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# Conversion Rate towards a Syncytium-Inducing (SI) Phenotype during Different Stages of Human Immunodeficiency Virus Type 1 Infection and Prognostic Value of SI Phenotype for Survival after AIDS Diagnosis

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The presence of syncytium-inducing (SI) human immunodeficiency virus type 1 (HIV-1) variants is predictive for accelerated progression to AIDS. This study showed that a 4-year survival with AIDS also occurred significantly more often for patients who lacked SI variants. However, multivariate Cox analysis excluded the predictive value of SI viruses for rapid death as being independent from low $CD4^+$ T cell counts. Incidence of appearance of SI variants was increased in persons with $CD4^+$ T cell counts $<500/\mu L$ but remained constant in the strata of $CD4^+$ T cell counts $<500/\mu L$, excluding the possibility that loss of immune control is the only prerequisite for the development of SI HIV-1 variants.

The asymptomatic phase of infection with human immunodeficiency virus type 1 (HIV-1) and the period between AIDS diagnosis and death can vary among individuals. This variation seems to be determined by immunologic, viral, and host genetic factors that have been used as prognostic markers [1–5]. Differences among HIV-1 isolates in biologic properties, such as syncytium-inducing (SI) capacity, replication rate, and cytotropism, have indeed been described (reviewed in [6]). Whereas non–syncytium-inducing (NSI) variants can be detected throughout HIV-1 infection, SI variants generally develop during HIV-1 infection in ~50% of infected persons, preceding an accelerated $CD4^+$ T cell decline. HIV-1 SI phenotype is independently predictive for more rapid disease progression [7], even in asymptomatic persons with $CD4^+$ T cell counts $<200/\mu L$ [3]. In cross-sectional comparisons, the isolation of SI variants was associated with low $CD4^+$ T cell numbers [7–9], although the

first detection of SI variants can occur within a broad range of $CD4^+$ T cell numbers [8].

We studied patients who progressed to AIDS to determine whether those who had only NSI HIV-1 variants differed significantly from those who also had SI variants with respect to $CD4^+$ T cell counts at the time of diagnosis, AIDS-defining events, and mortality. Moreover, to better understand the basis of HIV-1 phenotype evolution in the pathogenesis of AIDS, we analyzed the conversion rate of NSI to SI variants in relation to deterioration of the immune system before and after AIDS diagnosis.

## Subjects and Methods

*Subjects.* All patients who attended the Academic Medical Centre in Amsterdam and were diagnosed as having AIDS (according to the 1987 definition [10]) between 1 December 1985 and 1 January 1988 were included in this study ($n = 218$). Patients were followed until death or until 1 January 1994. In addition, we studied the NSI to SI conversion rate among HIV-1–positive asymptomatic participants in the Amsterdam Cohort Study. At 3-month intervals, clinical, immunologic, virologic, and epidemiologic data were collected, and serum and peripheral blood mononuclear cells (PBMC) were cryopreserved. For this study, all participants during the period October 1984 until November 1995, who had only NSI variants on at least one occasion and at least three different measurements of $CD4^+$ T cell counts ($n = 380$) or three measurements of T cell reactivity (as measured by response to phytohemagglutinin [PHA] stimulation, $n = 348$) were included.

*Virus phenotyping.* Cryopreserved patient PBMC ($10^6$) from the time of AIDS diagnosis and from time points near the end of follow-up were thawed and cocultivated with $10^6$ MT-2 cells according to standard procedures [7] to assess the presence of SI HIV-

1 variants. From April 1992, the presence of SI HIV-1 was determined every 3 months among the asymptomatic participants from the Amsterdam cohort. Cryopreserved PBMC samples were used to determine the time of phenotype conversion before April 1992.

*CD4+ cell numbers and T cell function.* CD4+ cell numbers were determined by flow cytofluorometry. The functional capacity of T cells was measured as the ability to proliferate in response to stimulation by PHA in vitro.

*Serum HIV-1 antigen.* Sera were tested for HIV-1 p24 antigen in a commercial solid-phase immunoassay (Abbott, Abbott Park, IL) according to the manufacturer's instructions.

*Statistical analysis.* Software of Number Crunching Statistical Systems (version 5.0; NCSS, Kaysville, UT) was used for statistical analysis. CD4+ T cell count, zidovudine treatment, p24 antigenemia, and HIV-1 phenotype were included in a stepwise forward Cox proportional hazard analysis to calculate the contribution of covariates on survival after AIDS diagnosis. The Mann-Whitney test and the paired samples $t$ test were used to compare CD4+ cell number. The $\chi^2$ test was used to compare groups with and without SI variants for AIDS-defining symptoms, serum HIV-1 antigen status, and treatment.

## Results

Viable frozen PBMC samples at the time of AIDS diagnosis were available from 202 patients. SI variants were isolated from 90 (47%) of 192 patients whose samples were successfully cocultured.

The groups of AIDS patients with and without SI variants were comparable with respect to age and p24 antigenemia. For persons with non-Hodgkin's lymphoma as the AIDS-defining illness ($n = 8$), only NSI variants were isolated. SI variants were more frequently isolated from patients with Kaposi's sarcoma (25.6% among SI carriers vs. 17% among NSI carriers, $P = .01$) and from patients with opportunistic infections other than *Pneumocystis carinii* pneumonia or *Candida* esophagitis (27.8% among SI carriers vs. 15.7% among NSI carriers, $P = .04$). At the time of AIDS diagnosis, SI carriers had significantly lower CD4+ cell numbers than did NSI carriers (median, 80 vs. 160; $P < .001$, Mann-Whitney test).

No significant difference in the proportion of patients ($n = 107$) receiving zidovudine was observed between the groups of NSI and SI carriers (56.9% and 54.4%, respectively), and almost all started treatment shortly after AIDS diagnosis (median, 3.3 months, range −7.5 to 62.2).

At the end of the study, 192 patients (88%) had died, 25 patients (11%) were lost to follow-up, and 1 patient was censored. The cumulative incidence for survival of patients with and without SI variants was determined according to the Kaplan-Meier method (figure 1). Both survival curves were quite comparable in the first year after AIDS diagnosis but diverged after 2 years, resulting in 11.6% survival of AIDS patients with NSI variants compared with 1.3% of those with SI variants after 48 months ($P = .01$).

Time-fixed Cox proportional hazard modeling was used to determine the laboratory variables at AIDS diagnosis that predicted survival in this study population. In univariate models, both the presence of SI variants (relative risk [RR] = 1.5, 95%
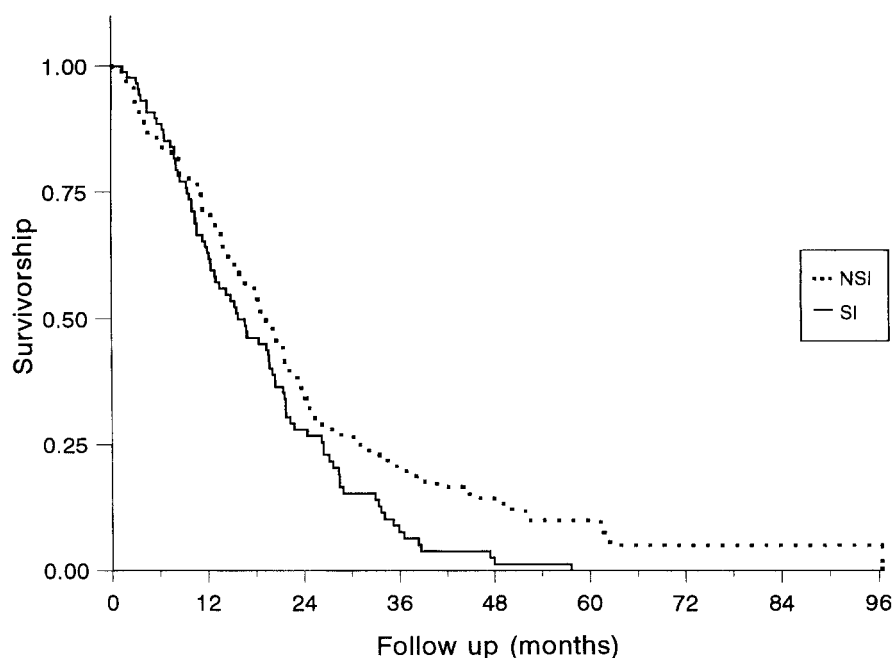


**Figure 1.** Survival time according to HIV phenotype. AIDS patients ($n = 192$) were stratified by presence or absence of syncytium-inducing (SI) and non–SI (NSI) HIV-1 variants at time of AIDS diagnosis.

confidence interval [CI] = 1.1–2.0, $P = .02$; $n = 90$) and CD4+ cell number <100/$\mu$L (RR = 1.8, 95% CI = 1.3–2.5, $P = .0004$; $n = 90$) at AIDS diagnosis were predictive for decreased survival, whereas antiretroviral treatment was significantly associated with prolonged survival (RR = 0.5, 95% CI = 0.4–0.7, $P < .0001$; $n = 113$). This decreased RR for death among antiretroviral-treated persons was also observed in the separate groups of patients with NSI and SI variants (RR = 0.5 and 0.4, respectively). The presence of p24 antigenemia in this study was not associated with decreased survival. In a multivariate analysis among 176 persons from whom CD4+ T cell numbers and HIV-1 phenotypes were available at the time of AIDS diagnosis, CD4+ cell numbers <100/$\mu$L and zidovudine treatment were found to be independent markers for decreased and increased survival, respectively (CD4 cells <100/$\mu$L, RR = 1.8, 95% CI = 1.3–2.4, $P = .0003$; zidovudine, RR = 0.5, 95% CI = 0.3–0.7, $P < .0001$). HIV-1 phenotype, however, did not predict survival independent of low CD4+ cell counts. Additional multivariate analysis using zidovudine treatment and isolation of SI variants revealed that if only those two parameters were included in the model, both could be used as independent predictors for survival (RR = 0.3 and 1.5, respectively).

With respect to phenotype evolution, for 58 persons who developed AIDS and who had NSI HIV-1 variants (median CD4+ cell count, 160; range 100–270), virus phenotype data were available at a later time point as well. In 8 (13.8%) persons, phenotype conversion from NSI to SI was observed after AIDS diagnosis. Taking into account the cumulative years (111.2) of follow-up after AIDS diagnosis for all 58 persons during the period they had only the NSI HIV-1 variant, there was an average phenotype conversion rate of 7.2% per NSI-year. This is comparable with the phenotype conversion rate of 8.1% in a cohort of 192 asymptomatic HIV-1–positive men with significantly higher CD4+ T cell counts at baseline (median CD4+ cell count, 455; range, 310–620) [7].

To test the hypothesis that deterioration of the immune system is not accompanied by an increased rate of phenotype conversion, we analyzed the SI conversion rate in relation to CD4+ T cell counts using the Kaplan-Meier method. To be able to analyze as many participants as possible during a maximal range of CD4+ T cell counts, we determined the follow-up time within each CD4 stratum for each individual participant. In this analysis, cases were defined as participants with conversion from NSI to SI in a specific stratum; participants were censored within a specific CD4 stratum when they entered the next CD4 cell category or when lost to follow-up. The NSI to SI conversion rate when CD4+ T cell counts were 1000–750, 750–500, 500–250, and 250–0/$\mu$L are shown in figure 2A. A clear difference in conversion rate could be observed between CD4 cell strata above and below 500 CD4+ T cell counts/$\mu$L ($P < .0001$). In contrast, the average time for phenotype conversion when CD4+ T cell counts were 500–250 was comparable to that

observed in persons with CD4+ T cell counts of 250–0 (figure 2A).

Comparable results were obtained after stratification of cohort participants according to the functional capacity of T cells, as determined after in vitro stimulation with PHA (figure 2B). Also, in this analysis, persons in the strata with normal to high T cell reactivity (8000–12,000 and >12,000, respectively) showed a decreased NSI to SI conversion rate compared with persons in the strata with significantly reduced T cell reactivity (0–4000 and 4000–8000).

Also, a selection of samples with both low T cell functional capacity and low absolute CD4+ T cell counts did not reveal a significantly further increased NSI to SI conversion rate with further deterioration of the immune system (data not shown).

## Discussion

We analyzed the role of HIV-1 biologic phenotype in the survival of patients after AIDS diagnosis. During the first 2 years after AIDS diagnosis, no virus phenotype–dependent differences in survival were observed. An explanation for this observation might be that NSI carriers with a relatively short survival after AIDS diagnosis have high-virulence viruses, whereas those with a longer survival carry relatively low-virulence NSI viruses [11]. In univariate Cox analysis over a 4-year period, the presence of SI variants correlated significantly with reduced survival, which might be explained by the strong association between SI variants and low CD4+ T cell counts [12]. As shown before [7, 12], individuals who developed AIDS and had SI variants had lower CD4+ T cell numbers at the time of AIDS diagnosis, which might explain why opportunistic infections more frequently were the AIDS-defining events in those with SI than NSI variants. We have no explanation why non-Hodgkin's lymphoma was more frequently diagnosed in individuals who developed AIDS with only NSI variants.

We previously demonstrated that SI viruses can emerge at relatively high CD4+ T cell counts and low virus loads [9], thus excluding the possibility that the emergence of SI viruses is only the consequence of and not contributing to impaired immunity and increased viral replication, assuming that CD4 T cell counts are a measure of immune function. Here we observed an increased SI conversion rate in individuals with CD4+ T cell counts <500/$\mu$L and T cell reactivity upon PHA stimulation of <8000 cpm. Apparently, a certain degree of immune impairment favors the appearance of SI variants. Alternatively, or in addition, viral replication (and thus the generation of new virus variants) might be limited in individuals who still have high CD4+ T cell counts.

If, on average, virus load and turnover increase with decreasing CD4+ T cell numbers, a continuous increase in the SI conversion rate in progressing patients might be foreseen. However, no difference in the emergence of SI variants was seen between individuals with CD4+ T cell counts of 500–250/$\mu$L
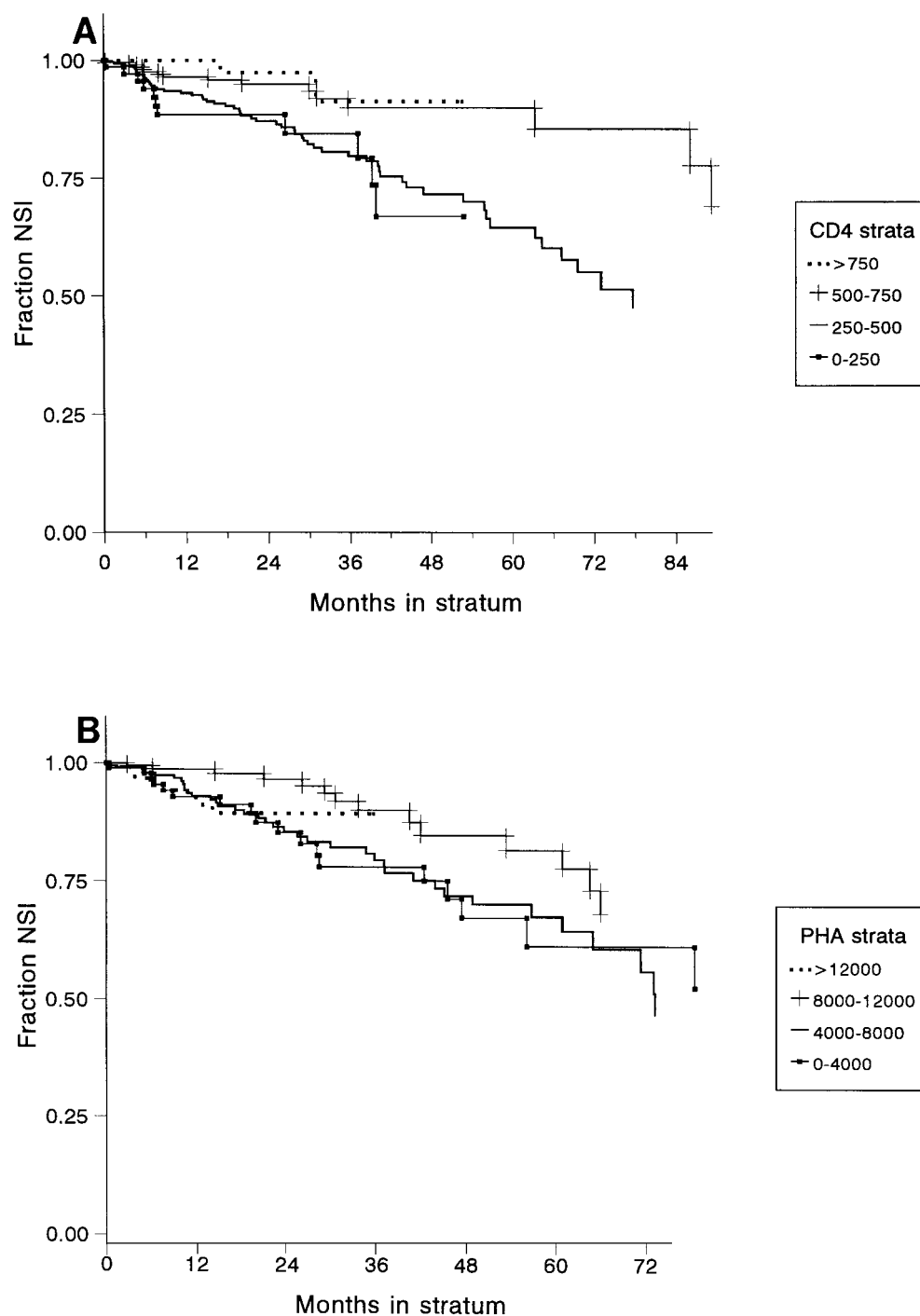
**Figure 2.** *A*, Non–syncytium-inducing (NSI) to syncytium-inducing (SI) conversion rate according to stratification of CD4$^+$ T cell counts. All data on CD4$^+$ T cell counts ($n = 11{,}067$) from 380 cohort participants from whom at least 1 visit with only NSI variants was available were used to determine NSI to SI conversion rate during period with CD4$^+$ T cell counts of 1000–750 ($n = 119$), 750–500 ($n = 240$), 500–250 ($n = 292$), or 250–0 ($n = 75$) CD4$^+$ T cell counts/$\mu$L. *B*, NSI to SI conversion rate according to phytohemagglutinin (PHA) response stratification. All PHA data ($n = 9743$) from 348 cohort participants from whom at least 1 visit with only NSI variants was available were used to determine NSI to SI conversion rate during period with PHA response counts of 16,000–12,000 ($n = 114$), 12,000–8000 ($n = 202$), 8000–4000 ($n = 208$), or 4000–0 ($n = 96$) cpm. Fraction NSI on *y* axis indicates % of persons who, at given time point, had not converted to SI variant.

and 250–0/µL or with T cell reactivity of 8000–4000 and 4000–0 cpm, implicating that other factors may influence the SI conversion rate as well. One possible explanation might be that late-stage NSI variants are as fit as SI viruses, thus complicating the selection of newly emerging SI viruses.

On the other hand, the expression of SI and NSI discriminating coreceptors as well as the expression of SDF-1, RANTES, MIP1α, and MPI1ß that interfere with SI and NSI HIV-1 replication [13–15] might very well be an important selection pressure for the emergence of SI variants. Whether genetic differences for these receptors and/or the chemokine expression patterns do influence the phenotypic evolution of the HIV-1 quasispecies remains to be established.

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