0.32 [95% CI, 0.20–0.51]; $P < .0001$; for group 2, IRR, 0.29 [95% CI, 0.20–0.41]; $P < .0001$; for group 3, IRR, 0.35 [95% CI, 0.19–0.70]; $P = .0024$). The median $CD4^+$ cell count over the course of the entire follow-up period was significantly higher in patients who had started CART in all groups: in group 1, 410 cells/μL (IQR, 270–586 cells/μL) for patients who had started CART and 355 cells/μL (IQR, 242–496 cells/μL) for those who had not; in group 2, 456 cells/μL (IQR, 330–625 cells/μL) for patients who had started CART and 396 cells/μL (IQR, 300–531 cells/μL) for those who had not; and in group 3, 510 cells/μL (IQR, 400–670 cells/μL) for patients who had started CART and 459 cells/μL (IQR, 374–590 cells/μL) for those who had not ($P < .0001$).

Figure 1 shows the incidence of OIs in each group according to the latest $CD4^+$ cell count. In groups 1 and 2, there was a clear pattern of decreasing incidence of the OIs as the current

Table 1. Baseline characteristics of EuroSIDA patients (groups 1–3)

Characteristic	Group ($CD4^+$ cell count threshold)		
	1 (≥100 cells/μL) ($n = 9219$)	2 (≥200 cells/μL) ($n = 7934$)	3 (≥300 cells/μL) ($n = 7838$)
All			
Male	7003 (76.0)	5937 (74.8)	5958 (76.0)
Female	2216 (24.0)	1997 (25.2)	1880 (24.0)
MSM	3947 (42.8)	3338 (42.1)	3518 (44.9)
IDU	2212 (24.0)	1896 (23.9)	1741 (22.2)
Heterosexual	2446 (26.5)	2185 (27.5)	2070 (26.4)
Other risk factors	614 (6.7)	515 (6.5)	509 (6.5)
White	7921 (85.9)	6802 (85.7)	6790 (86.6)
Other race	1298 (14.1)	1132 (14.3)	1048 (13.4)
Patients with a prior diagnosis of AIDS			
Yes	2079 (22.6)	1203 (15.2)	1675 (21.4)
Receiving CART	3899 (42.3)	3309 (41.7)	3425 (43.7)
Receiving ART	3172 (34.4)	2663 (33.6)	2558 (32.6)
No ART[a]	393 (4.3)	330 (4.2)	318 (4.1)
ART naive	1755 (19.0)	1632 (20.6)	1537 (19.6)
$CD4^+$ cell count			
Baseline, median (IQR), cells/μL	310 (193–447)	342 (251–470)	389 (333–495)
Nadir, median (IQR), cells/μL	187 (90–299)	210 (104–315)	200 (85–316)
HIV-1 RNA load, median (IQR), $\log_{10}$ copies/mL	2.70 (1.88–4.05)	2.70 (1.70–3.92)	2.62 (1.70–3.63)
Age, median (IQR), years	37.0 (31.7–44.1)	37.0 (31.7–44.2)	37.7 (32.3–44.8)
Date of first $CD4^+$ cell count above threshold, median (IQR)	11/97 (2/96–12/01)	3/98 (12/96–1/02)	3/99 (5/97–2/02)

NOTE. Data are no. (%), unless otherwise indicated. AIDS, $CD4^+$ cell count, HIV-1 RNA load, and age were defined at first $CD4^+$ cell count above the threshold. HIV-1 RNA load data were available for 6380 patients in group 1 (69.2%), 5861 patients in group 2 (73.9%), and 6376 patients in group 3 (81.3%). HIV-1 RNA load <400 $\log_{10}$ copies/mL was for 2782 (43.6%), 2712 (46.3%), and 3396 (53.3%) patients, respectively. ART, antiretroviral therapy; CART, combination antiretroviral therapy; IDU, injection drug user; IQR, interquartile range; MSM, men who have sex with men.

[a] Patients started some ART (ART/CART) but were receiving no ART at baseline.

Table 2. Baseline characteristics of patients according to whether they developed opportunistic infections (OIs).

Characteristic	Group 1			Group 2			Group 3		
	No OI	OI, $CD4^+$ cell count ⩾100 cells/μL	*P*	No OI	OI, $CD4^+$ cell count ⩾200 cells/μL	*P*	No OI	OI, $CD4^+$ cell count ⩾300 cells/μL	*P*
Patients	9148 (99.2)	71 (0.8)	…	7809 (98.4)	125 (1.6)	…	7802 (99.5)	36 (0.5)	…
Baseline date, median (IQR)	11/97 (2/96–12/01)	11/96 (5/94–2/97)	<.0001	3/98 (1/97–1/02)	12/95 (5/94–1/98)	<.0001	3/99 (5/97–2/02)	4/97 (8/95–12/98)	.0002
$CD4^+$ cell count, median (IQR), cells/μL									
Baseline	310 (194–448)	196 (124–290)	<.0001	343 (251–477)	320 (251–392)	.026	389 (333–495)	394 (353–496)	.55
Nadir	188 (90–299)	122 (57–243)	.0039	210 (104–315)	238 (120–32)	.34	200 (84–315)	308 (210–372)	.0006
Baseline HIV-1 RNA load, median (IQR), $\log_{10}$ copies/mL	2.70 (1.87–4.05)	3.66 (2.86–4.37)	.034	2.70 (1.70–3.92)	3.35 (2.60–4.14)	.014	2.60 (1.70–3.62)	3.51 (2.19–4.84)	.015
Age, median (IQR), years	37.0 (31.7–44.1)	34.8 (30.7–39.3)	.029	37.0 (31.7–44.1)	36.8 (31.2–45.5)	.79	37.7 (32.4–44.8)	36.7 (30.3–41.2)	.14
Treatment									
CART	3890 (42.5)	9 (12.7)	<.0001	3288 (42.1)	21 (16.8)	<.0001	3417 (43.8)	8 (22.2)	.040
ART[a]	3129 (34.2)	43 (60.6)		2614 (33.5)	49 (39.2)		2543 (32.6)	15 (41.7)	
No ART[b]	390 (4.3)	3 (4.2)		322 (4.1)	8 (6.4)		317 (4.1)	1 (2.8)	
ART naive	1739 (19.0)	16 (22.5)		1585 (20.3)	47 (3.6)		1525 (19.6)	12 (33.3)	
DSP	2857 (31.2)	32 (45.1)	.012	2411 (30.9)	40 (32.0)	.79	96 (1.2)	2 (5.6)	.020
Prior diagnosis of AIDS	2048 (22.4)	31 (43.7)	<.0001	1184 (15.2)	19 (15.2)	.99	1672 (21.4)	3 (8.3)	.056
Exposure group									
IDU	2190 (23.9)	22 (31.0)	.17	1854 (23.7)	42 (33.6)	.010	1728 (22.2)	13 (36.1)	.044
Other	6958 (76.1)	49 (69.0)		5595 (76.3)	83 (66.4)		6074 (77.8)	23 (63.9)	

NOTE. Data are no. (%), unless otherwise indicated. All characteristics are baseline data (date of the first $CD4^+$ cell count above the threshold). ART, antiretroviral therapy; CART, combination antiretroviral therapy; DSP, disease-specific prophylaxis; IDU, injection drug user.

[a] Any ART that was not CART

[b] Patients who had previously received ART but were not receiving any treatment at baseline.

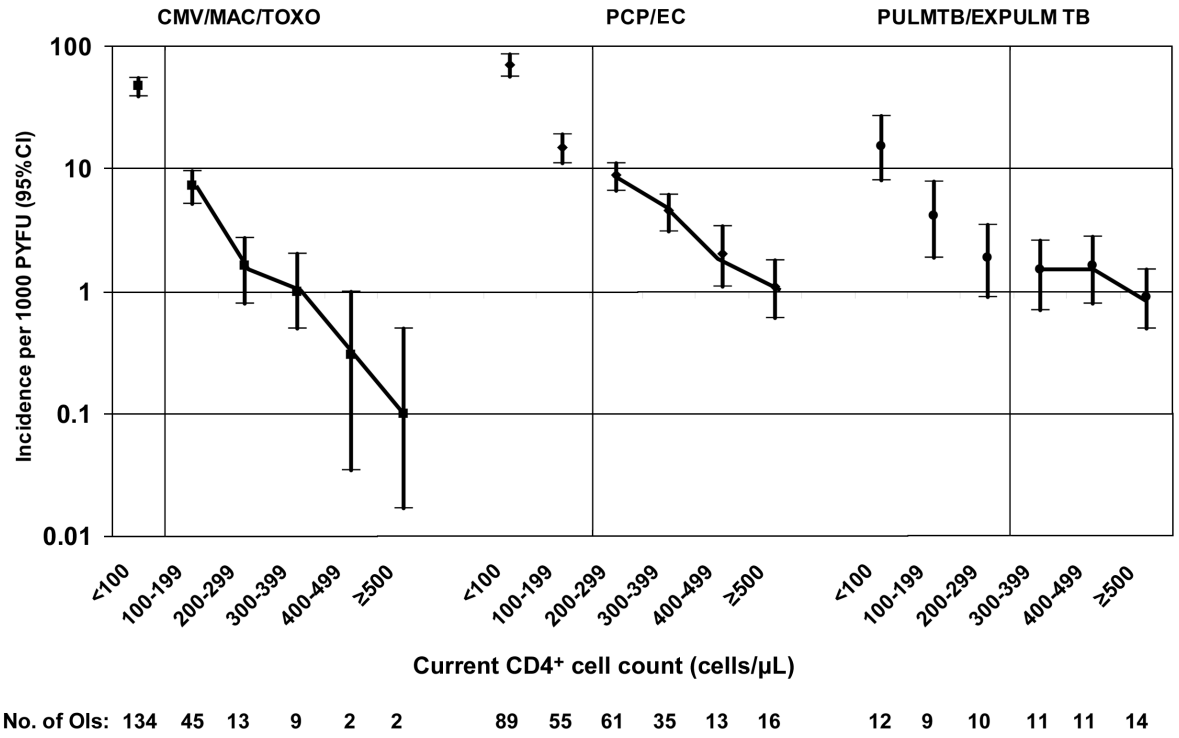

Figure 1. Incidence of opportunistic infections (OIs), according to the latest $CD4^+$ cell count. CI, confidence interval; CMV, cytomegalovirus retinitis; MAC, *Mycobacterium avium* complex; EC, esophageal candidiasis; PCP, *Pneumocystis jirovecii* pneumonia; PULM/EXPULM TB, pulmonary/extrapulmonary tuberculosis; PYFU, person-years of follow-up; TOXO, cerebral toxoplasmosis.

$CD4^+$ cell count increased, both above and below the threshold. For group 3, the incidence of TB was significantly higher when the $CD4^+$ cell count decreased to <300 cells/μL. However, as the current $CD4^+$ cell count increased to above this level, there were few changes in the incidence of TB, although a trend toward an inverse correlation between current $CD4^+$ cell count and TB incidence was observed.

The median $CD4^+$ cell count at diagnosis was 175 cells/μL (IQR, 120–266 cells/μL) in group 1, 302 cells/μL (IQR, 251–385 cells/μL) in group 2, and 444 cells/μL (IQR, 360–569 cells/μL) in group 3, which is <2 times the predefined threshold. The median time between last $CD4^+$ cell count and the diagnosis of an OI in all groups was 2 months (IQR, 1–5 months). Median HIV-1 RNA levels at the time of diagnosis were higher in group 1 (3.88 [IQR, 3.02–4.87]) than in group 2 (3.62 [IQR, 2.45–4.69]) and in group 2 than in group 3 (3.53 [IQR, 2.30–4.75]) (data were available for 37, 76, and 28 patients, respectively). The median time between last HIV-1 RNA measurement and the diagnosis of an OI was 1 month (IQR, 0–4 months) in all groups.

Predictors for the development of an OI. The results of the univariate and multivariate analyses are shown in table 3. In the main analysis, adjustment for HIV-1 RNA level was not performed, because this test was not routinely performed across Europe until 1997, and including this variable would have reduced the power of the analyses.

In group 1, a lower $CD4^+$ cell count was associated with a higher risk of CMV retinitis, MAC infection, or TOXO (for a 50% lower $CD4^+$ cell count, IRR, 5.37 [95% CI, 3.71–7.77]; $P < .0001$). In addition, patients with any prior AIDS-defining conditions had higher incidences of CMV retinitis, MAC infection, or TOXO (IRR, 2.91 [95% CI, 1.74–4.85]; $P < .0001$). Patients who had started CART had a significantly reduced incidence of CMV retinitis, MAC infection, or TOXO (IRR, 0.47 [95% CI, 0.27–0.77]; $P = .0025$). After adjustment, older patients had a significantly lower incidence of CMV retinitis, MAC infection, or TOXO (per 10 years older, IRR, 0.71 [95% CI, 0.53–0.95], $P = .021$). Although patients with a longer cumulative time of $CD4^+$ cell count below the threshold had a significantly higher incidence of CMV retinitis, MAC infection, or TOXO in the univariate analysis, this was not significant in the multivariate model.

In group 2, the strongest predictive factor for the development of an OI was current $CD4^+$ cell count, as was seen in group 1. Patients with a 50% lower current $CD4^+$ cell count had a significantly higher incidence of PCP or EC (IRR, 4.28 [95% CI, 2.98–6.14]; $P < .0001$). Furthermore, patients starting either CART (IRR, 0.37 [95% CI, 0.26–0.54]; $P < .0001$) or any

Table 3. Incidence rate ratios (IRRs) for specific opportunistic infections above the given threshold (includes only parameters significant in the univariate analysis; $P < .1$).

	Univariate analysis		Multivariate analysis	
Risk factors	IRR (95% CI)	*P*	IRR (95% CI)	*P*
Group 1				
Age	0.76 (0.58–1.00)	.048	0.71 (0.53–0.95)	.021
Nadir CD4$^+$ cell count	1.10 (0.99–1.21)	.079	1.03 (0.88–1.21)	.69
Time from nadir CD4$^+$ cell count	0.73 (0.57–0.93)	.011	0.79 (0.60–1.05)	.091
Time with CD4$^+$ cell count less than threshold	1.27 (1.03–1.56)	.025	0.86 (0.65–1.13)	.27
Current CD4$^+$ cell count	5.95 (4.19–8.44)	<.0001	5.37 (3.71–7.77)	<.0001
Prior diagnosis of AIDS	3.04 (1.90–4.85)	<.0001	2.91 (1.74–4.85)	<.0001
Started CART	0.32 (0.20–0.51)	<.0001	0.47 (0.29–0.77)	.0025
Started some ART	2.26 (1.16–4.42)	.017	1.68 (0.82–3.42)	.16
IDU	1.63 (0.99–2.70)	.056	1.16 (0.69–1.94)	.58
Group 2				
Current CD4$^+$ cell count	4.64 (3.26–6.61)	<.0001	4.28 (2.98–6.14)	<.0001
Started CART	0.29 (0.20–0.41)	<.0001	0.37 (0.26–0.54)	<.0001
Started some ART	0.71 (0.49–1.02)	.064	0.61 (0.42–0.88)	.0092
IDU	1.92 (1.33–2.79)	.0006	1.51 (1.04–2.21)	.031
From Eastern Europe	3.12 (1.38–7.09)	.0065	1.98 (0.83–4.73)	.13
Group 3				
Nadir of the CD4$^+$ cell count	0.66 (0.48–0.91)	.011	0.83 (0.60–1.16)	.29
Time from nadir of the CD4$^+$ cell count	0.65 (0.47–0.90)	.0096	0.78 (0.57–1.07)	.12
Time with CD4$^+$ cell count less than threshold	0.77 (0.59–1.00)	.046	0.91 (0.68–1.21)	.50
Started CART	0.36 (0.19–0.70)	.0024	0.49 (0.25–0.96)	.037
IDU	2.38 (1.21–4.70)	.012	2.12 (1.07–4.21)	.032

NOTE. Age, per 10 years older; nadir and current CD4$^+$ cell count, per 50% lower; time from nadir and with CD4$^+$ cell count less than threshold, per 12 months. ART, antiretroviral therapy; CART, combination antiretroviral therapy; CI, confidence interval; IDU, injection drug user.

ART (IRR, 0.61 [95% CI, 0.42–0.88]; $P = .0092$) had a significantly reduced incidence of PCP or EC. The incidence of PCP or EC was 1.51 times higher in IDUs than in non-IDUs (95% CI, 1.04–2.21; $P = .031$). Further analysis showed that the increased incidence of PCP or EC in IDUs was mainly due to an increased incidence of EC rather than of PCP (data not shown).

In the multivariate model for group 3, the only predictors for the development of TB were starting CART and HIV-1 risk group; patients starting CART (IRR, 0.49 [95% CI, 0.25–0.96]; $P = .037$) had a significantly reduced incidence of TB, and IDUs had a significantly higher incidence of TB, compared with non-IDUs (IRR, 2.12 [95% CI, 1.07–4.21]; $P = .032$).

For the subset of patients with HIV-1 RNA levels available, subanalyses were performed to investigate whether the incidence of OIs was related to current HIV-1 RNA level after adjustment for the same variables presented in table 3. In all groups, a 1-log higher current HIV-1 RNA load was associated with an increased incidence of disease (for group 1, IRR, 1.50 [95% CI, 1.15–1.95]; $P = .0025$; for group 2, IRR, 1.68 [95% CI, 1.40–2.02]; $P < .0001$; for group 3, IRR, 1.89 [95% CI, 1.40–2.54]; $P < .0001$). After adjustment for current HIV-1 RNA level, there was no longer a reduced incidence of any of the OIs associated with starting CART in groups 1–3 (for group 1, IRR, 1.27 [95% CI, 0.59–1.73]; $P = .54$; for group 2, IRR, 1.25 [95% CI, 0.75–2.08]; $P = .38$; for group 3, IRR, 1.04 [95% CI, 0.46–2.35]; $P = .93$). Additional adjustment for weight, in a further reduced subset of patients, showed no relationship between current weight and the development of OIs in groups 1 and 2. In group 3, after adjustment for the variables shown in table 3 and current HIV-1 RNA load, a 5-kg weight loss was associated with a 20% increased incidence of TB (IRR, 1.20 [95% CI, 1.01–1.43]; $P = .044$).

DISCUSSION

The results of the present study show that current CD4$^+$ cell count was the strongest predictor of the development of OIs at higher-than-expected CD4$^+$ cell counts in patients with moderately to severely impaired immune function, as measured by

$CD4^+$ cell count (groups 1 and 2). Furthermore, we found a strong inverse correlation between the use of CART and the development of OIs at higher-than-expected $CD4^+$ cell counts that was independent of current $CD4^+$ cell count. In group 3, patients infected via injection drug use had an increased risk of TB. Neither nadir nor baseline $CD4^+$ cell counts nor the duration of severe immunodeficiency predicted the development of OIs in multivariate analyses.

To the best of our knowledge, this is the first study of the risk of OIs at higher-than-expected $CD4^+$ cell counts, and the analyses suggest that the risk of OI is a continuum of progressively lower risk the higher the $CD4^+$ cell count gets—similar to the situation in patients with $CD4^+$ cell counts within the expected range (figure 1) [10–12]. Although this continuum exists for all OIs, the differences in risk according to latest $CD4^+$ cell count was less pronounced for TB than for the other OIs studied. The explanation for this difference could be that contraction of TB is more dependent on exposure group than on immunological status in patients who do not have severely impaired immune function. This is consistent with previous findings that IDUs develop TB more frequently than do persons in other exposure groups [13, 14].

The $CD4^+$ cell count thresholds chosen for groups 1–3 were all above the 75th percentile for development of the different OIs as identified in a study by Mocroft et al. [1]. OIs were grouped according to the range of $CD4^+$ cell count at which the risk of occurrence is highest and the initiation of the disease-specific prophylaxis is recommended at the same $CD4^+$ cell count level within each of the groups (except for TB, for which guidelines do not suggest a certain $CD4^+$ cell count threshold) [4].

Our results show a beneficial effect of CART on the incidence of OIs at higher-than-expected $CD4^+$ cell counts after controlling for the beneficial effect of CART on the number of $CD4^+$ cells. These results are consistent with those of other studies, which showed that the beneficial effects of CART on reducing mortality and morbidity among HIV-1–infected patients cannot be fully explained by how this medication affects the number of $CD4^+$ cells [2, 15–17]. The fact that some patients developed OIs after starting CART suggests that, despite the restoration of $CD4^+$ cell counts, there remains a deficit in immune function that cannot be captured by the level of $CD4^+$ cells alone. However, the positive effect of CART was eliminated after adjustment for HIV-1 RNA level, which suggests that the decreased incidence of OIs after the initiation of CART can be further explained by decreasing HIV-1 RNA load.

One of the study limitations, however, was that a limited number of patients received CART, and there was not sufficient power to repeat the analyses only for patients who started CART. There was no correlation between the calendar date of diagnosis of an OI in any group and the $CD4^+$ cell count at diagnosis (data not shown), which suggests that the OIs diagnosed in each group after the widespread introduction of CART were not diagnosed at different levels of immunodeficiency. Longer prospective follow-up and a larger sample size are required to compare the risk of disease progression in treated and untreated patients at the same levels of $CD4^+$ cells and with suppressed or unsuppressed HIV-1 RNA. The limited power and difficulties in diagnosis [18] also meant that it was not possible to evaluate the number of events in which immune reconstitution syndrome played a significant role in patients starting CART. Another limitation is that included centers are generally centers of excellence, and the cohort might not be representative of all HIV-1–infected adults in Europe, especially in Eastern Europe. Patients enrolled in the cohort are assumed to be more compliant to therapy than the general population. Thus, it is likely that we are underestimating the incidence of OIs at higher than expected $CD4^+$ cell counts, particularly that of TB in IDUs. Furthermore, we have no data on current drug use, which may be an important surrogate for a lifestyle in which the risk of contracting TB is high.

Previously, a EuroSIDA study [19] and a study by Law et al. [5] reported a clear trend of increasing $CD4^+$ cell counts at the time of AIDS diagnosis over time from before to after the initiation of CART, although these analyses did not suggest that the increase was due to an increasing risk of a particular OI for a given $CD4^+$ cell count [19]. However, few studies have investigated factors associated with the development of OIs at higher-than-expected $CD4^+$ cell counts. In a EuroSIDA study by Miller et al. [20], there was an association between disease progression (to AIDS or death) and nadir of the $CD4^+$ cell count among patients with baseline $CD4^+$ cell counts of $\geqslant$200 cells/μL. In that analysis, a time-updated (current) $CD4^+$ cell count was not included. In contrast, our analysis did not demonstrate an association between the degree of previous immunosuppression and the risk of developing an OI.

In a nested case-control study, Hennessey et al. [21] investigated the development of AIDS at $CD4^+$ cell counts of >300 cells/μL. That study showed that, during the pre-CART era, the HIV-1 RNA level prior to a diagnosis of AIDS was significantly associated with a faster rate of disease progression, whereas no association was observed for immunological factors. The sample size in the study, however, was small (33 cases of AIDS, 52% of which were defined by the presence of Kaposi sarcoma). In our study, a clinically more-relevant analytic approach was used in a larger sample (232 OIs), with considerably longer prospective follow-up.

In summary, the present results provide important clinical information about the risk of OIs at higher-than-expected $CD4^+$ cell counts. The incidence of OIs, particularly in groups 1 and 2, continued to decrease the further the $CD4^+$ cell count increased from the threshold value. Thus, although OIs in groups 1 and 2 occurred infrequently at higher-than-expected $CD4^+$

cell counts, the incidence was considerably smaller in patients whose current $CD4^+$ cell count was twice that of the threshold level. In group 3, our results suggest that TB may be present in a person with a history of injection drug use and with clinical symptoms suggestive of TB.

Our data may be used as an argument in favor of the initiation and/or optimization of CART at higher $CD4^+$ cell counts. However, it needs to be recognized that the decision of when to start CART should be based on a complex set of arguments, including potential benefits versus potential harm for earlier rather than deferred initiation. Further investigation and a better understanding of the factors associated with disease progression in patients with acceptable immune function will improve the management of HIV-1–infected patients with respect to the early recognition of OIs, the possible use of OI prophylaxis, and the use of CART, all of which may help to further reduce morbidity and mortality caused by chronic HIV-1 infection.

EUROSIDA STUDY GROUP MEMBERS

Members of the multicenter EuroSIDA study group are as follows (names of national coordinators are in italics): Argentina: *M. Losso* and A. Duran (Hospital J. M. Ramos Meji'a, Buenos Aires); Austria: *N. Vetter* (Pulmologisches Zentrum der Stadt Wien, Vienna); Belarus: *I. Karpov* and A. Vassilenko (Belarus State Medical University, Minsk); Belgium: *N. Clumeck,* S. De Wit, and B. Poll (Saint-Pierre Hospital, Brussels) and R. Colebunders (Institute of Tropical Medicine, Antwerp); Czech Republic: *L. Machala* and H. Rozsypal (Faculty Hospital Bulovka, Prague) and D. Sedlacek (Charles University Hospital, Plzen); Denmark: *J. Nielsen,* J. Lundgren, T. Benfield, and O. Kirk (Hvidovre Hospital, Copenhagen), J. Gerstoft, T. Katzenstein, A.-B. E. Hansen, and P. Skinhøj (Rigshospitalet, Copenhagen), and C. Pedersen (Odense University Hospital, Odense); Estonia: *K. Zilmer* (Tallinn Merimetsa Hospital, Tallinn); France: *C. Katlama* (Hôpital de la Pitié-Salpétière, Paris), J.-P. Viard (Hôpital Necker–Enfants Malades, Paris), P.-M. Girard (Hôpital Saint Antoine, Paris), T. Saint-Marc (Hôpital Edouard Herriot, Lyon), P. Vanhems (University Claude Bernard, Lyon), C. Pradier (Hôpital de l'Archet, Nice), and F. Dabis (Université Victor Segalen Bordeaux); Germany: M. Dietrich and C. Manegold (Bernhard-Nocht-Institut for Tropical Medicine, Hamburg), J. van Lunzen and H.-J. Stellbrink (Eppendorf Medizinische Kernklinik, Hamburg), S. Staszewski and M. Bickel (J. W. Goethe University Hospital, Frankfurt), F.-D. Goebel (Medizinische Poliklinik, Munich), G. Fätkenheuer (Universität Köln, Cologne), *J. Rockstroh* (Universitäts Klinik Bonn, Bonn), and R. Schmidt (Mediziniche Hochschule, Hannover); Greece: *J. Kosmidis,* P. Gargalianos, G. Xylomenos, and J. Perdios (Athens General Hospital, Athens) and G. Panos, I. Karydis, A. Filandras, and E. Karabatsaki (1st IKA Hospital, Athens); Hungary: *D. Banhegyi* (Szent Lásló Hospital, Budapest); Ireland: *F. Mulcahy* (St. James's Hospital, Dublin); Israel: *I. Yust,* M. Burke, and D. Turner (Ichilov Hospital, Tel Aviv), S. Pollack and G. Hassoun (Rambam Medical Center, Haifa), Z. Sthoeger (Ben Ari Institute of Clinical Immunology, Rehovot), and S. Maayan (Hadassah Hospital, Jerusalem); Italy: *A. Chiesi,* and M. Lichtner (Istituto Superiore di Sanità, Rome), G. Antonucci and F. Iacomi (Istituto Nazionale Malattie Infettive, Rome), R. Esposito, R. Borghi, and A. Bedini (Università di Modena, Modena), C. Arici (Ospedale Riuniti, Bergamo), R. Pristerá (Ospedale Generale Regionale, Bolzano), F. Mazzotta and A. Gabbuti (Ospedale S. Maria Annunziata, Florence), A. Chirianni and E. Montesarchio (Presidio Ospedaliero A.D. Cotugno, Naples), V. Vullo (Università di Roma La Sapienza, Rome), P. Narciso, M. Zaccarelli, and P. Franci (Ospedale Spallanzani, Rome), A. Lazzarin and R. Finazzi (Ospedale Spallanzani, Rome), and A. D'Arminio Monforte (Ospedale L. Sacco, Milan); Latvia: *L. Viksna* (Infectology Centre of Latvia, Riga); Lithuania: *S. Chaplinskas* (Lithuanian AIDS Centre, Vilnius); Luxembourg: *R. Hemmer* and T. Staub (Centre Hospitalier, Luxembourg); The Netherlands: *P. Reiss* (Academisch Medisch Centrum bij de Universiteit van Amsterdam, Amsterdam); Norway: *J. Bruun,* A. Maeland, and V. Ormaasen (Ulleval Hospital, Oslo); Poland: *B. Knysz* and J. Gasiorowski (Medical University, Wroclaw), A. Horban (Centrum Diagnostyki i Terapii AIDS, Warsaw), D. Prokopowicz and A. Wiercinska-Drapalo (Medical University, Bialystok), A. Boron-Kaczmarska and M. Pynka (Medical University, Szczecin), M. Beniowski and E. Mularska (Osrodek Diagnostyki i Terapii AIDS, Chorzow), and H. Trocha (Medical University, Gdansk); Portugal: *F. Antunes* and E. Valadas (Hospital Santa Maria, Lisbon), K. Mansinho (Hospital de Egas Moniz, Lisbon), and F. Maltez (Hospital Curry Cabra); Romania: *D. Duiculescu* (Spitalul de Boli Infectioase si Tropicale Dr. Victor Babes, Bucuresti), A. Streinu-Cercel (Institutul de Boli Infectioase Prof. Dr. Matei Bal, Bucuresti); Russia: *A. Rakhmanova* (Botkin Hospital of Infectious Diseases, St. Petersburg) and E. Vinogradova (St. Petersburg AIDS Centre); Serbia and Montenegro: *J. Jevtovic* (The Institute for Infectious and Tropical Diseases, Belgrade); Slovakia: *M. Mokrás* and D. Staneková (Derrer Hospital, Bratislava); Spain: *J. González-Lahoz,* M. Sánchez-Conde, T. García-Benayas, L. Martin-Carbonero, and V. Soriano (Hospital Carlos III, Madrid), B. Clotet, A. Jou, J. Conejero, and C. Tural (Hospital Germans Trias i Pujol, Badalona), J. M. Gatell and J. M. Miró (Hospital Clinic i Provincial, Barcelona), P. Domingo, M. Gutierrez, G. Mateo, and M. Antonia Sambeat (Hospital de la Santa Creu i Sant Pau, Barcelona); Sweden: *A. Blaxhult* (Karolinska Hospital, Stockholm), A. Karlsson (Sødersjukhuset, Stockholm), and P. Pehrson (Huddinge Sjukhus, Stockholm); Switzerland: *B. Ledergerber* and R. Weber (University Hospital, Zürich), P. Francioli and A. Telenti (Centre Hospitalier Universitaire Vaudois, Lausanne), B. Hirschel and V. Soravia-Dunand

(Hospital Cantonal Universitaire de Geneve, Geneve), and H. Furrer (Inselspital Bern, Bern); Ukraine: *N. Chentsova* and E. Kravchenko (Kyiv Centre for AIDS, Kyiv); and United Kingdom: *S. Barton* (St. Stephen's Clinic, Chelsea and Westminster Hospital, London), A. M. Johnson and D. Mercey (University College Campus, Royal Free and University College London Medical School, London), A. Phillips, M. A. Johnson, and A. Mocroft (Royal Free Campus, Royal Free and University College Medical School, London), M. Murphy (Medical College of Saint Bartholomew's Hospital, London), J. Weber and G. Scullard (Imperial College School of Medicine at St. Mary's, London), M. Fisher (Royal Sussex County Hospital, Brighton), and R. Brettle (Western General Hospital, Edinburgh).

Virology group: B. Clotet and L. Ruiz (Central Virology Coordinators), plus ad hoc virologists from participating sites in the EuroSIDA Study.

Steering Committee: F. Antunes, A. Blaxhult, N. Clumeck, J. Gatell, A. Horban, A. Johnson, C. Katlama, B. Ledergerber (chair), C. Loveday, A. Phillips, P. Reiss, and S. Vella.

Coordinating center staff: J. Lundgren (project leader), I. Gjørup, O. Kirk, N. Friis-Møller, A. Mocroft, A. Cozzi-Lepri, W. Bannister, M. Ellefson, A. Borch, D. Podlekareva, C. Holkmann Olsen, and J. Kjær.

References

1. Mocroft A, Youle M, Phillips AN, et al. The incidence of AIDS-defining illnesses in 4883 patients with human immunodeficiency virus infection. Royal Free/Chelsea and Westminster Hospitals Collaborative Group. Arch Intern Med **1998**; 158:491–7.
2. Mocroft A, Ledergerber B, Katlama C, et al. Decline in the AIDS and death rates in the EuroSIDA study: an observational study. Lancet **2003**; 362:22–9.
3. Hanson DL, Chu SY, Farizo KM, Ward JW. Distribution of CD4+ T lymphocytes at diagnosis of acquired immunodeficiency syndrome-defining and other human immunodeficiency virus-related illnesses. The Adult and Adolescent Spectrum of HIV Disease Project Group. Arch Intern Med **1995**; 155:1537–42.
4. Centers for Disease Control and Prevention. Guidelines for preventing opportunistic infections among HIV-infected persons—2002 recommendations of the US Public Health Service and the Infectious Diseases Society of America. MMWR Morb Mortal Wkly Rep **2002**; 51:1–46.
5. Law MG, de Winter L, McDonald A, Cooper DA, Kaldor JM. AIDS diagnoses at higher CD4 counts in Australia following the introduction of highly active antiretroviral treatment. AIDS **1999**; 13:263–9.
6. Jacobson MA, Zegans M, Pavan PR, et al. Cytomegalovirus retinitis after initiation of highly active antiretroviral therapy. Lancet **1997**; 349: 1443–5.
7. Bartlett JG, Gallant J, eds. 2004 medical management of HIV infection. Baltimore, MD: John Hopkins Medicine Health Publishing Business Group, **2004**:333–5.
8. Phair J, Munoz A, Detels R, Kaslow R, Rinaldo C, Saah A. The risk of *Pneumocystis carinii* pneumonia among men infected with human immunodeficiency virus type 1. Multicenter AIDS Cohort Study Group. N Engl J Med **1990**; 322:161–5.
9. 1993 revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. MMWR Recomm Rep **1992**; 41:1–19.
10. Moore RD, Chaisson RE. Natural history of opportunistic disease in an HIV-infected urban clinical cohort. Ann Intern Med **1996**; 124:633–42.
11. Thiebaut R, Chene G, Jacqmin-Gadda H, et al. Time-updated CD4+ T lymphocyte count and HIV RNA as major markers of disease progression in naive HIV-1-infected patients treated with a highly active antiretroviral therapy: the Aquitaine cohort, 1996–2001. J Acquir Immune Defic Syndr **2003**; 33:380–6.
12. Lundgren JD, Mocroft A, Gatell JM, et al. A clinically prognostic scoring system for patients receiving highly active antiretroviral therapy: results from the EuroSIDA study. J Infect Dis **2002**; 185:178–87.
13. Kirk O, Gatell JM, Mocroft A, et al. Infections with *Mycobacterium tuberculosis* and *Mycobacterium avium* among HIV-infected patients after the introduction of highly active antiretroviral therapy. EuroSIDA Study Group JD. Am J Respir Crit Care Med **2000**; 162:865–72.
14. The Antiretroviral Therapy Cohort Collaboration. Incidence of tuberculosis among HIV-infected patients receiving highly active antiretroviral therapy in Europe and North America. Clin Infect Dis **2005**; 41:1772–82.
15. Egger M, Hirschel B, Francioli P, et al. Impact of new antiretroviral combination therapies in HIV infected patients in Switzerland: prospective multicentre study. Swiss HIV Cohort Study. BMJ **1997**; 315: 1194–9.
16. Egger M, May M, Chene G, et al. Prognosis of HIV-1-infected patients starting highly active antiretroviral therapy: a collaborative analysis of prospective studies. Lancet **2002**; 360:119–29.
17. Lawrence J, Mayers DL, Hullsiek KH, et al. Structured treatment interruption in patients with multidrug-resistant human immunodeficiency virus. N Engl J Med **2003**; 349:837–46.
18. Lipman M, Breen R. Immune reconstitution inflammatory syndrome in HIV. Curr Opin Infect Dis **2006**; 19:20–5.
19. Mocroft A, Katlama C, Johnson AM, et al. AIDS across Europe, 1994–98: the EuroSIDA study. Lancet **2000**; 356:291–6.
20. Miller V, Mocroft A, Reiss P, et al. Relations among CD4 lymphocyte count nadir, antiretroviral therapy, and HIV-1 disease progression: results from the EuroSIDA study. Ann Intern Med **1999**; 130:570–7.
21. Hennessey KA, Giorgi JV, Kaplan AH, et al. AIDS onset at high CD4+ cell levels is associated with high HIV load. AIDS Res Hum Retroviruses **2000**; 16:103–7.