HIV-1 Dual Infection Is Associated With Faster $CD4^+$ T-Cell Decline in a Cohort of Men With Primary HIV Infection

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Background. In vitro, animal, and mathematical models suggest that human immunodeficiency virus (HIV) co- or superinfection would result in increased fitness of the pathogen and, possibly, increased virulence. However, in patients, the impact of dual HIV type 1 (HIV-1) infection on disease progression is unclear, because parameters relevant for disease progression have not been strictly analyzed. The objective of the present study is to analyze the effect of dual HIV-1 infections on disease progression in a well-defined cohort of men who have sex with men.

Methods. Between 2000 and 2009, 37 men who had primary infection with HIV-1 subtype B, no indication for immediate need of combination antiretroviral therapy (cART), and sufficient follow-up were characterized with regard to dual infection or single infection and to coreceptor use. Patients were followed to estimate the effect of these parameters on clinical disease progression, as defined by the rate of $CD4^+$ T-cell decline and the time to initiation of cART.

Results. Four patients presented with HIV-1 coinfection; 6 patients acquired HIV-1 superinfection, on average 8.5 months from their primary infection; and 27 patients remained infected with a single strain. Slopes of longitudinal $CD4^+$ T-cell counts and time-weighted changes from baseline were significantly steeper for patients with dual infection compared with patients with single infection. Multivariate analysis showed that the most important parameter associated with $CD4^+$ T-cell decline over time was dual infection ($P = .001$). Additionally, patients with HIV-1 coinfection had a significantly earlier start of cART ($P < .0001$).

Conclusions. Dual HIV-1 infection is the main factor associated with $CD4^+$ T-cell decline in men who have untreated primary infection with HIV-1 subtype B.

Human immunodeficiency virus type 1 (HIV-1) coinfections (ie, infection with a second virus strain before seroconversion) and superinfections (ie, infection with a second virus strain after seroconversion) are reported with increasing frequency. Superinfections were first reported in 2002 [1–3], and a serial superinfection case was published in 2005 [4]. In many cases, infection with >1 virus strains was associated with disease progression. However, HIV-1 superinfections have also been reported in long-term nonprogressors [5–7], making it unclear whether superinfection is harmful to the individual. The clinical effects of HIV-1 coinfections are also largely unknown.

Infection of cats with feline immunodeficiency virus suggest that both co- and superinfection lead to the rapid outgrowth of viruses with increased replication capacity and virulence [8], as is also suggested by a general mathematical model describing the evolution of parasitic virulence [9]. Another mathematical model suggested that superinfection with a more virulent HIV-1 strain leads to faster disease progression [10]. In vitro superinfection of T-cell lines with different HIV-1 strains resulted in an increase in virus production and cell mortality [11]. Analysis of 2 HIV-superinfected patients showed that

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the second strain had greater replicative fitness [12], which could lead to greater virulence. Furthermore, superinfection could compromise the patient's immune system by potentiating immune escape [3]. Immune escape, as well as increased replicative fitness or drug resistance, could rapidly be acquired by the virus through recombination between the 2 strains after dual infection. Recombination events expand the evolutionary potential of HIV-1 and pose one of the dangers of dual infection, both for the individual patient as well as for the epidemic [13].

The reported clinical effects of dual HIV infections are largely anecdotal. In fact, many HIV-1 superinfections were detected retrospectively because of unfavorable changes in clinical parameters. Disease outcome was studied in dual HIV-1–infected patients from 3 cohorts, in whom dual infection was associated with rapid disease progression in 5 individuals [14]. However, these patients were infected with HIV-1 well before 2000 and were not matched for sex, HIV subtype, HLA type, or CCR5 genotype, while the seroconversion date and date of superinfection were approximated. Studies have shown that recent HIV-1 strains are increasing in replicative fitness [15] and in virulence by adapting to protective HLA-I types [16, 17] and by escaping from antibody neutralization [18].

To study the effect of dual HIV infections in recent years and to control for other variables relevant to disease progression, we assessed disease progression in a prospective study involving 37 well-characterized patients with primary HIV infection (PHI).

MATERIALS AND METHODS

Patients

Thirty-seven patients with PHI who were seen at the Academic Medical Center in Amsterdam, the Netherlands, between 2000 and 2009 were selected from the Primo-SHM cohort, a multicenter prospective cohort study in the Netherlands with an embedded randomized trial that investigates the natural course of HIV-1 infection and the effects of early combination antiretroviral therapy (cART) in patients with PHI [19]. PHI was defined as negative or indeterminate results of a Western blot combined with either positive results of a test for p24 antigen or a detectable HIV-1 RNA concentration, or as negative results of an HIV screening test $\leq$6 months before seroconversion. At their first visit to the Academic Medical Center, eligible patients were invited to join the Primo-SHM cohort and, if included, were randomized with regard to the study arm (ie, to receive early treatment or not). Inclusion criteria for the present analysis were laboratory evidence of PHI with subtype B (as determined by genotyping of the *pol* gene), male sex, no immediate indication for cART, and at least 2 years of follow-up, unless 2 $CD4^+$ T-cell counts of $\leq 350 \times 10^6$ cells/mL were reached and/or cART was started before that time. Eight enrolled patients, who were later all determined to be and remain infected with a single strain, presented with $CD4^+$ T-cell counts of $<350 \times 10^6$ cells/mL, but low $CD4^+$ T-cell counts were not an exclusion criterion. In most cases, $CD4^+$ T-cell counts increased spontaneously after the first measurement. Clinical parameters, including treatment, $CD4^+$ T-cell count, and plasma HIV-1 load, were collected at enrollment and during follow-up visits every 3 months. Follow-up was terminated at the start of cART or at the end of the study (1 September 2010). The research protocol was approved by the medical ethical committee of the Academic Medical Center, and all participants gave written informed consent.

Assays

Plasma viral load measurements were performed using the Versant HIV-1 RNA 3.0 assay (Bayer Diagnostics Division) from January 2000 to February 2007; from February 2007 onward, the Abbott RealTime HIV-1 assay (Abbott Molecular) was used. The lower limits of detection of these assays were 50 and 40 HIV-1 copies/mL, respectively. Sequences of genes encoding HIV-1 protease/reverse transcriptase were generated using the ViroSeq HIV-1 genotyping kit, version 2 (Celera Diagnostics). HLA typing was performed and CCR5 haplotypes were analyzed for the presence of the CCR5-Δ32 deletion allele as described elsewhere [20].

Polymerase Chain Reaction Amplification and Sequence Analysis

RNA was isolated from 200 μL blood plasma, using silica and guanidine thiocyanate [21]. One-fifth of the isolate was then used to reverse-transcribe, amplify, clone, and sequence the *env* V3-V4 fragment (nucleotide 6949-7519 of the HXB2 reference sequence [GenBank accession no. K03455]) [22]. At least 16 clones were analyzed per sample.

Phylogenetic Analysis

Sequences were aligned with the ClustalW sequence alignment tool implemented in BioEdit Sequence Alignment Editor, version 7.0.9 [23]. Alignments were manually adjusted to preserve in-frame insertions and deletions. Phylogenetic analyses were performed with the MEGA 5 software package [24] and with the parallel version of MrBayes 3.1 [25], as described elsewhere [22].

Definition of HIV Co- or Superinfection

HIV-1 coinfection was defined as having 2 separate *env* sequence clusters in a phylogenetic tree in the first sample obtained during PHI. HIV-1 superinfection was defined as infection with a second HIV-1 strain that clustered separately in a phylogenetic tree at a time point after PHI. For all patients, a second sample from the end of the follow-up period was analyzed to investigate whether an HIV-1 superinfection had occurred after the PHI period. In case superinfection was identified in the second (ie, last) sample, all earlier samples from that patient were amplified and sequenced to accurately determine the moment of superinfection. Although

it is possible that a superinfection is missed when analyzing only 1 follow-up sample and when only *env* region sequences are cloned [26], we identified 90% of dual infections correctly with this strategy.

Coreceptor Usage

HIV-1 *env* clones were genotyped for CCR5/CXCR4 coreceptor tropism with the Geno2pheno algorithm [27].

Statistical Analysis

Variables were compared between the group with single infection and the group with dual infection, and for some analyses, the group with dual infection was separated into co- and superinfected subgroups. Mann–Whitney test was used to compare continuous variables, and proportions were compared using the Fischer exact test. Clinical progression was defined as the time between PHI and 1 of the 2 end points of the study: a $CD4^+$ T-cell count of <350 cells/mL on 2 consecutive occasions or initiation of cART. Clinical progression was compared across the groups, using Kaplan–Meier plots and log-rank tests.

Longitudinal slopes of $CD4^+$ T-cell count in individual patients were calculated by linear regression, and time-weighted changes from baseline were calculated by linear trapezoidal integration. Parameters associated with the slope of the $CD4^+$ T-cell count in univariate analyses with *P* values of <.1 were included in multivariate analyses (general linear model; full factorial). Linear mixed models with patient as a random factor and time from baseline as a fixed covariate were used to compare the changes from baseline of the $CD4^+$ T-cell count between the patient groups and to estimate the longitudinal slopes of $CD4^+$ T-cell count in the patient groups. For plasma viral load, $\log_{10}$-transformed values were used. Linear mixed models and general linear models were performed using PASW Statistics 18 [28], and all other statistical tests were performed using GraphPad Prism 5.01 [29]. All statistical tests were 2-sided. *P* values of <.05 were considered statistically significant.

RESULTS

Patient Characteristics

Patient characteristics are summarized in Table 1. All 37 patients were men who have sex with men (MSM), and 36 (97%) were white. Twenty-four (65%) reported symptoms compatible with

Table 1. Patient Characteristics at Study Enrollment

Characteristic	Overall (n = 37)	Single Infection (n = 27)	Dual Infection (n = 10)	*P*[a]
Age, years, median (IQR)	37.0 (29.0–42.0)	36.0 (29.0–42.0)	39.0 (24.8–41.3)	.86
Acute retroviral syndrome				
Yes	64.9 (24)	66.7 (18)	60.0 (6)	.72
No	35.1 (13)	33.3 (9)	40.0 (4)	
Plasma viral load, $\log_{10}$ copies/mL, median (IQR)	5.24 (2.25–7.15)	5.51 (3.34–7.15)	4.52 (2.25–5.89)[b]	.05
CCR5-Δ32 genotype				
Homozygous wild-type	91.9 (34)	88.9 (24)	100.0 (10)	.55
Heterozygous CCR5-Δ32	8.1 (3)	11.1 (3)	0	
HLA-A haplotype[c]				
Homozygous	73.3 (22)	78.3 (18)	57.1 (4)	.34
Heterozygous	26.7 (8)	21.7 (5)	42.9 (3)	
HLA-B haplotype[d]				
Alleles associated with slow progression[e]	27.6 (8)	22.7 (5)	42.9 (3)	.36
Alleles associated with rapid progression[e]	34.5 (10)	31.8 (7)	42.9 (3)	.66
HLA-C haplotype[c]				
Homozygous	80.0 (24)	78.3 (18)	85.7 (6)	1.00
Heterozygous	20.0 (6)	21.7 (5)	14.3 (1)	

Data are % (No.) of patients, unless otherwise indicated.

Abbreviation: IQR, interquartile range.

[a] Mann–Whitney tests were used for continuous variables, and Fischer exact tests were used for proportions.

[b] The difference in plasma viral load seen between patients with single infection and those with dual infection (*P* = .05) at enrollment could be attributed to a difference between patients with single infection and those with future superinfection (*P* = .03), as no difference between patients with single infection and those with coinfection was observed (*P* = .58).

[c] Data are for 30 patients (23 with single infection and 7 with dual infection).

[d] Data are for 29 patients (22 with single infection and 7 with dual infection).

[e] According to Goulder and Watkins [30], HLA-B alleles associated with slow progression are HLA-B*1302, HLA-B*2705, HLA-B*5101, HLA-B*5701/02/03, HLA-B*5801, and HLA-B*8101; HLA-B alleles associated with rapid progression are HLA-B*1801, HLA-B*3502/03, and HLA-B*5802.

acute retroviral syndrome. Follow-up was, on average, 33 months (range, 9–80 months), and 19 patients (51%) reached a study end point before the intended follow-up of 2 years. Four patients had HIV-1 coinfection, 6 were superinfected with a second HIV-1 strain on average 8.5 months (range, 3.5–15.5 months) after the PHI, and 27 remained infected with a single HIV-1 strain until the end of follow-up. Three patients with dual infection were identified earlier [12, 31, 32]. The remaining 7 patients were discovered during additional analysis of samples with a high degenerate base code count in the genotyping sequence [31] or were found during the present study. Although all PHI occurred with subtype B strains, 1 patient was coinfected with a subtype G strain, while 2 superinfections occurred with CRF01_AE, a third with CRF02_AG, and a fourth with a subtype A strain (Figure 1).

Patient age, the occurrence of acute retroviral syndrome during PHI, HLA-I haplotype, and host CCR5 genotype did not differ significantly between those with single infection and those with dual infection (Table 1). All strains associated with PHI were predicted to use the CCR5 coreceptor; 1 exception involved the first infecting strain in a patient, in whom superinfection later emerged, who presented with an exceptionally low plasma viral load [12]. However, as the Geno2pheno algorithm is known to have only approximately 75% sensitivity for CXCR4-using viruses [34], some might have been missed.

At enrollment, a difference in plasma viral load that was marginally nonsignificant was seen between patients with single infection and those with dual infection ($P = .05$). This could be attributed to a difference between patients with single infection and those with future superinfection ($P = .03$) but not to the difference between patients with single infection and those with coinfection ($P = .58$), although the number of patients was low (Table 1). Patients who were going to

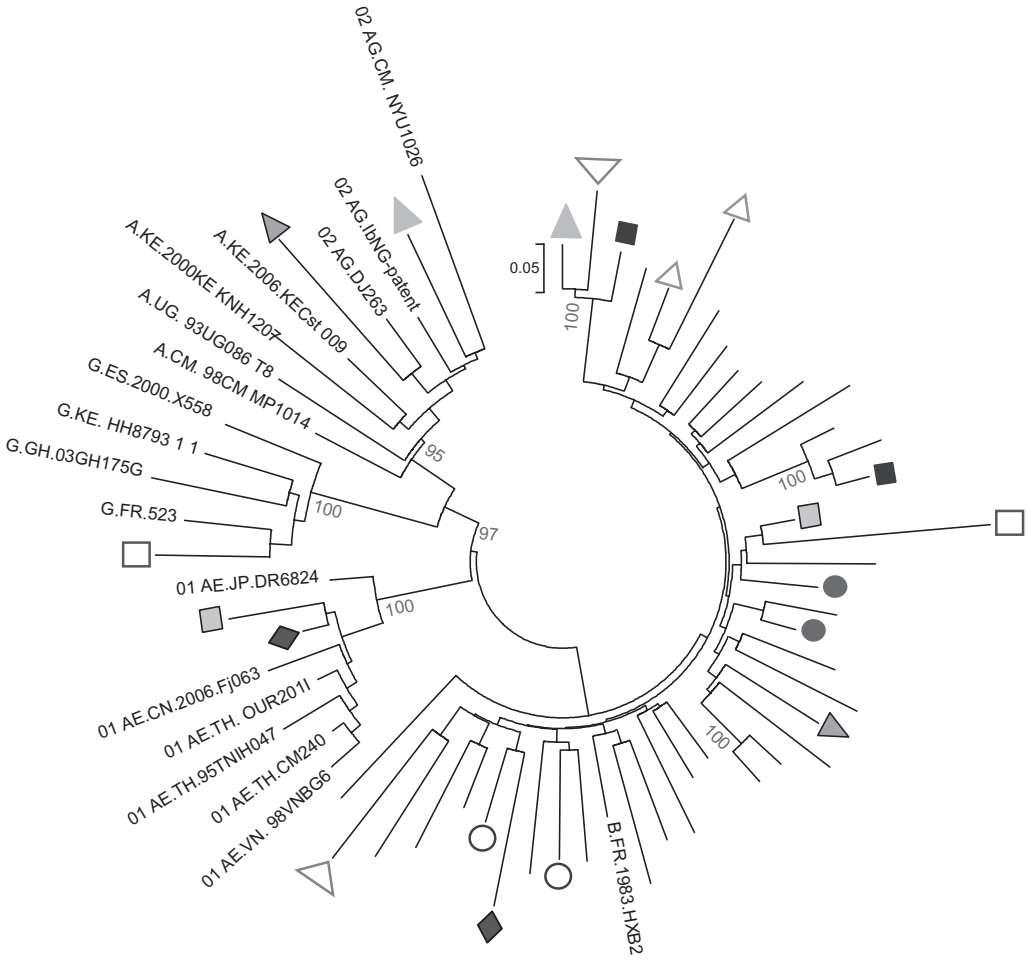

Figure 1. Phylogenetic tree of human immunodeficiency virus type 1 (HIV-1) *env* sequences, generated with the neighbor-joining method implemented in MEGA 5 [24] and bootstrap resampling with 1000 replicates. Shown are *env* sequences from 27 patients infected with a single HIV-1 strain and 2 *env* sequences each from the 10 patients with dual infection (open symbols depict sequences from coinfected patients, and closed symbols depict sequences from superinfected patients). Bootstrap values >85 are indicated. Reference sequences were from the Los Alamos database [33]. HIV-1 *env* sequences have been deposited in the GenBank database with accession numbers JN647642-JN647688.

experience HIV-1 superinfection at a later time point had a lower plasma viral load at baseline. At the study end point, there was no significant difference in plasma viral load between the groups ($P = .51$).

Disease Progression and CD4$^+$ T-Cell Count Dynamics

Time to reach CD4$^+$ T-cell levels of $\leq 350 \times 10^6$ cells/mL was not significantly different between patients with dual infection and those with single infection ($P = .88$) (Figure 2*A*, left panel), possibly because 4 of 10 patients with dual infection were censored, as cART was initiated at higher CD4$^+$ T-cell counts. Splitting patients with dual infection into those with coinfection and those with superinfection did not show differences either (Figure 2*A*, right panel).

Next, we looked at start of cART, which is driven not only by CD4$^+$ T-cell count, but also by clinical symptoms. Of the 37 patients, 31 patients started cART within 2 years. Six started before their CD4$^+$ T-cell counts reached $\leq 350 \times 10^6$ cells/mL: 4 had dual infection, and 2 had single infection ($P = .035$). Seven patients (6 with single infection and 1 with superinfection) started according to treatment guidelines, and 18 patients started treatment at lower CD4$^+$ T-cell counts (average count, 219 cells/mL [range, 100–320 cells/mL]; average treatment delay, 13.5 months [range, 1–33 months]). Of the latter patients, 14 had single infection (mean treatment delay, 15.4 months), and 4 had dual infection (mean treatment delay, 7.0 months), suggesting that dual infections are associated with more constitutional symptoms. However, an AIDS-defining opportunistic illness was not

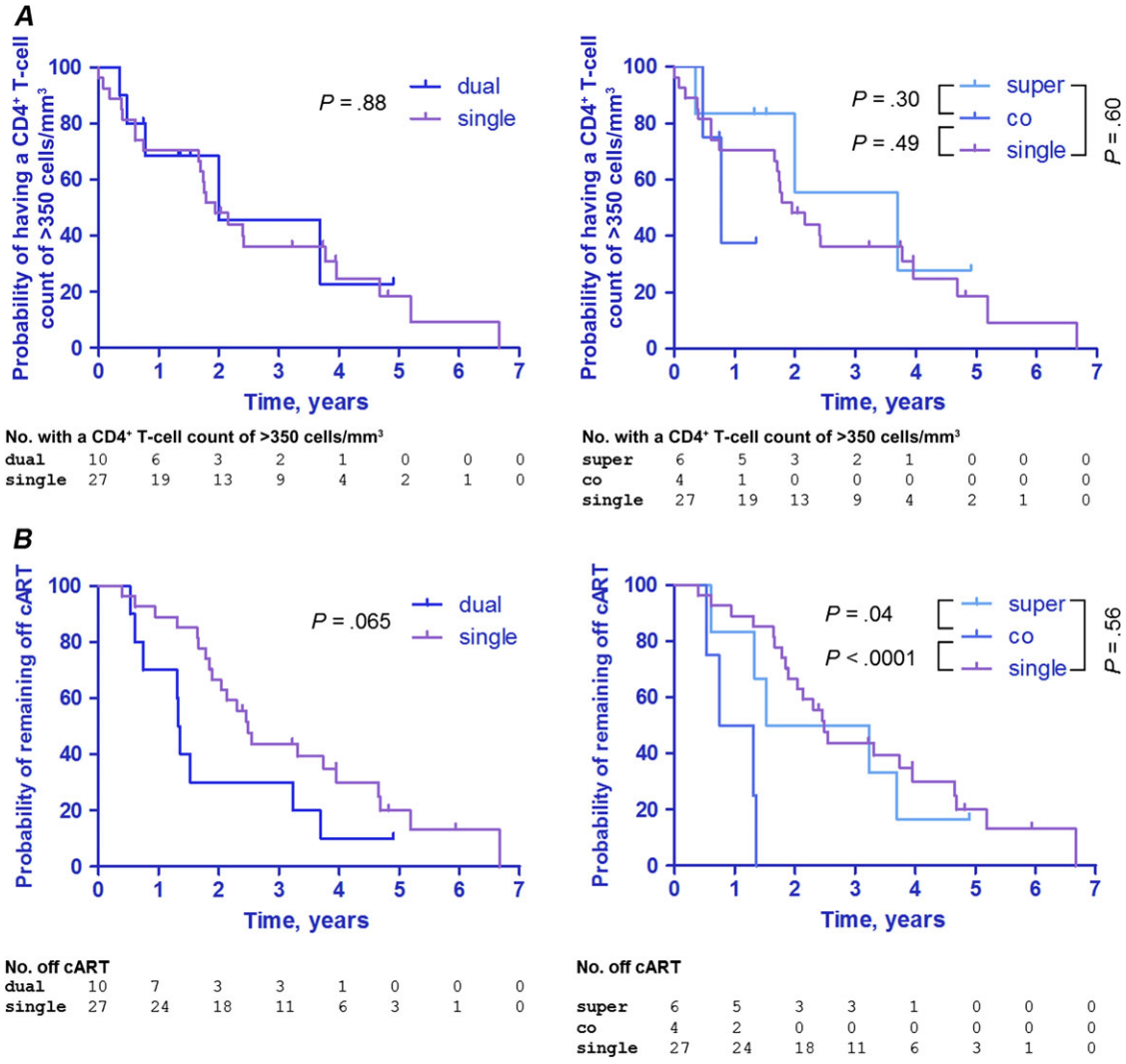

Figure 2. Kaplan–Meier analysis of time to reach a repeated CD4$^+$ T-cell count of <350 cells/mL (*A*) or to start of cART (*B*) according to single or dual infection (left panels) or single, super-, or coinfection (right panels). *P* values were calculated using the log-rank test. Abbreviation: cART, combination antiretroviral therapy.

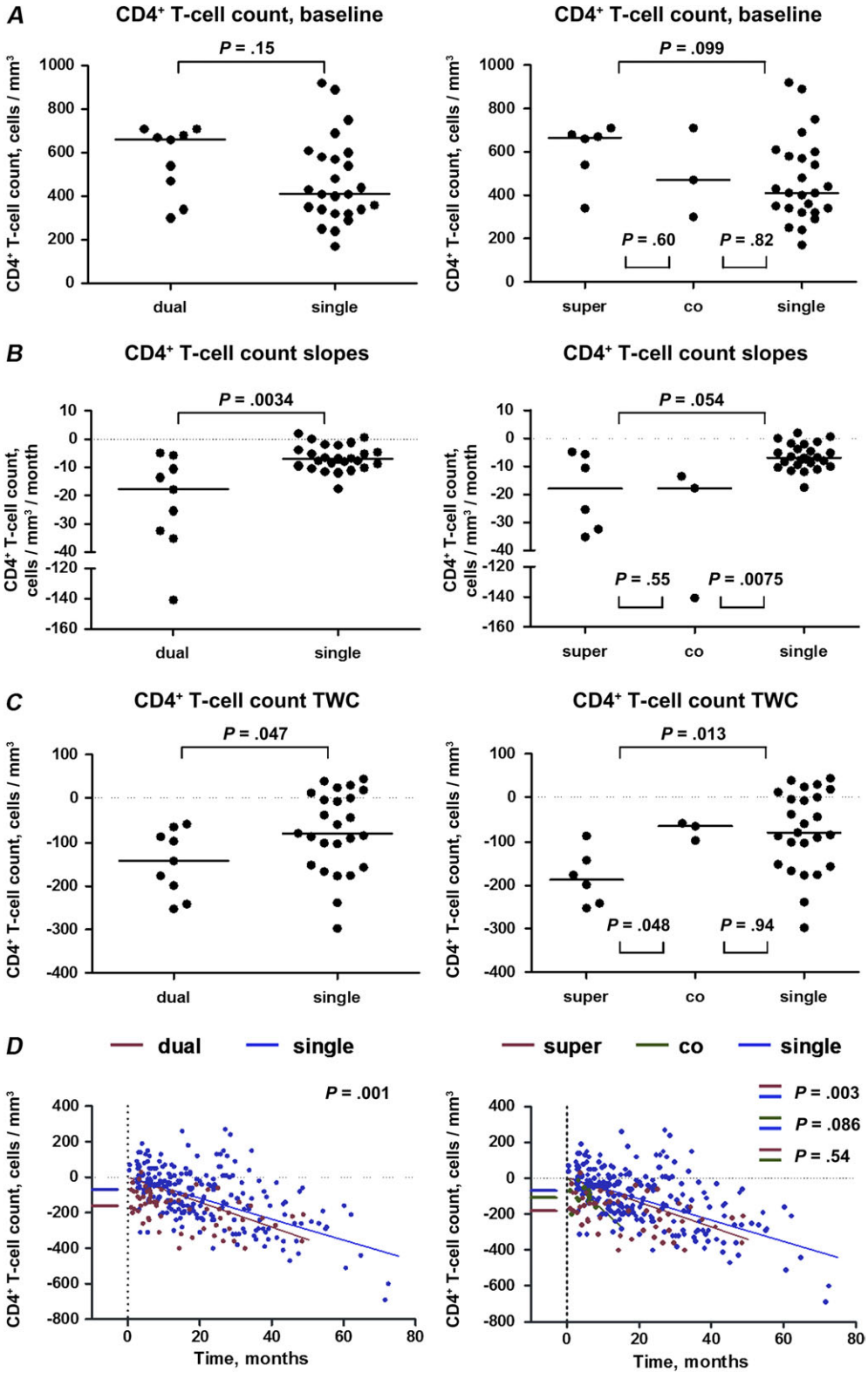

Figure 3. Effects of dual infection on CD4$^+$ T-cell count. *A*, CD4$^+$ T-cell counts at the adjusted baseline. *B*, Linear regression slopes of CD4$^+$ T-cell counts relative to the adjusted baseline. *C*, Time-weighted changes (TWCs) of CD4$^+$ T-cell counts from adjusted baseline. Horizontal lines show the median values; groups were compared using the Mann–Whitney test (*A*–*C*). *D*, Changes in CD4$^+$ T-cell counts relative to adjusted baseline as estimated by linear mixed modeling. Dots on the graphs represent changes in CD4$^+$ T-cell counts from adjusted baseline at all follow-up time points. Colored horizontal lines on the *y*-axes depict the median changes from the adjusted baseline in the corresponding patient groups. *P* values of comparisons of changes from baseline between the groups were calculated by fitting linear mixed models. The diagonal lines show the longitudinal slopes of CD4$^+$ T-cell count, as estimated by the linear mixed modeling.

Table 2. Variables Associated With CD4$^+$ T-Cell Count Slope

Variable	CD4$^+$ T-Cell Count Slope[a]	*P*[b]	*P* (multivariate)[c]
Age in years at infection (n = 34)	−0.16 ± 0.48; 0.00	.74	
Acute retroviral syndrome (n = 34)			
Yes (n = 22)	−8.2 (−11.6 to −3.4)	.94	
No (n = 12)	−7.2 (−12.7 to −5.3)		
Dual infection (n = 34)			
Yes (n = 9)	−17.8 (−33.8 to −8.1)	.003	.001
No (n = 25)	−6.9 (−9.7 to −2.9)		
Plasma viral load, $\log_{10}$ copies/mL, by time			
6 mo after infection (n = 34)	0.70 ± 6.44;0.00	.91	
12 mo after infection (n = 32)	−3.65 ± 1.89;0.11	.06	.59
CCR5-Δ32 genotype (n = 34)			
Homozygous (n = 31)	−8.3 (−11.9 to −5.2)[d]	.045	.48
Heterozygous (n = 3)	−2.1 (−4.6 to −1.8)		
HLA-A haplotype (n = 27)			
Homozygous (n = 7)	−6.9 (−13.5 to −5.2)	.87	
Heterozygous (n = 20)	−8.5 (−11.4 to −2.5)		
HLA-B haplotype, by progression speed			
Alleles associated with slow progression (n = 26)			
Yes (n = 7)	−7.6 (−10.6 to −5.2)	.84	
No (n = 19)	−8.3 (−11.9 to −4.6)		
Alleles associated with rapid progression (n = 26)			
Yes (n = 8)	−11.7 (−23.4 to −7.4)	.02	.006
No (n = 18)	−7.1 (−10.4 to −2.0)		
HLA-C haplotype (n = 27)			
Homozygous (n = 6)	−9.2 (−21.3 to −0.9)	.84	
Heterozygous (n = 21)	−7.6 (−11.3 to −4.7)		

[a] For associations of CD4$^+$ T-cell count slope with continuous variables, linear regression slopes and R^2 values are shown; for associations of CD4$^+$ T-cell count slope with discrete variables, medians and quartiles of CD4$^+$ T-cell count slope in each group are shown.

[b] Determined by linear regression for continuous variables, and by the Mann–Whitney test for discrete variables.

[c] Determined by fitting the general linear model.

[d] Cells/mL per month.

documented for any patient. At the time of cART initiation, the treating physician was unaware of the patient's infection status (ie, single or dual), which could otherwise possibly have influenced the decision to start treatment. Analysis of the time to start of cART revealed a significantly earlier start of therapy between patients with single infection and those with coinfection ($P < .0001$; Figure 2*B*, right panel), but not between patients with single infection and those with either superinfection ($P = .56$) or dual infection ($P = .065$; Figure 2*B*, left panel).

However, time to the achievement of CD4$^+$ T-cell counts of $\leq 350 \times 10^6$ cells/mL and time to initiation of cART in superinfected patients should be interpreted with caution, as superinfected patients were initially infected with a single strain and remained infected with a single strain for a substantial portion of follow-up. To accurately compare disease progression between patients with superinfection and those with single infection, we set an adjusted baseline at 8.5 months after infection (the mean time to superinfection in the superinfected patients) for patients with single infection and those with coinfection. Baseline for superinfected patients was set at their identified moment of superinfection. For 1 patient with coinfection and 2 with single infection, no CD4$^+$ T-cell counts after adjusted baseline could be included, as these patients started cART before that point.

Whereas the CD4$^+$ T-cell counts at adjusted baseline were not significantly different between patients with dual infection and those with single infection ($P = .15$), longitudinal slopes after adjusted baseline and the time-weighted change from the adjusted baseline of CD4$^+$ T-cell counts were significantly different ($P = .003$ and $P = .047$, respectively) (Figure 3*A*–*C*, left panels), indicating that dual infection is associated with faster disease progression. Time-weighted changes in CD4$^+$ T-cell counts were also significantly different between patients with superinfection and those with single infection ($P = .013$), whereas the difference in the slopes of CD4$^+$ T-cell counts between the groups was marginally nonsignificant ($P = .054$) (Figure 3*B* and 3*C*, right panels). The CD4$^+$ T-cell count slopes for coinfected patients were

significantly different from those for patients infected with a single strain ($P = .0075$). It was not possible to calculate $CD4^+$ T-cell count slopes before and after a superinfection event, as superinfections occurred mostly close to the acute phase of infection, with $CD4^+$ T-cell counts not yet having reached set-point values.

Next, we estimated changes in $CD4^+$ T-cell counts relative to adjusted baseline during the follow-up period by fitting the linear mixed models, taking into account correlations of repeated measurements within the individuals (Figure 3*D*). Median changes from adjusted baseline were -180×10^6 cells/mL for patients with superinfection, -110×10^6 cells/mL for patients with coinfection, and -70×10^6 cells/mL for patients with single infection ($P = .001$ for the comparison between patients with dual infection and those with single infection, $P = .003$ for those with superinfection vs those with single infection, and $P = .09$ for those with coinfection vs those with single infection). Linear mixed modeling was also used to estimate the longitudinal slopes of $CD4^+$ T-cell counts in the patient groups. As shown in Figure 3*D*, the slope was steeper for patients with dual infection than for those with single infection, and was especially steep for patients with coinfection. This analysis confirmed the earlier observed association of dual infection with accelerated disease progression.

Finally, we looked at whether the observed effect of dual infection on disease progression could be confounded by other variables. As shown in Table 2, neither age at infection, occurrence of acute retroviral syndrome, nor homozygosity for HLA-A or -C haplotypes was significantly associated with the slope of the $CD4^+$ T-cell count, in contrast to CCR5-Δ32 heterozygosity and presence of an HLA-B allele associated with rapid progression ($P = .045$ and $P = .02$, respectively) [30]. Plasma viral load at month 12 after infection was associated with $CD4^+$ T-cell count slope, although the P value for the association was marginally greater than the threshold for statistical significance ($P = .06$, by linear regression). In a multivariate model, dual infection and HLA-B haplotype remained significantly associated with the $CD4^+$ T-cell count slope ($P = .001$ and $P = .006$, respectively; Table 2).

DISCUSSION

We evaluated the effect of co- or superinfection versus single infection on disease progression in a Dutch MSM cohort with subtype B PHI acquired between 2000 and 2009. Multivariate analysis showed that dual HIV-1 infection, resulting either from co- or superinfection, was the most important factor influencing $CD4^+$ T-cell decline over time and was the major disease-accelerating characteristic in this cohort, suggesting that the viral virulence is increased in these patients, as predicted by model systems [8, 9, 35]. Unfavorable HLA-B alleles were also significantly associated with a more rapid decline in $CD4^+$ T-cell count, which is in agreement with findings from previous studies [30]. Of the patients with dual HIV-1 infection, those with coinfection had the most rapid decline in $CD4^+$ T-cell count. In line with these findings, patients with HIV-1 coinfection started cART significantly earlier compared with patients with single infection. Patients acquiring HIV-1 superinfection also had a decline in $CD4^+$ T-cell count that was significantly greater than that for with individuals with single infection but less so than that for patients with coinfection. Most likely, competition between virus strains is more severe when the moment of infection with the second strain is closer to the first infection point.

The strength of this study is that disease progression was studied in a well-characterized, recently infected cohort of patients with PHI. The limitations are that, in this cohort, 4 of the 6 superinfections occurred with a non-B HIV-1 subtype. However, none of the subtypes involved have been associated with increased disease progression. Second, because this study has been performed in a cohort with PHI in which early superinfections occurred, it is unclear whether the findings are also applicable to patients who acquire HIV-1 superinfection in a later phase of the infection. It is possible that susceptibility to a second HIV-1 infection is increased during the acute phase, as cellular immunity is less developed at that time, and that the second infection is also more damaging during this phase. Of note, 65% of patients presented with an acute retroviral syndrome, which is a strong predictor of HIV-1 disease progression [36], suggesting that our results may not be generalized to unrecognized PHI. Finally, as dual HIV-1 infections are infrequent events and PHI is usually not recognized, the sample size of our study is relatively small, so that replication of these results in an independent cohort would strengthen our findings.

In conclusion, this study demonstrates that recent dual HIV-1 infection is independently associated with increased disease progression. Our findings suggest that serosorting (eg, practicing unsafe sex only with partners who have the same HIV serostatus) should be discouraged, especially in patients with recognized PHI, as superinfections increase disease progression, shown by a faster decline in $CD4^+$ T-cell counts in this study. Finally, temporary cART during PHI might have clinical benefits, although randomized data are not yet available [37]. Apart from potential other benefits, an additional advantage of (temporary) treatment might be the prevention of early HIV-1 superinfection.

Note

Potential conflicts of interest. J. M. P. has served as a consultant for ViiV, Bristol-Myers Squibb, and Abbott, and has received payment for lectures from Merck Sharp & Dohme (MSD) and for meeting expenses from Abbott, MSD, Boehringer, Gilead, and Bristol-Myers Squibb. All other authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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