

CD4/CD8 ratio and CD8 counts predict CD4 response in HIV-1-infected drug naive and in patients on cART

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Abstract

Plasma HIV viral load is related to declining CD4 lymphocytes. The extent to which CD8 cells, in addition to RNA viral load, predict the depletion of CD4 cells is not well characterized so far. We examine if CD8 cell count is a prognostic factor for CD4 cell counts during an HIV infection.

A longitudinal analysis is conducted using data from the Swiss HIV cohort study collected between January 2000 and October 2014. Linear mixed regression models were applied to observations from HIV-1-infected treatment naive patients (NAIVE) and cART-treated patients to predict the short-term evolution of CD4 cell counts. For each subgroup, it was quantified to which extent CD8 cell counts or CD4/CD8 ratios are prognostic factors for disease progression.

In both subgroups, 2500 NAIVE and 8902 cART patients, past CD4 cells are positively ($P < 0.0001$) and past viral load is negatively ($P < 0.0001$) associated with the outcome. Including additionally past CD8 cell counts improves the fit significantly ($P < 0.0001$) and increases the marginal explained variation 31.7% to 40.7% for the NAIVE and from 44.1% to 50.7% for the cART group. The past CD4/CD8 ratio (instead of the past CD8 level) is positively associated with the outcome, increasing the explained variation further to 41.8% for NAIVE and 51.9% for cART.

Abbreviations: AIDS = acquired immune deficiency syndrome; patient group with AIDS, ART = antiretroviral therapy, BIC = Bayesian information criterion for model selection, cART = combined antiretroviral therapy; patient group receiving standard combined antiretroviral therapy, CD4 = CD4 lymphocyte cell counts measured per μL in blood plasma by flow cytometry, CD8 = CD8 lymphocyte cell counts measured per μL in blood plasma by flow cytometry, HCV = hepatitis C co-infection, HET = heterosexual patient group, HIV-1 = human immunodeficiency virus of subtype 1, IDU = patient group of intravenous drug users, $\log(\text{CD4}/\text{CD8})$ = logarithmic (\log_{10}) transformed CD4 to CD8 ratio, $\log\text{-RNA}$ = logarithmic (\log_{10}) transformed RNA measurements, M1 = model 1, M2 = model 2, M3 = model 3, MSM = patient group of men who have sex with men, NAIVE = patient group with an untreated HIV-1 infection, NRTI = nucleoside reverse-transcriptase inhibitors, RNA = number of RNA copies per mL blood plasma, SHCS = Swiss HIV cohort study.

Keywords: CD4/CD8 ratio, CD8 cell counts, disease progression, linear mixed model, longitudinal data, prediction

1. Introduction

An untreated human immunodeficiency virus of subtype 1 (HIV-1) infection is characterized by declining CD4 target cells which is associated with the viral load level. Over time, viral load levels in general tend to increase and CD4 levels continue to decline with subsequent cellular immunodeficiency leading to an acquired immune deficiency syndrome (AIDS) and ultimately death.^[1,2] Successful antiretroviral treatment (ART) results in sustained

suppression of HIV-1 plasma RNA levels below the detection limit of currently available assays. Today's combined antiretroviral therapy (cART) is a combination of at least 3 different substances consisting of a non-nucleoside reverse transcriptase, a boosted protease or an integrase inhibitor with a combined 2 drug nucleoside/nucleotide backbone.^[3]

Past research based on randomized trials and cohort studies mainly focused on the HIV-1 plasma RNA load and CD4 cell

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count interactions over time^[4–6] and the restoration of the CD4 cell counts.^[7–9] However, already in the early times of HIV research it was suggested to include additional immune-activation measures such as CD8 lymphocyte cell counts, CD4/CD8 ratios or CD4 and CD8 percentages.^[10–13] A negative correlation between changes in CD4 and CD8 cell counts during an intensification of the antiretroviral therapy was reported.^[14] In the Swiss HIV cohort study (SHCS), larger changes in CD4 cell counts were found to be negatively associated with CD8 cell counts measured at baseline for HIV-1 patients receiving antiretroviral therapy.^[15] For HIV-1-infected treatment naive patients, CD8 counts increase, whereas CD4 counts decline^[16] but only viral load and CD4 counts and not CD8 cell counts, were considered to be the most relevant predictors for disease progression.^[17] Time to normalization of the CD4/CD8 ratio, defined as 2 subsequent measurements with a ratio between 1 and 1.2 was found to be negatively associated with its baseline value^[18,19] but only a minority of HIV-1-infected individuals under antiretroviral therapy normalize their CD4/CD8 ratio,^[18–20] in particular if treatment was started at low CD4 counts.^[3,15,21] Low CD4/CD8 ratios were also found to be associated with increased morbidity and mortality of non-AIDS-related death causes.^[19,22,23]

These studies all hint toward a possibly important role of CD8 cell counts during an HIV infection. However, up to now an analysis of the time-dependent relationship between changing CD8 and CD4 lymphocytes based on a cohort study is lacking. Furthermore, there is a large inter-patient variation in disease progression, in CD4 recovery under therapy and in CD4/CD8 normalization, depending on a multitude of factors such as viral and host factors.^[24–26] Here, by taking patient-specific variation into account, we examined whether past CD8 cell counts contain additional information to determine future CD4 cell counts and investigated this effect separately, for treatment naive individuals and for patients receiving cART.

2. Methods

2.1. Study population

The SHCS,^[27] established in 1988, includes HIV-1-infected persons >18 years, living in Switzerland. The SHCS has been approved by the ethical committees of all participating institutions, and written informed consent has been obtained from all participants (www.shcs.ch). The SHCS schedules regular follow-up visits every 6 months, whereas the common clinical follow-up interval is 3 months, at which CD4 and CD8 lymphocyte cell counts and plasma HIV-1 viral load are measured. The lymphocyte cell counts per μL blood were measured by flow cytometry. Since the year 2000 all assays used for HIV-1 RNA detection had a detection limit of 50 copies per mL or lower. For this study, the RNA detection limit was set at 50 RNA copies/mL of plasma, independent of the applied assays and all values below this limit, or without detection, were set to 25 copies/mL. Data were extracted from the October 2014 update of the SHCS database. Observations prior to the year 2000 were excluded in order to guarantee comparable assay technology used to measure plasma RNA load and that an established cART was available to all patients. We extracted from the database 280,554 lymphocyte cell counts and 325,984 RNA measurements obtained from 11,899 patients.

The study population was divided into 2 subgroups, 1 covers all observations obtained from patients with an untreated HIV-1 infection (NAIVE), observed as long as they did not start cART.

The second group includes observations from patients receiving available standard cART (cART). Accordingly the same patient may be included in both groups, which is the case for 1797 patients or 71.9% of the NAIVE study population.

The following exclusion criteria were applied in the order illustrated in Fig. 1: Lymphocytes and RNA are not always measured at the same time, so we matched results of the 2 laboratory analyses if the time difference was <8 days and the date of the RNA analysis was kept. Observations that were excluded because no corresponding lymphocyte or RNA measurement within an 8 days interval was available were assumed to be missing at random. If a patient quit or interrupted cART therapy, all follow-up observations were omitted, independent of a likely therapy resumption. A therapy interruption is assumed to have an impact on future CD4 cell counts. Therefore, observations are only included up to the therapy interruption and censored afterwards. As it was shown that hepatitis C co-infection (HCV) influences CD4 cell counts, all patients with indetermined HCV status were excluded.^[28] A missing HCV test is assumed to be missing at random. Moreover, all observations with a follow-up time between 2 subsequent measurements of >12 months were excluded, as the information of past lymphocyte cell counts and past RNA measurements for future CD4 counts was assumed to diminish over time. If a patient met all of the above inclusion criteria he additionally had to have at least 3 measurements of CD4 and CD8 cell counts as well as RNA blood viral load, observed at 3 different occasions.

2.2. Statistical methods and analysis

The hypothesis that past CD4/CD8 ratios predict current CD4 cell counts was examined by linear mixed regression models for longitudinal data^[29] for each patient subgroup. The outcome in each model is the square root transformed CD4 cell count^[4,30] observed at the current follow-up visit. We estimated for both patient groups 3 models for which we included different combinations of suitably transformed CD4 and CD8 cell counts, observed at the preceding follow-up visit, as predictors. In this way, the influence of CD4 and CD8 lymphocytes, which are lagged by 1 follow-up visit, on the outcome is examined. The lag between 2 subsequent follow-up visits corresponds on average to a time period of 3 months. From here on predictors are called lagged if, relative to the observed CD4 cell count outcome, they were observed at the preceding follow-up visits. In the first model formulation (M1), we included the lagged square root transformed CD4 cell counts and the lagged log₁₀ transformed RNA measurement as predictors, both observed at the preceding of 2 subsequent follow-up visits. In the second model (M2), we added the lagged square root transformed CD8 cell counts as additional predictor. In the third approach (M3), we replaced the lagged CD8 cell counts by the natural log transformed, lagged CD4/CD8 ratio.

In order to assess which of the 3 models is most suitable for each patient group, we compared the marginal R^2 ,^[31,32] which is the proportion of explained variation by the predictors as proportion of the overall variation. Additionally the models were compared by a version of the Bayesian information criterion (BIC), modified for linear mixed models.^[33] The modified BIC penalizes the inclusion of model parameters and lower values indicate that the corresponding model captures more information and thus should be preferred.

Time was set to zero at cohort entry for the NAIVE and at therapy initiation for the cART subgroup. The time scale was

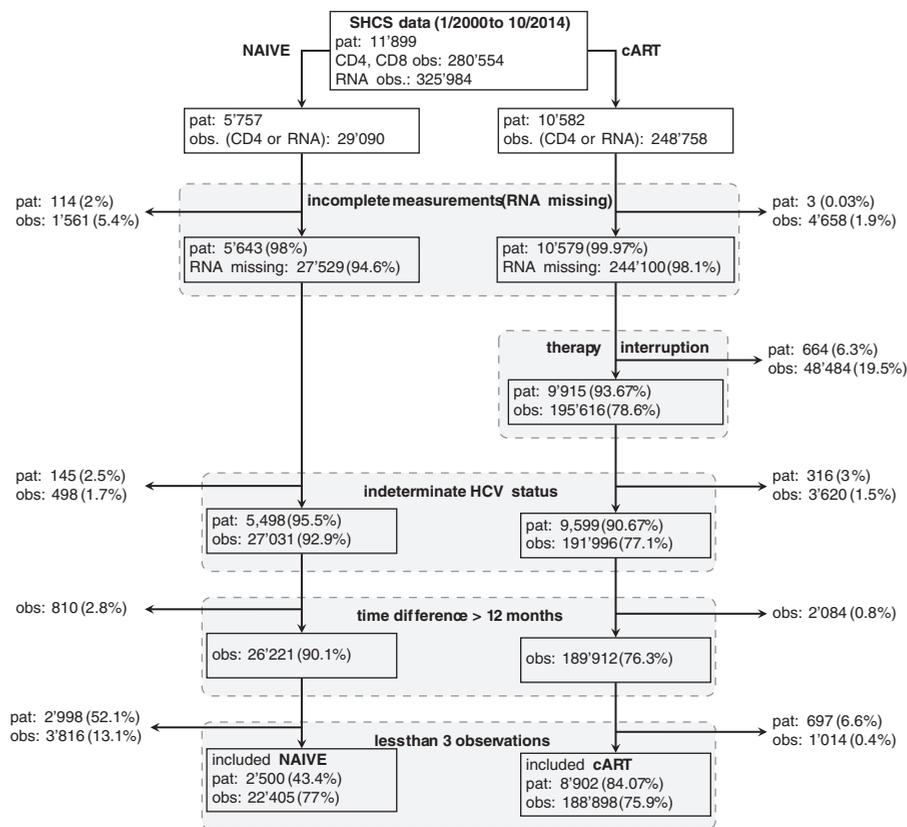


Figure 1. SHCS data and study population with number of observations (obs.) and number of patients (pat.). Percentages (%) are based on the initial NAIVE or cART data given on the second level in the diagram. cART = combined antiretroviral therapy; patient group receiving standard combined antiretroviral therapy, NAIVE = patient group with an untreated HIV-1 infection, SHCS = Swiss HIV cohort study.

standardized to 3 month intervals, as this corresponds to the common clinical follow-up period in the SHCS. For the cART group, time since therapy start was square root transformed,^[34] as this allowed us to capture the sharp increase in CD4 cell counts after therapy initiation.^[35] We also included AIDS (yes, no) and age, which are time-dependent, the transmission group (transmission) and the status of a hepatitis C co-infection (HCV), both observed at baseline, as predictors in all models. The probable HIV transmission^[36] is a categorical predictor with 6 groups: men who have sex with men (MSM), male and female intravenous drug users (IDU-male, IDU-female), heterosexual males and females (HET-male, HET-female), and a group for which the transmission path is not further specified (other). The HCV status has 3 categories: HCV negative, patients with inactive and patients with replicating (active) HCV. For the cART patient group, we additionally included the time period prior to cART during which an individual was receiving mono or dual regimens with nucleoside reverse-transcriptase inhibitors (NRTI).

In order to address patient-specific heterogeneity at baseline we included a random intercept in each model and heterogeneity between patients in the CD4 cell time course is taken into account by a random slope, which allows for patient-specific deviations from the average time course.^[4] The random effect structure is the same for both subgroups and all 3 model specifications. An analysis of the model residuals did not provide evidence for any longitudinal structure or other dependency. All statistical analyses and data preparation were done in R version 3.3.0 (2016–05–03). All linear mixed models were fitted with the software package NLME^[37] version 3.1–119.

3. Results

3.1. Patient characteristics

According to the above inclusion criteria 2500 patients were eligible in the NAIVE subgroup with 22,405 observations, whereas 8902 patients were included in the cART subgroup with 188,898 observations (see Fig. 1). The patient characteristics for both subgroups are shown in Table 1. The upper part in Table 1 shows the patient characteristics for predictors observed at baseline and the lower part for time-dependent, longitudinal predictors based on all included observations.

Patients in the NAIVE, compared to the cART subgroup, had lower CD4 cell counts (NAIVE 457, cART 497), higher CD8 cell counts (NAIVE 952, cART 796), a lower CD4/CD8 ratio (NAIVE 0.48, cART 0.62), and higher log-transformed RNA levels (NAIVE 4.13, cART 1.40). The cART group had more follow-up visits per patient (NAIVE 7, cART 17), as usually most of the patients start cART rather quickly and continuously stay under therapy for the remaining observation time, thus patients are also older at baseline in the treated group (NAIVE 36, cART 39). The proportion of patients having AIDS was higher in the cART group (NAIVE 4.9%, cART 24.1%). All other characteristics (follow-up time, transmission, HCV) were similar for both subgroups.

3.2. Model comparison and predictive value of CD8 lymphocytes

The first 2 columns in Table 2 give the marginal R^2 for the 3 models and for each patient group. For the NAIVE subgroup,

Table 1**Patient characteristics for baseline (upper part) and longitudinal (lower part) characteristics of the study population for both subgroups.**

Baseline characteristics	NAIVE	(n = 2500)	cART	(n = 8902)
CD4 at baseline	491	(376, 670)	367	(231, 539)
CD8 at baseline	904	(654, 1265)	846	(601, 1185)
CD4/CD8 at baseline	0.55	(0.38, 0.8)	0.41	(0.25, 0.67)
log (RNA) at baseline	4.23	(3.58, 4.8)	1.95	(1.40, 3.11)
AIDS at baseline	46	(1.8%)	1843	(20.7%)
Age at baseline	36	(30, 42)	39	(33, 46)
Transmission				
MSM	1181	(47.2%)	3723	(41.8%)
IDU-male	208	(8.3%)	841	(9.4%)
IDU-female	94	(3.8%)	423	(4.8%)
HET-male	415	(16.6%)	1602	(18.0%)
HET-female	506	(20.2%)	1892	(21.3%)
other	96	(3.8%)	421	(4.7%)
HCV				
HCV negative	2001	(80.0%)	7056	(79.3%)
HCV inactive	109	(4.4%)	367	(4.1%)
HCV active	390	(15.6%)	1479	(16.6%)
Longitudinal characteristics	NAIVE	(n = 22405)	cART	(n = 188898)
CD4	457	(346, 609)	497	(338, 688)
CD8	952	(694, 1300)	796	(573, 1096)
CD4/CD8	0.48	(0.34, 0.69)	0.62	(0.40, 0.92)
log (RNA)	4.13	(3.50, 4.66)	1.4	(1.40, 1.40)
Follow-up visits per patient	7	(4, 12)	17	(9, 31)
Follow-up time (months)	3.5	(3.0, 5.6)	3.3	(2.8, 5.3)

Data are patient numbers (with %) for discrete and median (with the first and third quartile) for continuous predictors. Lymphocyte cell counts and ratio are untransformed, the RNA is log₁₀-transformed. AIDS = acquired immune deficiency syndrome; patient group with AIDS, cART = combined antiretroviral therapy; patient group receiving standard combined antiretroviral therapy, CD4 = CD4 lymphocyte cell counts measured per μL in blood plasma by flow cytometry, CD8 = CD8 lymphocyte cell counts measured per μL in blood plasma by flow cytometry, HCV = hepatitis C co-infection, HET = heterosexual patient group, IDU = patient group of intravenous drug users, MSM = patient group of men who have sex with men, NAIVE = patient group with an untreated HIV-1 infection, RNA = number of RNA copies per mL blood plasma.

the marginal R^2 increases from 31.7% for M1, to 40.7% for M2 after inclusion of the lagged CD8 cell counts. For cART the increase is from 44.1% to 50.7%. Including the lagged CD4/CD8 ratio instead of the lagged CD8 cell counts, increases the R^2 measure even more to 41.8% for the NAIVE and to 51.9% for the cART group. The modified BIC for the 3 models and each patient group is shown in the last 2 columns of Table 2. The modified BIC is decreasing from the model M1 to M3 for both patient groups. Thus, consistent with the marginal R^2 , also the information criterion prefers the model which includes the CD4/CD8 ratio over the other 2 models.

Figure 2 illustrates the relationship between the lagged lymphocyte predictors, observed at the first follow-up visit and the outcome, observed at the second of 2 subsequent follow-up visits. Figure 2 depicts the relationship for the population mean in the NAIVE (top) and cART (bottom) group. Square root

transformed lymphocyte cell counts were back transformed to absolute cell count values and the log-transformation of the CD4/CD8 ratio was also reversed. The lower and upper limits for the predictors in Fig. 2 were set to the 2.5% and 97.5% quantiles of all observation (0.13–1.8 for the lagged CD4/CD8 ratio and 114–1184 for the lagged CD4). The contour lines of the plots in the first 2 rows indicate the predicted CD4 cell count at the next follow-up visit, for a given combination of lagged CD4/CD8 ratio and lagged CD4 cell count. All other predictors in model M2 and M3 were set to a constant value: time since cohort entry for NAIVE or since therapy start for cART was set to 3 months, the age to 40 years, we assumed a median viral load equal to 4.1 (corresponding to 13,380 copies per mL blood) for NAIVE and a suppressed RNA level for cART (equal to a viral load of 1.4 or 25 copies per mL blood), which occurs for 76.4% of the observations in this group. The categorical predictors,

Table 2**Marginal R^2 and modified BIC for both patient subgroups and 3 models including different lagged longitudinal predictors.**

	Marginal R^2		Modified BIC	
	NAIVE	cART	NAIVE	cART
M1: CD4 + RNA	31.7%	44.1%	95,594	865,332
M2: CD4 + CD8 + RNA	40.7%	50.7%	95,060	862,400
M3: CD4 + CD4/CD8 + RNA	41.8%	51.9%	94,962	861,192

All models additionally include an intercept and were adjusted for AIDS, age, transmission, HCV and for time since cohort entry for NAIVE and time since therapy start plus NRTI at baseline for cART (see also Table 3).

BIC = Bayesian information criterion for model selection, cART = combined antiretroviral therapy; patient group receiving standard combined antiretroviral therapy, CD4 = CD4 lymphocyte cell counts measured per μL in blood plasma by flow cytometry, CD8 = CD8 lymphocyte cell counts measured per μL in blood plasma by flow cytometry, NAIVE = patient group with an untreated HIV-1 infection, RNA = number of RNA copies per mL blood plasma.

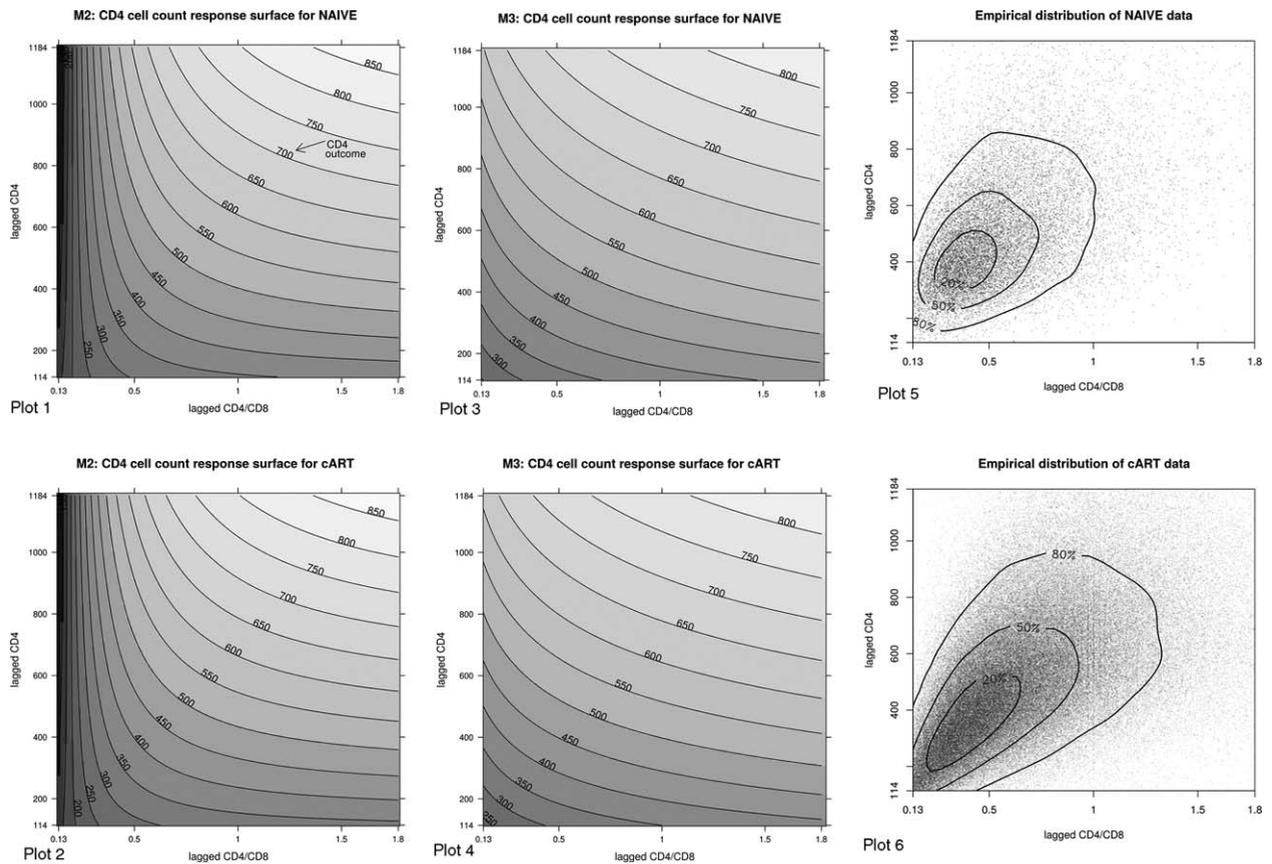


Figure 2. Relation between lagged CD4/CD8 ratio predictor (abscissa), lagged CD4 cell counts predictor (ordinate), and the predicted CD4 cell count outcome (contour lines) for the population mean in NAIVE (top) and cART (bottom) subgroups in model M2 (left) and M3 (middle) and the empirical distribution of the data (right) for which the contour lines indicate the location of 80%, 50%, and 20% of the data. cART = combined antiretroviral therapy; patient group receiving standard combined antiretroviral therapy, NAIVE = patient group with an untreated HIV-1 infection.

transmission and HCV, were set to the reference category and NRTI duration was set to zero.

Figure 2 demonstrates that for model M2 and M3 the relationship between the lymphocyte predictors and the outcome is similar for untreated and cART patients. However, the 2 models give considerably different predictions. The plots for cART and NAIVE based on model M2 (left column) show converging contour lines in the upper left corner. This leads to almost vertical contour lines for Model M2, if the CD4/CD8 ratio is below 0.25, implying a CD8 count which is 4 times larger than the CD4 cell count. The vertical contour lines mean that in this range the CD4 lymphocyte level has, according to Model M2, no more impact on the prediction of CD4 at the next follow-up visit. Model M3 in contrast, which instead of the CD8 cell counts includes the CD4/CD8 ratio as predictor, does not have this feature of quickly converging contour lines (middle column). In model M3 the level of CD4 lymphocytes is still an important predictor, even if the CD4/CD8 ratio is <0.25.

The right column in Fig. 2 shows the empirical bivariate distribution of CD4 cell counts and the CD4/CD8 ratio for NAIVE and cART patients. The contour lines indicate where 70%, 50% and 10% of the observations are located. For the NAIVE subgroup 8.3% and for cART 9.7% of the observed data are below a CD4/CD8 ratio equal to 0.25. The empirical distribution in Fig. 2 underpins the fact, that the differences between model M2 and M3 are relevant, as there is a substantial

proportion of observations situated in the bottom left corner, where the models differ most.

3.3. Estimates of regression coefficients

The upper part in Table 3 shows the regression coefficients together with a 95% confidence interval (CI) for the intercept and the longitudinal predictors, based on model M3 only which according to the criteria in Table 2 and Fig. 2 is the preferred model. The intercept represents the population mean of the square root transformed CD4 cell counts at baseline (18.02 NAIVE, 15.56 cART). The regression coefficient for time reflects the population mean course of the outcome since cohort entry for the NAIVE (-0.10) and since therapy start for cART (0.34). These estimates are in agreement with the established finding that for untreated HIV infected patients the CD4 count declines, whereas an antiretroviral therapy causes an increase. The lagged square root CD4 cell count, observed at the preceding follow-up visit, is positively associated with the outcome and has a similar coefficient for both subgroups (0.36 NAIVE and 0.39 cART). The negative coefficient for the plasma viral load (-0.46 NAIVE and -0.27 cART) is in line with the acknowledged decline of CD4 cell counts for higher viral loads. The lagged log-transformed CD4/CD8 ratio is positively associated with the outcome for both subgroups (1.79 NAIVE and 1.65 cART). As the CD4/CD8 ratio is log-transformed, this implies a positive effect if CD4 exceeds

Table 3
Coefficient estimates with 95% confidence intervals (CI) and P-values for model M3 and for both patient subgroups (NAIVE and cART).

	NAIVE			cART		
	Coefficient	95% CI	P	Coefficient	95% CI	P
Intercept	18.02	17.43 to 18.61	<0.0001	15.56	15.32 to 15.80	<0.0001
Time	-0.10	-0.11 to -0.10	<0.0001			<0.0001
Square root time			<0.0001	0.34	0.32 to 0.36	<0.0001
Square root CD4	0.36	0.35 to 0.38	<0.0001	0.39	0.39 to 0.40	<0.0001
log RNA	-0.46	-0.53 to -0.40	<0.0001	-0.27	-0.29 to -0.25	<0.0001
log CD4/CD8	1.79	1.66 to 1.93	<0.0001	1.65	1.60 to 1.70	<0.0001
AIDS at follow-up visit	-0.88	-1.28 to -0.48	<0.0001	-1.01	-1.12 to -0.90	<0.0001
Age at follow-up visit	-0.01	-0.02 to 0.00	0.0018	-0.03	-0.03 to -0.02	<0.0001
NRTI at baseline				-0.02	-0.02 to -0.01	0.00013
Transmission			0.01			<0.0001
MSM (reference)	0.00			0.00		
IDU-male	-0.14	-0.61 to 0.32		-1.13	-1.39 to -0.88	
IDU-female	-0.12	-0.71 to 0.46		-0.75	-1.05 to -0.44	
HET-male	-0.32	-0.60 to -0.05		-0.64	-0.79 to -0.49	
HET-female	-0.4	-0.66 to -0.15		-0.47	-0.61 to -0.32	
Other	-0.04	-0.55 to 0.46		-0.34	-0.60 to -0.08	
HCV			0.47			<0.0001
Negative (reference)	0.00			0.00		
Inactive	-0.08	-0.59 to 0.44		0.04	-0.26 to 0.34	
Active	-0.13	-0.49 to 0.23		-0.44	-0.64 to -0.23	

Upper part shows longitudinal predictors and the lower part time constant predictors for AIDS and age at follow-up visit, NRTI at baseline, transmission, and HCV.

AIDS = acquired immune deficiency syndrome; patient group with AIDS, cART = combined antiretroviral therapy; patient group receiving standard combined antiretroviral therapy, CD4 = CD4 lymphocyte cell counts measured per μL in blood plasma by flow cytometry, CD8 = CD8 lymphocyte cell counts measured per μL in blood plasma by flow cytometry, HCV = hepatitis C co-infection, HET = heterosexual patient group, IDU = patient group of intravenous drug users, MSM = patient group of men who have sex with men, NAIVE = patient group with an untreated HIV-1 infection, NRTI = nucleoside reverse-transcriptase inhibitors, RNA = number of RNA copies per mL blood plasma.

the CD8 cell count, but a negative effect if the ratio is below 1. A patient with an imbalanced immune system, for whom the CD4 is below the CD8 cell count, has a lower prediction for the CD4 cells at the next follow-up visit, compared to a patient with equal CD4 count but with a higher CD4/CD8 ratio.

The regression coefficients for the remaining predictors are shown in the lower part of Table 3. In contrast to the predictors in the upper part, these predictors are not time dependent and have an effect on the predicted CD4 cell count level only. However, they do not imply differences in the decline or relapse of the outcome, as they do not include any interactions with time-dependent longitudinal predictors reported in the upper part of Table 3. If a patient had AIDS at the time of observation, the CD4 cell count will be lower. Also the patient's age and the duration of NRTI prior to cART have a negative level effect on the outcome. The estimates for the transmission group provides evidence that the heterosexual transmission categories (HET-male, HET-female) are different from the reference category (MSM) in the NAIVE group as the upper bound of the 95% CI is negative. For the cART group, the 95% CIs imply that, compared to the reference category (MSM), all other transmission categories have a lower CD4 cell count level. For the HCV predictor in the NAIVE group, there is no evidence for a difference between the 3 categories. However, in the cART group, the corresponding *P*-value is small, implying that at least 1 HCV category has a different CD4 level compared to the HCV negative reference category, which probably concerns the active HCV category. The Supplementary Material provides results for model M1 and M2, as well as additional information.

4. Discussion

Based on a large dataset from the Swiss HIV cohort study we have shown that CD8 cell counts contain crucial predictive

information for the HIV disease progression in drug naive patients and as well for the CD4 cell count recovery in patients receiving cART. Both lymphocyte cell subtypes, CD4 and CD8, as well as the RNA viral load, have been identified as important prognostic factors for the CD4 cell count over the next months. We could show that the model which includes the lagged levels of CD4 and CD8 as 2 separate predictors was inferior compared to an approach which included the lagged CD4/CD8 ratio instead of the CD8 level. These findings persisted, also if we applied alternative transformations to the lagged lymphocyte predictors, such as the log instead of the square root transformation. The CD4/CD8 ratio can be interpreted as a measure for the imbalance of the patient's immune system which captures essential information, additional to the cell count levels of both lymphocyte subtypes. The CD8 cell count is a marker for immune activation, which has been found to be an important factor for disease progression.^[38,39]

The relationship between the lagged CD4 cell count predictor, the lagged CD4/CD8 ratio predictor and the CD4 cell count at the next follow-up visit were found to be surprisingly similar for untreated patients and patients under a cART. This was illustrated by the response surface for the CD4 cell counts in Fig. 2 and the comparable estimates between NAIVE and cART for the lymphocyte predictors shown in Table 3.

Furthermore, we could quantify the relative importance of the 2 lymphocyte subtypes and the viral load as prognostic factors. It was previously found that the viral load only explains a low proportion (below 10%) of the variation for changes in CD4 cell counts over 1 year.^[6] Such a statement crucially depends on the outcome and time period for which the prediction is made. We found that even for NAIVE patients lagged CD8 cell counts are more important than lagged viral loads in explaining the variation in future CD4 cell counts. If the lagged RNA predictor is omitted then the marginal R^2 reduces from 31.7% to 27.2% in

the approach which includes lagged CD4 as predictor. For the cART group the marginal R^2 is even increasing for the same model formulation, if the viral load predictor is omitted. This reflects the fact that the association between CD4 cell counts and viral load is fundamentally different for treatment naive and patients under therapy. The improved marginal R^2 and also the difference in the regression coefficients of the lagged RNA for the NAIVE and cART group, supports the argument that although the viral load is a relevant predictor for future CD4 cell counts, it explains perhaps only a small proportion of the observed variation. As the plasma RNA load is often suppressed below the assay detection limit under cART we also examined the possible influence of recurrent detectable plasma RNA viral loads (“blips”), but no evidence for an additional effect was found.

By choosing a linear mixed model for the longitudinal SHCS data we could incorporate patient specific variation for the CD4 cell count at baseline and for its patient-specific time course. The applied model simplified the observation pattern, ignoring interval censored data and assumed regular follow-up times, although in the SHCS data there is notable variation for the time between 2 follow-up visits (see Supplementary Material, <http://links.lww.com/MD/B352>). Of course, causal pathways between the quantities of interest, CD4, CD8 and RNA, are inherently difficult to assess and not possible to estimate without a minimal set of assumptions. Nevertheless, we have derived a statistical model, which allowed us to address the clinically important question of how the patient’s immune system will evolve given the current state. It would be of interest to examine the sensitivity of the results with respect to the underlying assumptions. For example, an alternative analysis would incorporate the underlying rather than the observed lymphocyte and viral load measurements as predictors.^[40] These aspects may constitute different sources of a potential bias. As mentioned above the simplification of the temporal dependencies, the ignorance of a measurement error for laboratory analyses but also the characteristics of the sampling process, for example, sampling from different centers and regions, may implicitly introduce a bias in the analysis of this cohort study. To address these aspects, more investigations would be required and would result in more complex statistical models, which was beyond the scope of the presented analysis.

With this statistical analysis of a large HIV cohort, we could confirm the findings by other studies, which attributed an important role to CD8 cells for describing the HIV-1 disease progression. We were able to show that the CD4/CD8 ratio is an important time-dependent prognostic factor, for treatment naive and cART-treated patients. The SHCS data could be used for further investigations concerning the role of CD8 during an HIV-1 infection, which could reveal more details about the connections between HIV-1 disease progression and variation for the time needed to normalize the CD4/CD8 ratio. Elaborating such associations in more detail, also for interactions of other risk factors,^[41,42] would support the understanding of the mechanisms leading to the high variation in different individual immune responses.

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<http://www.shcs.ch/180-health-care-providers>). Members of the Swiss HIV Cohort Study are: Aubert V, Battegay M, Bernasconi E, Böni J, Braun DL, Bucher HC, Burton-Jeangros C, Calmy A, Cavassini M, Dollenmaier G, Egger M, Elzi L, Fehr J, Fellay J, Furrer H (Chairman of the Clinical and Laboratory Committee), Fux CA, Gorgievski M, Günthard H (President of the SHCS), Haerry D (deputy of “Positive Council”), Hasse B, Hirsch HH, Hoffmann M, Hösli I, Kahlert C, Kaiser L, Keiser O, Klimkait T, Kouyos R, Kovari H, Ledergerber B, Martinetti G, Martinez de Tejada B, Marzolini C, Metzner K, Müller N, Nadal D, Nicca D, Pantaleo G, Rauch A (Chairman of the Scientific Board), Regenass S, Rudin C (Chairman of the Mother & Child Substudy), Schöni-Affolter F (Head of Data Centre), Schmid P, Speck R, Stöckle M, Tarr P, Trkola A, Vernazza P, Weber R, Yerly S. (version June 2015).

References

- [1] Mellors JW, Kingsley LA, Rinaldo CR, et al. Quantitation of HIV-1 RNA in plasma predicts outcome after seroconversion. *Ann Int Med* 1995; 122:573–9.
- [2] Mellors JW, Rinaldo CR, Gupta P, et al. Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. *Science* 1996;272:1167–70.
- [3] Günthard H, Aberg J, Eron J, et al. Antiretroviral treatment of adult HIV infection: 2014 recommendations of the International Antiviral Society—USA Panel. *J Am Med Assoc* 2014;312:410–25.
- [4] Boscardin JW, Taylor JM, Law N. Longitudinal models for AIDS marker data. *Stat Methods Med Res* 1998;7:13–27.
- [5] Thiébaud R, Jacqmin-Gadda H, Babiker A, et al. Joint modelling of bivariate longitudinal data with informative dropout and left-censoring, with application to the evolution of CD4+ cell count and HIV RNA viral load in response to treatment of HIV infection. *Stat Med* 2005; 24:65–82.
- [6] Rodríguez B, Sethi A, Cheruvu V, et al. Predictive value of plasma HIV RNA level on rate of CD4 t-cell decline in untreated HIV infection. *J Am Med Assoc* 2006;296:1498–506.
- [7] Kaufmann GR, Furrer H, Ledergerber B, et al. Characteristics, determinants, and clinical relevance of CD4 T cell recovery to <500 cells/L in HIV type 1-infected individuals receiving potent antiretroviral therapy. *Clin Infect Dis* 2005;41:361–72.
- [8] Battegay M, Nüesch R, Hirschel B, et al. Immunological recovery and antiretroviral therapy in HIV-1 infection. *Lancet Infect Dis* 2006;6: 280–7.
- [9] Khanna N, Opravil M, Furrer H, et al. CD4+ T cell count recovery in HIV type 1-infected patients is independent of class of antiretroviral therapy. *Clin Infect Dis* 2008;47:1093–101.
- [10] Taylor JMG, Fahey JL, Detels R, et al. CD4 percentage, CD4 number, and CD4: CD8 ratio in HIV infection: which to choose and how to use. *J Acquir Immune Defic Syndr* 1989;2:114–24.
- [11] Giorgi JV, Liu Z, Hultin LE, et al. Elevated levels of CD38+ CD8+ T cells in HIV infection add to the prognostic value of low CD4+ T cell levels: results of 6 years of follow-up. *J Acquir Immune Defic Syndr* 1993;6: 904–12.
- [12] Benito JM, López M, Lozano S, et al. CD38 expression on CD81 T lymphocytes as a marker of residual virus replication in chronically HIV-infected patients receiving antiretroviral therapy. *AIDS Res Hum Retroviruses* 2004;20:227–33.
- [13] Cohen Stuart JWT, Hazebergh MD, Hamann D, et al. The dominant source of CD4+ and CD8+ T-cell activation in HIV infection is antigenic stimulation. *J Acquir Immune Defic Syndr* 2000;25:203–11.
- [14] Kolber MA, Saenz MO, Tanner TJ, et al. Intensification of a suppressive HAART regimen increases CD4 counts and decreases CD8+ T-cell activation. *Clin Immunol* 2008;126:315–21.
- [15] Kaufmann G, Perrin L, Pantaleo G, et al. CD4 T-lymphocyte recovery in individuals with advanced HIV-1 infection receiving potent antiretroviral therapy for 4 years: the Swiss HIV cohort study. *Arch Int Med* 2003;163:2187–95.
- [16] Margolick JB, Munoz A, Donnenberg AD, et al. Failure of T-cell homeostasis preceding AIDS in HIV-1 infection. *Nat Med* 1995;1: 674–80.
- [17] Mellors JW, Munoz A, Giorgi JV, et al. Plasma viral load and CD4+ lymphocytes as prognostic markers of HIV-1 infection. *Ann Int Med* 1997;126:946–54.

- [18] Tinago W, Coghlan E, Macken A, et al. Clinical, immunological and treatment-related factors associated with normalised CD4+/CD8+ T-cell ratio: effect of naive and memory T-cell subsets. *PLoS One* 2014;9:e97011.
- [19] Mussini C, Lorenzini P, Cozzi-Lepri A, et al. CD4/CD8 ratio normalisation and non-AIDS-related events in individuals with HIV who achieve viral load suppression with antiretroviral therapy: an observational cohort study. *Lancet HIV* 2015;2:e98–106.
- [20] Leung V, Gillis J, Raboud J, et al. Predictors of CD4:CD8 ratio normalization and its effect on health outcomes in the era of combination antiretroviral therapy. *PLoS One* 2013;8:e77665.
- [21] Le T, Wright EJ, Smith DM, et al. Enhanced CD4+ T-cell recovery with earlier HIV-1 antiretroviral therapy. *N Engl J Med* 2013;368:218–30.
- [22] Serrano-Villar S, Sainz T, Lee SA, et al. HIV-infected individuals with low CD4/CD8 ratio despite effective antiretroviral therapy exhibit altered T cell subsets, heightened CD8+ T cell activation, and increased risk of non-AIDS morbidity and mortality. *PLoS Pathog* 2014;10:10.
- [23] Serrano-Villar S, Moreno S, Fuentes-Ferrer M, et al. The CD4:CD8 ratio is associated with markers of age-associated disease in virally suppressed HIV-infected patients with immunological recovery. *HIV Med* 2014;15:40–9.
- [24] Alizon S, von Wyl V, Stadler T, et al. Phylogenetic approach reveals that virus genotype largely determines HIV set-point viral load. *PLoS Pathogens* 2010;6:6.
- [25] Fellay J, Ge D, Shianna K, et al. Common genetic variation and the control of HIV-1 in humans. *PLoS Genet* 2009;5:e100791.
- [26] Fraser C, Lythgoe K, Leventhal GE, et al. Virulence and pathogenesis of HIV-1 infection: an evolutionary perspective. *Science* 2014;343:6177.
- [27] SHCS. Cohort profile: The Swiss HIV Cohort Study. *Int J Epidemiol* 2010;39:1179–89.
- [28] Greub G, Ledergerber B, Battegay M, et al. Clinical progression, survival, and immune recovery during antiretroviral therapy in patients with HIV-1 and hepatitis C virus coinfection: the Swiss {HIV} Cohort Study. *Lancet* 2000;356:1800–5.
- [29] Laird NM, Ware JH. Random-effects models for longitudinal data. *Biometrics* 1982;38:963–74. <http://www.jstor.org/stable/2529876>
- [30] Taffé P, May M. A joint back calculation model for the imputation of the date of HIV infection in a prevalent cohort. *Stat Med* 2008;27:4835–53.
- [31] Nakagawa S, Schielzeth H. A general and simple method for obtaining R^2 from generalized linear mixed-effects models. *Methods Ecol Evol* 2013;4:133–42.
- [32] Johnson PC. Extension of Nakagawa & Schielzeth's R^2 GLMM to random slopes models. *Methods Ecol Evol* 2014;5:944–6.
- [33] Pauler DK. The Schwarz criterion and related methods for normal linear models. *Biometrika* 1998;85:13–27.
- [34] Braun J, Held L, Ledergerber B. The Swiss HIV Cohort study. Accounting for baseline differences and measurement error in the analysis of change over time. *Stat Med* 2014;33:2–16.
- [35] Hammer SM, Squires KE, Hughes MD, et al. A controlled trial of two nucleoside analogues plus Indinavir in persons with human immunodeficiency virus infection and CD4 cell counts of 200 per cubic millimeter or less. *N Engl J Med* 1997;337:725–33.
- [36] Kouyos RD, von Wyl V, Yerly S, et al. Molecular epidemiology reveals long-term changes in HIV type 1 subtype B transmission in Switzerland. *J Infect Dis* 2010;201:1488–97.
- [37] J. Pinheiro, D. Bates, S. DebRoy, D. Sarkar, R Core Team, nlme: Linear and Non-linear Mixed Effects Models, r package version 3.1-112 (2013).
- [38] Giorgi JV, Majchrowicz MA, Johnson TD, et al. Immunologic effects of combined protease inhibitor and reverse transcriptase inhibitor therapy in previously treated chronic HIV-1 infection. *AIDS* 1998;14:1833–44.
- [39] Douek DC, Picker LJ, Koup RA. T cell dynamics in HIV-1 infection. *Ann Rev Immunol* 2003;21:265–304.
- [40] Muff S, Riebler A, Held L, et al. Bayesian analysis of measurement error models using integrated nested Laplace approximations. *J R Stat Soc C (Appl Stat)* 2015;64:231–52.
- [41] Panagiotakis SH, Soufla G, Baritaki S, et al. Concurrent CMV and EBV DNAemia is significantly correlated with a delay in the response to HAART in treatment-naïve HIV type 1-positive patients. *AIDS Res Hum Retroviruses* 2007;23:10–8.
- [42] Ferraz da Silva A, Giron L, Ramos da Silva S, et al. Human gammaherpesviruses viraemia in HIV infected patients. *J Clin Pathol* 2015;68:726–32.