

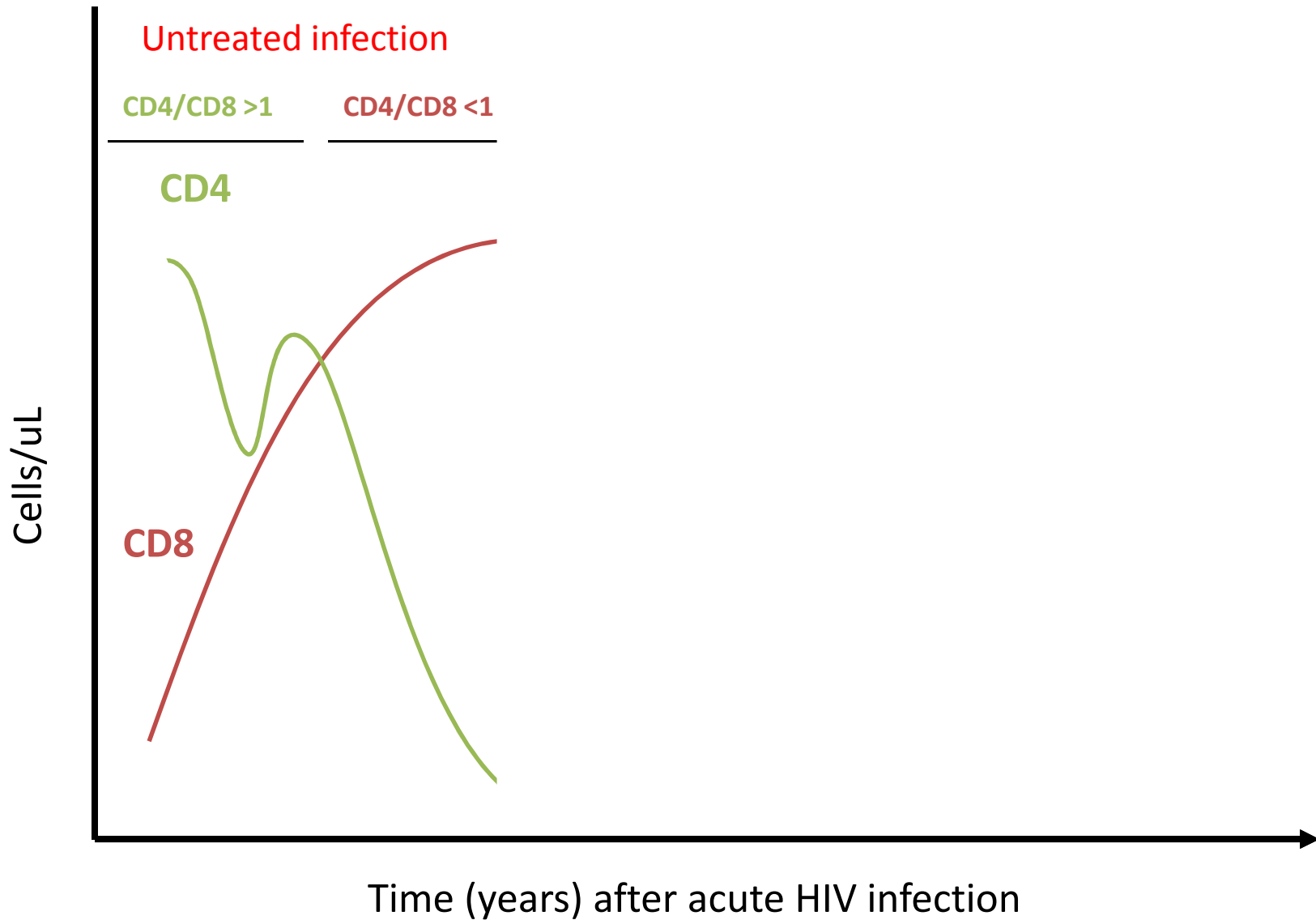
HIV-Infected Individuals with Low CD4/CD8 Ratio despite Effective Antiretroviral Therapy Exhibit Altered T Cell Subsets, Heightened CD8+ T Cell Activation, and Increased Risk of Non-AIDS Morbidity and Mortality

Sergio Serrano-Villar , Talia Sainz, Sulggi A. Lee, Peter W. Hunt, Elizabeth Sinclair, Barbara L. Shacklett, April L. Ferre, Timothy L. Hayes, Ma Somsouk, Priscilla Y. Hsue, Mark L. Van Natta, Curtis L. Meinert, Michael M. Lederman, Hiroyu Hatano, Vivek Jain, Yong Huang, Frederick M. Hecht, Jeffrey N. Martin, Joseph M. McCune, Santiago Moreno, Steven G. Deeks

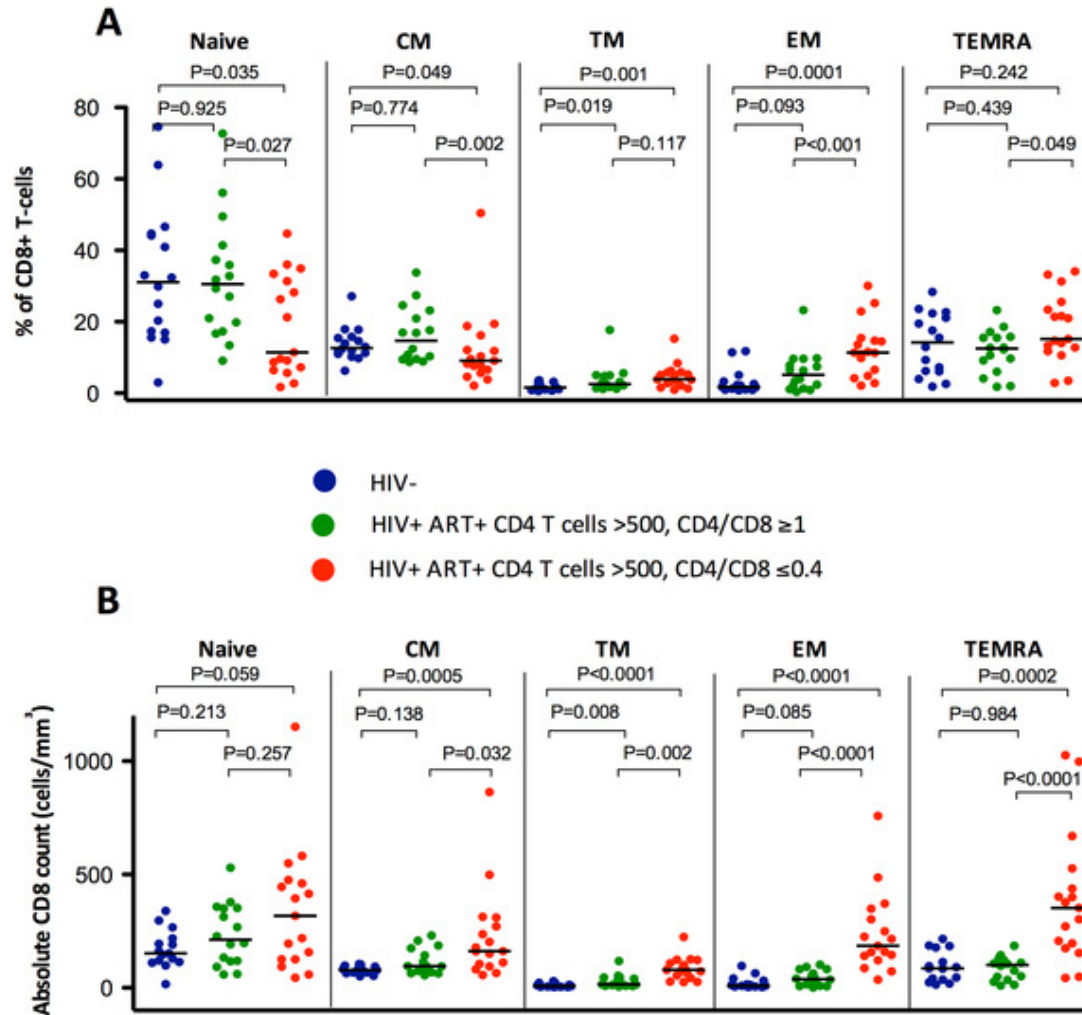
instituto ramón y cajal
de investigación sanitaria



CD4/CD8 Dynamics during HIV infection



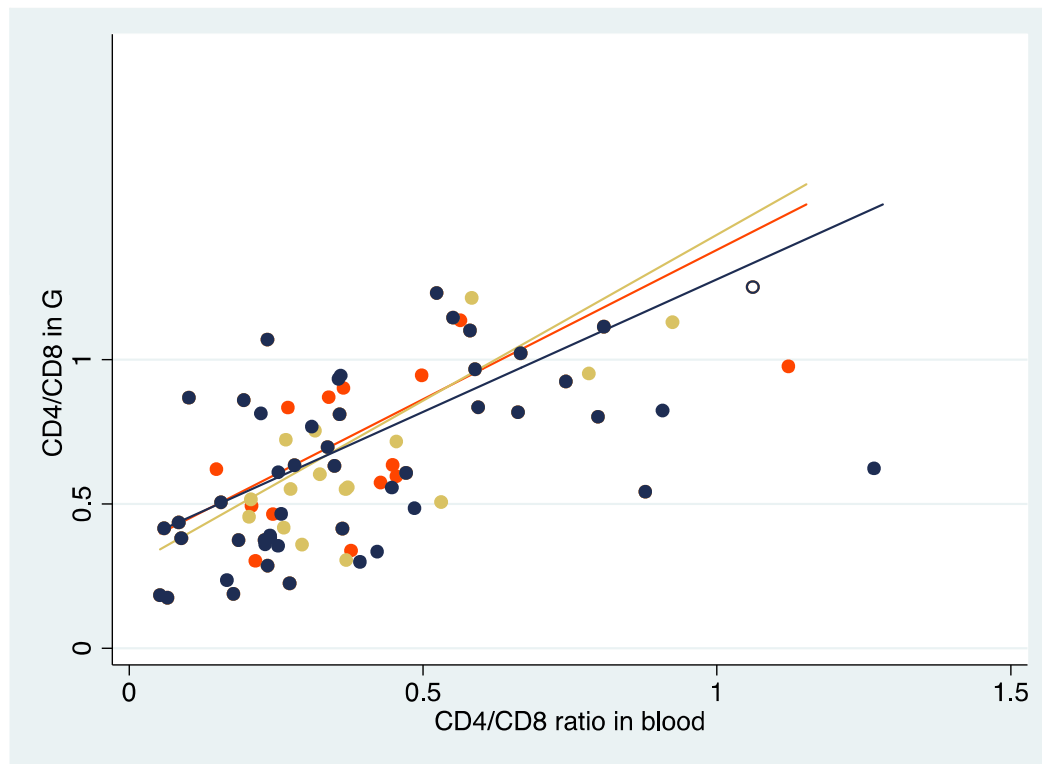
Percentages and absolute counts of CD8+ T cell maturation subsets among HIV-/CMV+ individuals and ART-suppressed HIV-infected patients with CD4 counts >500 cells/mm³ stratified by a normal or low CD4/CD8 ratio.



Serrano-Villar S, Sainz T, Lee SA, Hunt PW, Sinclair E, et al. (2014) 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* 10(5): e1004078. doi:10.1371/journal.ppat.1004078

<http://journals.plos.org/plospathogens/article?id=info:doi/10.1371/journal.ppat.1004078>

Correlations between the CD4/CD8 ratio in blood and in GALT in 32 subjects participating in the two clinical trials



Baseline
rho 0.680, P<0.0001
Week 24
rho 0.692, P<0.0001
Week 36
rho=0.649, P=0.0003

Each 10% increase
in the CD4/CD8
ratio in blood
predicted a 5.7%
increase in the
CD4/CD8 ratio in
GALT

Calculating mean change in variable A per change in variable B in long panels

```
. xtmixed logpcd4cd8_g logpcd4cd8 i.order || pid:, reml
```

Wald chi2(3) = 70.21
Prob > chi2 = 0.0000
Log restricted-likelihood = 31.186722

logpcd4cd8_g	Coef.	Std. Err.	z	P> z	[95% Conf. Interval]
logpcd4cd8	.5243698	.0630652	8.31	0.000	.4007644 .6479753
order					
2	.0048172	.0306126	0.16	0.875	-.0551823 .0648168
3	.0546903	.0342725	1.60	0.111	-.0124825 .1218632
_cons	.026068	.0323162	0.81	0.420	-.0372705 .0894065

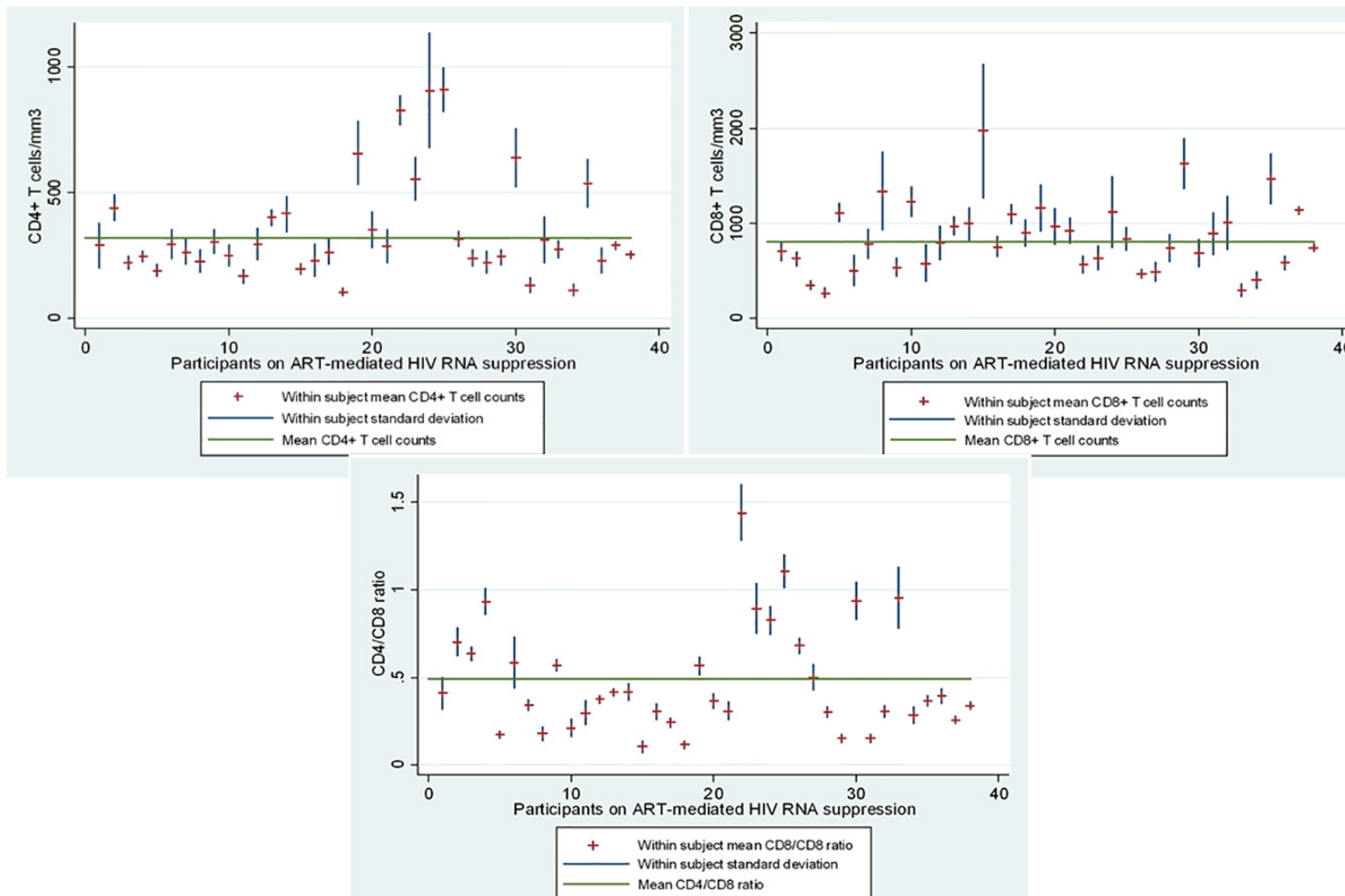
```
. nlcom 100*(exp(_b[logpcd4cd8]*log(1.10))-1)
```

_nl_1: 100*(exp(_b[logpcd4cd8]*log(1.10))-1)

logpcd4cd8_g	Coef.	Std. Err.	z	P> z	[95% Conf. Interval]
_nl_1	5.124774	.6318791	8.11	0.000	3.886314 6.363234

Interpretation: there is a 5% increase in A per 10% increase in B

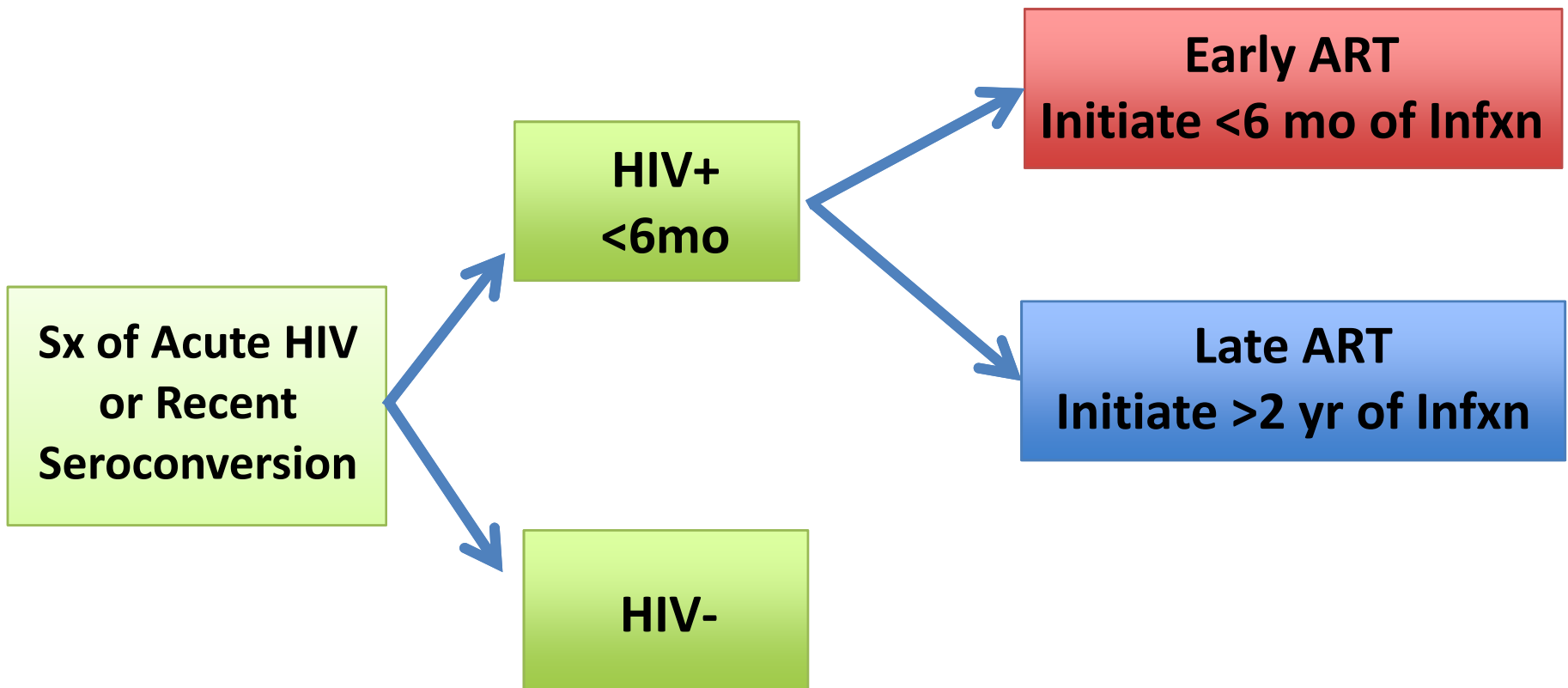
Coefficient of variation (within subject standard deviation (blue lines)/within subject mean (red plus symbols) for three different biomarkers



```
twoway (rspike inf_sdc4 sup_sdc4 numpacPBO) (scatter meancd4 numpacPBO,  
msymbol(plus)) (scatteri 318 0 318 38, recast(line))
```

Significantly lower for the CD4/CD8 ratio (12%) compared to CD4+ T cell counts (16%, $P = 0.017$) and for CD8+ T cell counts (18%, $P = 0.001$).

OPTIONS Cohort: Early vs. Late ART Initiation



	id	dob	group	groupcode	groupmerged	chronicunrx	chronicrx	aviremia	groupkitche
1	OPT1338	10/10/1977	Acute/Recent	1	1	0	0	0	Acute/Rec
2	OPT1338	10/10/1977	UnRx One Year	3	3	0	0	0	
3	OPT1338	10/10/1977	UnRx Late	4	3	1	0	0	Chronic Untrea
4	OPT1341	11/29/1967	Acute/Recent	1	1	0	0	0	Acute/Rec
5	OPT1341	11/29/1967	UnRx One Year	3	3	0	0	0	
6	OPT1341	11/29/1967	UnRx Late	4	3	1	0	0	Chronic Untrea
7	OPT1346	1/27/1949	Acute/Recent	1	1	0	0	0	Acute/Rec
8	OPT1346	1/27/1949	Late HAART Pre-HAART Baseline	4	3	1	0	0	Chronic Untrea
9	OPT1346	1/27/1949	Late HAART One Year RX	8	5	0	0	0	
10	OPT1346	1/27/1949	Late HAART Late RX	9	5	0	1	1	HAART ?
11	OPT1355	9/11/1980	Acute/Recent	1	1	0	0	0	Acute/Rec
12	OPT1355	9/11/1980	UnRx One Year	3	3	0	0	0	
13	OPT1355	9/11/1980	UnRx Late	4	3	1	0	0	Chronic Untrea
14	OPT1356	12/28/1970	Acute/Recent	1	1	0	0	0	Acute/Rec
15	OPT1356	12/28/1970	UnRx One Year	3	3	0	0	0	
16	OPT1356	12/28/1970	UnRx Late	4	3	1	0	0	Chronic Untrea
17	OPT1357	11/6/1967	Acute/Recent	1	1	0	0	0	Acute/Rec
18	OPT1357	11/6/1967	UnRx One Year	3	3	0	0	0	
19	OPT1357	11/6/1967	UnRx Late	4	3	1	0	0	Chronic Untrea
20	OPT1359	3/11/1964	Acute/Recent	1	1	0	0	0	Acute/Rec
21	OPT1359	3/11/1964	Early HAART One Year RX	6	4	0	0	0	
22	OPT1359	3/11/1964	Early HAART Late RX	7	4	0	1	1	HAART ?
23	OPT1360	2/21/1965	Acute/Recent	1	1	0	0	0	Acute/Rec
24	OPT1360	2/21/1965	Early HAART One Year RX	6	4	0	0	0	
25	OPT1360	2/21/1965	Early HAART Late RX	7	4	0	1	1	HAART ?
26	OPT1372	2/15/1950	Acute/Recent	1	1	0	0	0	Acute/Rec
27	OPT1372	2/15/1950	Early HAART One Year RX	6	4	0	0	0	
28	OPT1372	2/15/1950	Early HAART Late RX	7	4	0	1	1	HAART ?
29	OPT1374	9/2/1970	Acute/Recent	1	1	0	0	0	Acute/Rec
30	OPT1374	9/2/1970	UnRx One Year	3	3	0	0	0	
31	OPT1374	9/2/1970	UnRx Late	4	3	1	0	0	Chronic Untrea

Snapshots


```
. xtmixed cd4cd8 age sex basecd4 b4.momento##early2 if optionsmix==1 & early!=".&cmvstatusum==1|| idnum:, reml
note: 2.momento#1.early2 identifies no observations in the sample
```

```
Mixed-effects REML regression      Number of obs      =      215
Group variable: idnum              Number of groups   =       63

                                   Obs per group: min =       2
                                   avg =       3.4
                                   max =       4

                                   Wald chi2(9)      =      181.91
Log restricted-likelihood = -76.986956  Prob > chi2       =      0.0000
```

cd4cd8	Coef.	Std. Err.	z	P> z	[95% Conf. Interval]	
age	.0119125	.0052879	2.25	0.024	.0015484	.0222766
sex	-.4163314	.3403247	-1.22	0.221	-1.083355	.2506927
basecd4	.0005208	.0002023	2.57	0.010	.0001243	.0009173
momento						
1	-.0245236	.0750225	-0.33	0.744	-.171565	.1225177
2	-.4374237	.0692874	-6.31	0.000	-.5732245	-.301623
3	-.2155441	.0677131	-3.18	0.001	-.3482594	-.0828288
1.early2	.4139494	.1026112	4.03	0.000	.212835	.6150637
momento#early2						
1 1	-.4402655	.0902688	-4.88	0.000	-.617189	-.263342
2 1	0	(empty)				
3 1	.1464579	.0889053	1.65	0.099	-.0277933	.320709
_cons	.8291973	.7728747	1.07	0.283	-.6856094	2.344004

Stata FAQ

How can I graph the results of the margins command? (Stata 12)

Graphing results from the **margins** command can help in the interpretation of your model. Stata 12 introduced the **marginsplot** command which make the graphing process very easy. Let's start off with an easy example.

Example 1

The first example is a 3x2 factorial analysis of covariance. We will run the model using **anova** but we would get the same results if we ran it using **regression**.

```
use http://www.ats.ucla.edu/stat/data/hsbdemo, clear
anova write prog##female math read
```

```
. estimates store m1
```

```
.
end of do-file
```

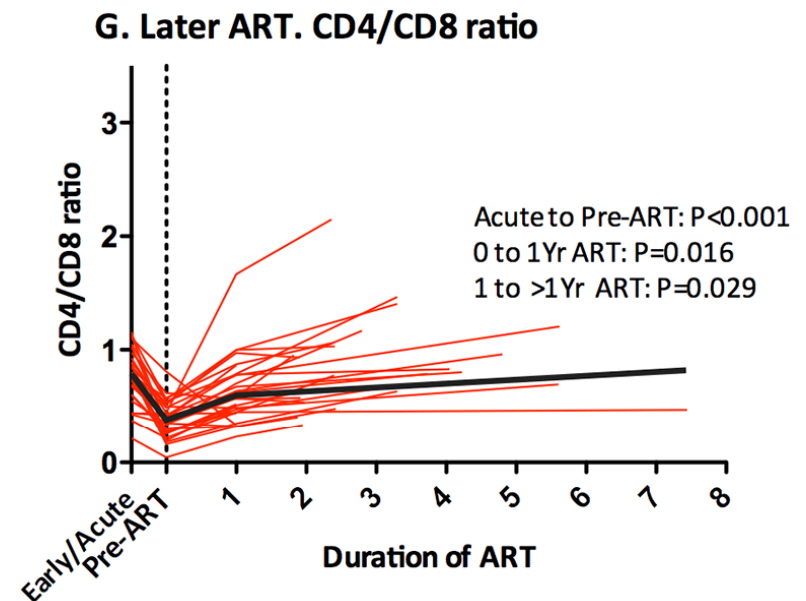
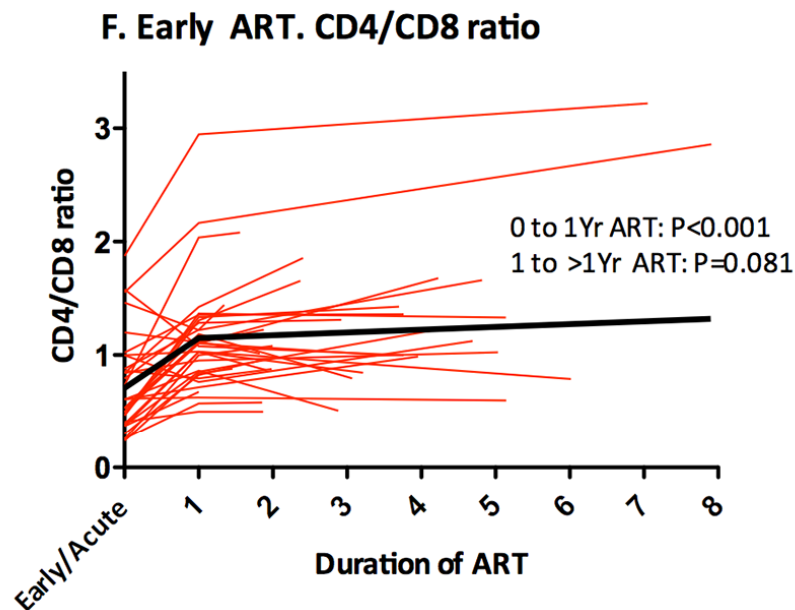
```
. margins momentoearly#early2, asbalanced post
```

```
Adjusted predictions          Number of obs   =          195
```

```
Expression   : Linear prediction, predict()
```

		Delta-method				
		Margin	Std. Err.	z	P> z	[95% Conf. Interval]
momentoearly#early2						
1	0	.7589109	.0782048	9.70	0.000	.6056322 .9121896
1	1	.7114771	.0737322	9.65	0.000	.5669646 .8559896
2	0	.6314659	.0782048	8.07	0.000	.4781872 .7847446
2	1	1.148721	.0758698	15.14	0.000	1.000019 1.297423
3	0	.8731835	.0884787	9.87	0.000	.6997685 1.046599
3	1	1.321746	.0737322	17.93	0.000	1.177233 1.466258

Impact of early or later ART CD4/CD8 ratio in the OPTIONS cohort.

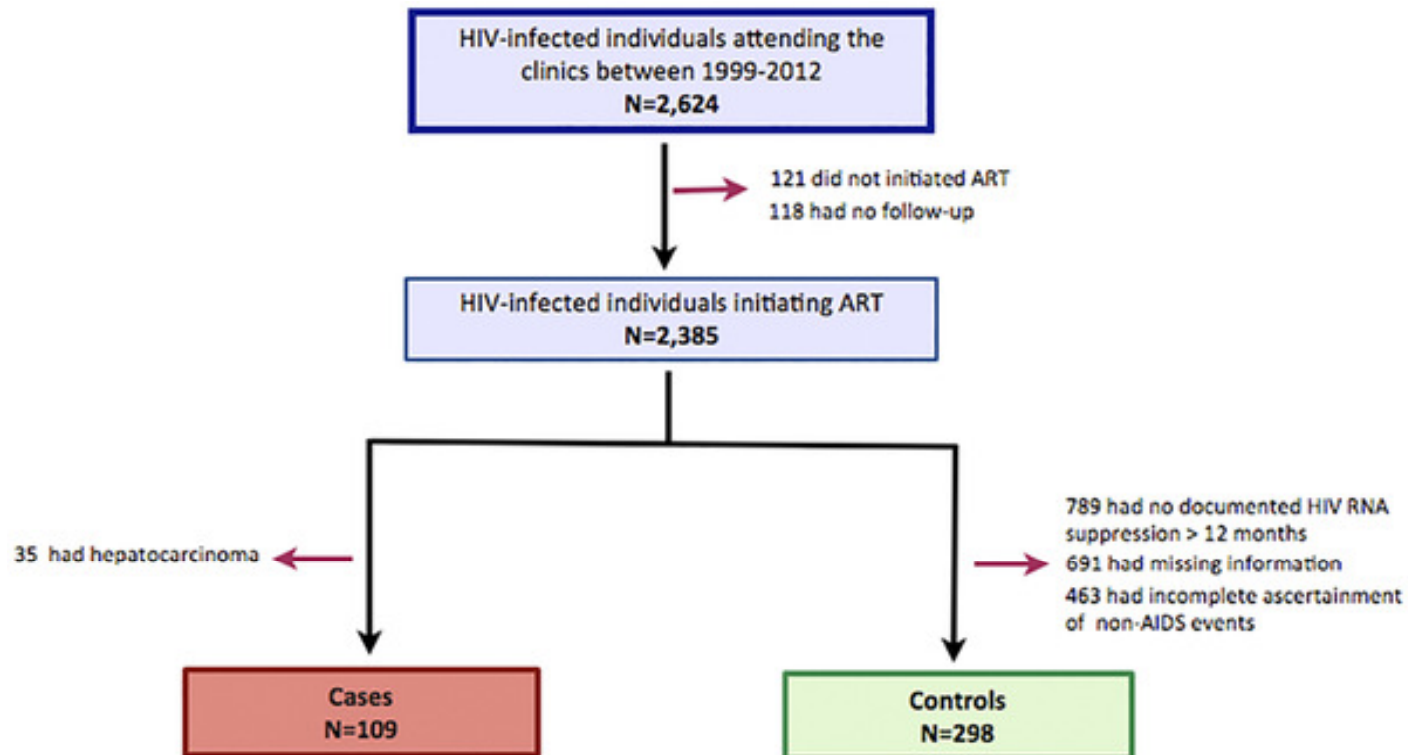


The greater effect of early ART compared to later ART on the CD4/CD8 ratio remained statistically significant after adjustment by age, gender, and baseline CD4+ T cell counts in the mixed-effects linear model. The mean CD4/CD8 ratio change predicted by the model was significantly higher among early ART initiators compared to later initiators after one year of ART (+0.44 vs. +0.25, respectively, $P < 0.001$), and after a median of 3 years of ART (+0.61 vs. +0.49, respectively, $P < 0.001$).

Serrano-Villar S, Sainz T, Lee SA, Hunt PW, Sinclair E, et al. (2014) 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* 10(5): e1004078. doi:10.1371/journal.ppat.1004078

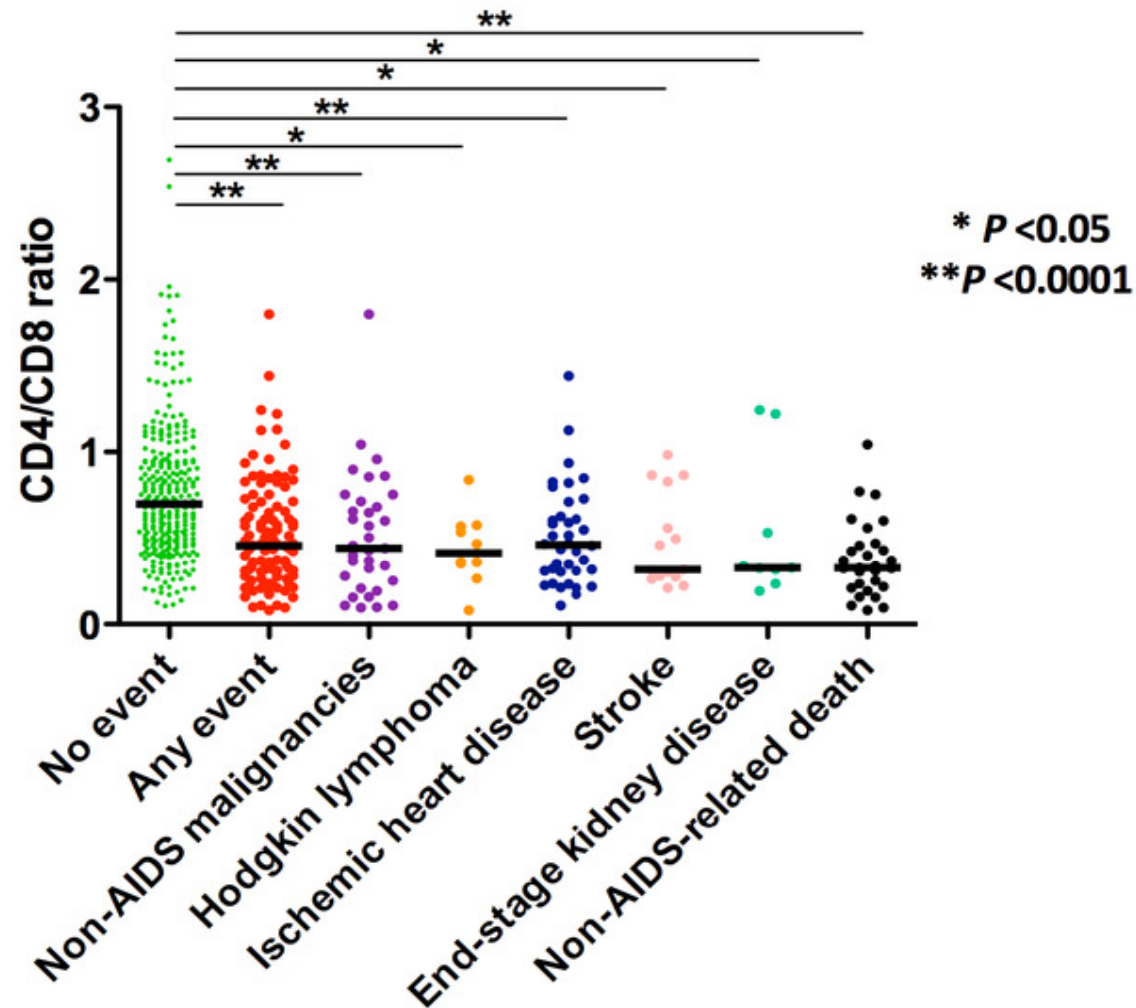
<http://journals.plos.org/plospathogens/article?id=info:doi/10.1371/journal.ppat.1004078>

Selection of study participants



Serrano-Villar S, Pérez-Elías MJ, Dronda F, Casado JL, Moreno A, et al. (2014) Increased Risk of Serious Non-AIDS-Related Events in HIV-Infected Subjects on Antiretroviral Therapy Associated with a Low CD4/CD8 Ratio. PLoS ONE 9(1): e85798. doi:10.1371/journal.pone.0085798
<http://journals.plos.org/plosone/article?id=info:doi/10.1371/journal.pone.0085798>

CD4/CD8 ratio according to the presence and type of event



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<http://journals.plos.org/plosone/article?id=info:doi/10.1371/journal.pone.0085798>

Madrid Cohort: Association with non-AIDS events

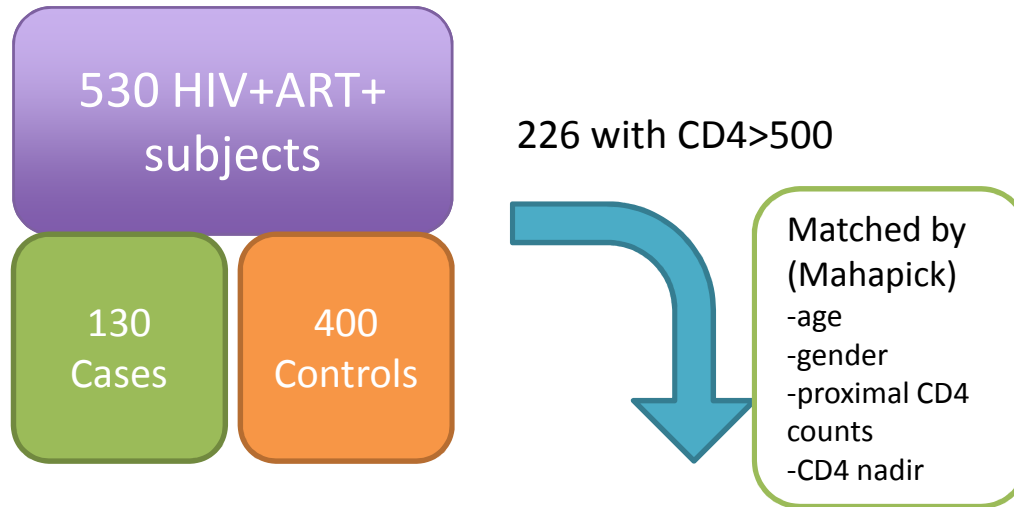


Table 5. General characteristics of participants in the Madrid cohort nested study

	Cases n=33	Controls n=33	P value
Male gender (No., %)	27 (82%)	27 (82%)	-
Age (years, IQR)	46 (41, 49)	43 (41, 47)	0.682
CD4+ Count (cells/mm ³ , IQR)	666 (580, 875)	714 (583, 837)	0.841
CD8+ Count (cells/mm ³ , IQR)	1239 (974, 1494)	897 (687, 1108)	0.002
CD4/CD8 ratio (IQR)	0.55 (0.44, 0.75)	0.81 (0.67, 0.94)	0.002
HIV RNA Level, log ₁₀ copies/mL	<1.6	<1.6	-
Nadir CD4+ Count (cells/mm ³ , IQR)	221 (80, 303)	213 (114, 289)	0.680
Cumulative ART exposure (cells/mm ³ , IQR)	10 (6, 12)	6 (5, 7)	0.001
HCV seropositivity (No, %)	7 (21%)	5 (15%)	0.523

Conditional regression logistic
(adjusted by ART exposure):
Per each CD4/CD8 qrt. decrease, five-fold increased odds of serious non-AIDS events
(OR: 4.8, 95% CI, 1.2, 19.5)

Cases: myocardial infarction (12 cases), solid-organ neoplasias (11 cases), stroke (5 cases), end-stage liver disease (4 cases) and end-stage renal disease (1 subject).

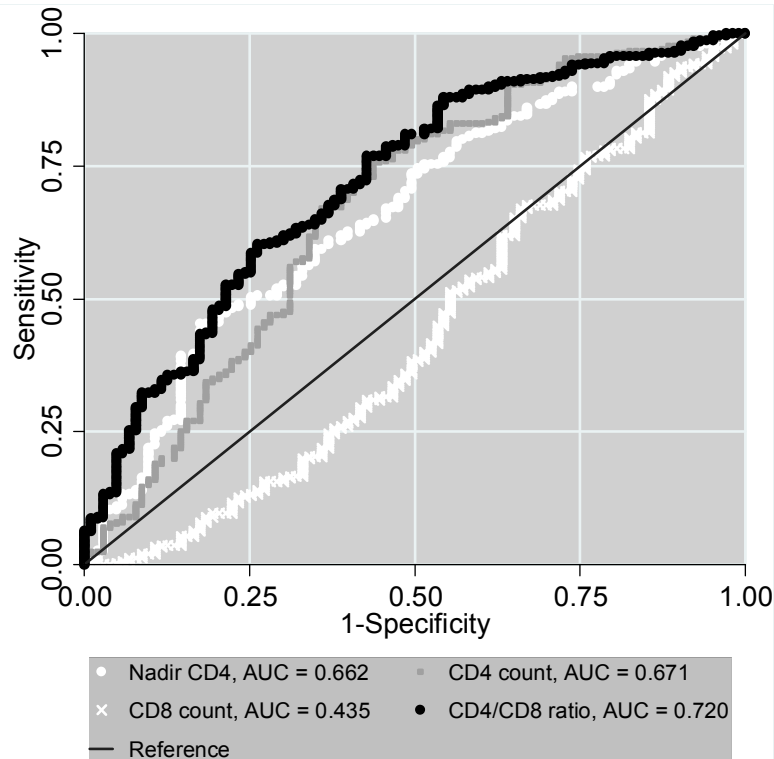
General characteristics of the study population

Study sample			
N = 407			
	Cases	Controls	P value
	N = 109	N = 298	
Sex, %			0.468
Male	77.9	81.1	
Female	22.1	18.8	
Age*	46 (43–49)	41 (34–47)	<0.0001
Geographic Origin, %			0.301
Western Europe	70.9	90.5	
South America	20.3	–	
Africa	5.8	9.5	
Other	3.1	–	
Risk category, %			0.098
Previous IDU	48.4	32.9	
Heterosexual	23.8	19.9	
Homosexual	21.3	40.8	
Other/Unknown	6.5	6.6	
CDC HIV Classification, %			0.008
A	27.5	61.2	
B	37.2	18.8	
C	33.3	19.9	
Cumulative ART exposure (years)*	9.7 (5.5–13.6)	3.9 (2.3–5.4)	<0.0001
CD4 nadir (cell/μL)*	124 (33–221)	219 (107–303)	<0.0001
CD4+ count (cell/μL)*	371 (225–586)	536 (407–678)	<0.0001
CD8+ count (cell/μL)*	880 (582–1185)	791 (588–1053)	0.007
CD4/CD8 ratio*	0.44 (0.30–0.68)	0.70 (0.48–0.94)	<0.0001

Serrano-Villar S, Pérez-Elías MJ, Dronda F, Casado JL, Moreno A, et al. (2014) Increased Risk of Serious Non-AIDS-Related Events in HIV-Infected Subjects on Antiretroviral Therapy Associated with a Low CD4/CD8 Ratio. PLoS ONE 9(1): e85798. doi:10.1371/journal.pone.0085798
<http://journals.plos.org/plosone/article?id=info:doi/10.1371/journal.pone.0085798>

Results

AUC: CD4/CD8 ratio Vs. Non-AIDS events



P < 0.0001
Best cut-off: 0.4

Logistic Regression: CD4/CD8 ratio (<0.4 vs. ≥0.4) association with non-AIDS events

	Adjusted OR	CI 95%	P
All	10.3	5.0 – 21.2	<0.0001
Subgroups			
CD4 >200 cels/μL	10.4	4.6 – 23.5	<0.0001
CD4 >350 cels/μL	17.5	5.7 – 53.3	<0.0001
CD4 >500 cels/μL	14.5	2.8 – 73.9	0.001
Nadir CD4 <200	12.7	4.7 – 34.7	<0.0001

Maximum model: age, sex, ART exposure, nadir CD4, CD4/CD8 ratio, CD4/CD8 ratio*CD4 nadir interaction. Only ART exposure was retained in the adjusted model as a potential confounder.

Adjusted CD4/CD8 ratio for non-AIDS mortality:

OR, 12.8; 95% CI, 3.6–45.1; P<0.0001

help for **mahapick**

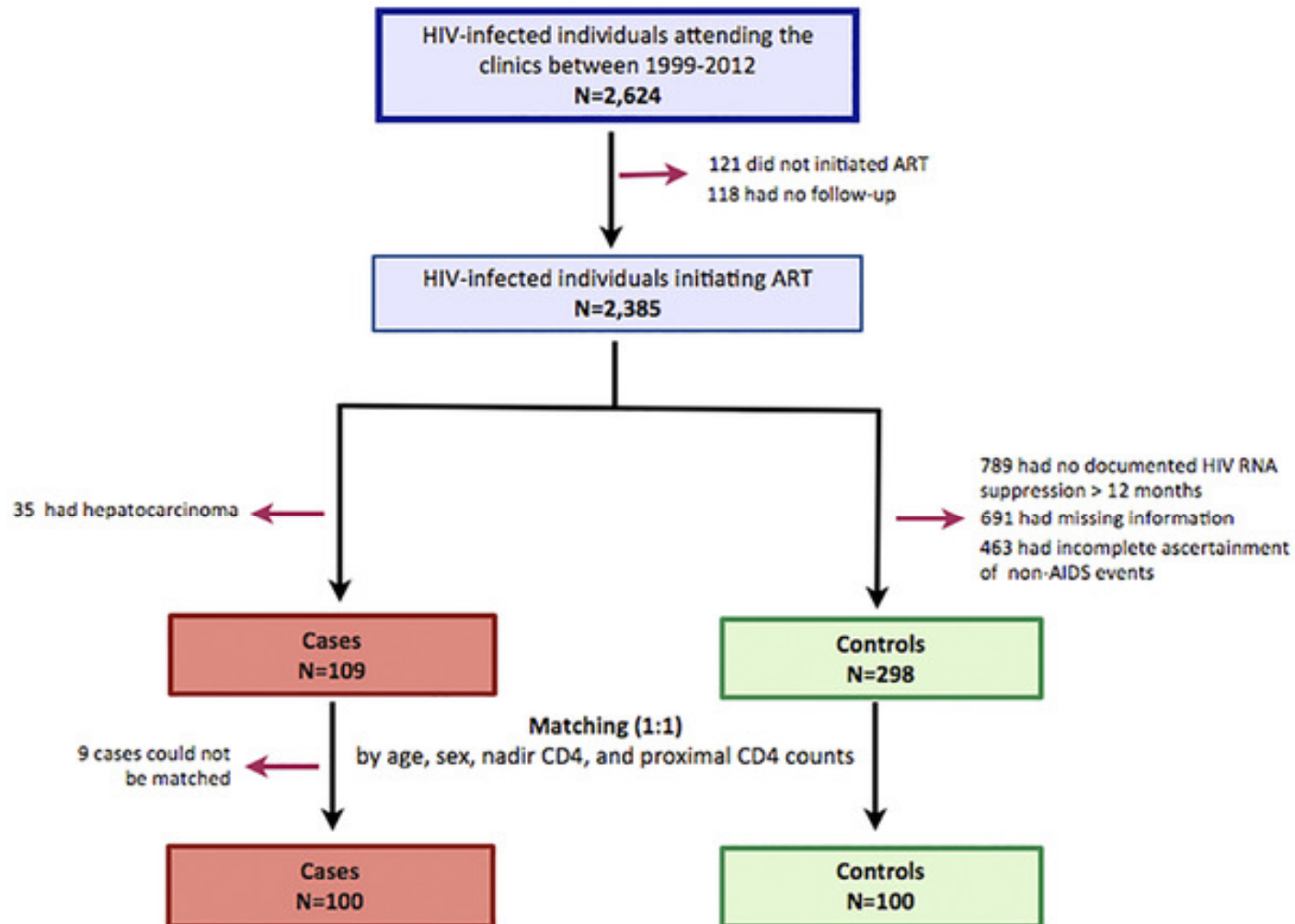
Select matching observations based on a Mahalanobis scoring

```
mahapick varlist [weight] , idvar(idvarname) treated(treatedvar) [  
  pickids(pickidvars) genfile(filename) replace  
  prime_id(prime_id_var) matchnum(matchnum_var) nummatches(#)  
  full matchon(matchonvars) sliceby(slicebyvars) clear fast  
  score scorevar(scorevarname) all unsguared euclidean  
  display(display_options) float nocovtrlimitation ]
```

Description

mahapick seeks matching observations for a set of "treated" observations, using a Mahalanobis distance measure which it calculates.

Selection of study participants



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<http://journals.plos.org/plosone/article?id=info:doi/10.1371/journal.pone.0085798>

General characteristics of the study population and in the nested case/control study.

	Study sample			Matched Study		
	N = 407			N = 200		
	Cases	Controls	P value	Cases	Controls	P value
	N = 109	N = 298		N = 100	N = 100	
Sex, %			0.468			1.0
Male	77.9	81.1		81.0	81.0	
Female	22.1	18.8		19.0	19.0	
Age*	46 (43–49)	41 (34–47)	<0.0001	46 (42–50)	46 (42–50)	1.0
Geographic Origin, %			0.301			0.154
Western Europe	70.9	90.5		80.0	93.0	
South America	20.3	–		15.0	–	
Africa	5.8	9.5		3.0	7.0	
Other	3.1	–		2.0	–	
Risk category, %			0.098			0.104
Previous IDU	48.4	32.9		47.6	29.2	
Heterosexual	23.8	19.9		28.6	19.8	
Homosexual	21.3	40.8		14.3	42.7	
Other/Unknown	6.5	6.6		9.5	8.3	
CDC HIV Classification, %			0.008			0.242
A	27.5	61.2		33.6	42.3	
B	37.2	18.8		31.1	27.4	
C	33.3	19.9		36.3	30.3	
Cumulative ART exposure (years)*	9.7 (5.5–13.6)	3.9 (2.3–5.4)	<0.0001	9.8 (5.4–13.5)	4.3 (2.4–6.2)	<0.0001
CD4 nadir (cell/μL)*	124 (33–221)	219 (107–303)	<0.0001	121 (40–231)	120 (46–233)	0.9105
CD4+ count (cell/μL)*	371 (225–586)	536 (407–678)	<0.0001	404 (260–588)	415 (173–589)	0.689
CD8+ count (cell/μL)*	880 (582–1185)	791 (588–1053)	0.007	925 (636–1245)	715 (501–978)	0.002
CD4/CD8 ratio*	0.44 (0.30–0.68)	0.70 (0.48–0.94)	<0.0001	0.46 (0.29–0.66)	0.62 (0.40–0.90)	0.001

Serrano-Villar S, Pérez-Elías MJ, Dronda F, Casado JL, Moreno A, et al. (2014) Increased Risk of Serious Non-AIDS-Related Events in HIV-Infected Subjects on Antiretroviral Therapy Associated with a Low CD4/CD8 Ratio. PLoS ONE 9(1): e85798. doi:10.1371/journal.pone.0085798
<http://journals.plos.org/plosone/article?id=info:doi/10.1371/journal.pone.0085798>

Conditional logistic regression in a case-control nested study

```
. clogit case logcd4cd8, group(setidtxt)
note: 2 groups (4 obs) dropped because of all positive or
      all negative outcomes.

Iteration 0:   log likelihood = -61.880912
Iteration 1:   log likelihood = -61.685324
Iteration 2:   log likelihood = -61.684833
Iteration 3:   log likelihood = -61.684833

Conditional (fixed-effects) logistic regression   Number of obs   =       179
                                                    LR chi2(1)      =         7.18
                                                    Prob > chi2     =         0.0074
Log likelihood = -61.684833                       Pseudo R2      =         0.0550
```

case	Coef.	Std. Err.	z	P> z	[95% Conf. Interval]	
logcd4cd8	-1.387275	.5518674	-2.51	0.012	-2.468915	-.3056347

Risk of serious non-AIDS events associated with a low CD4/CD8

Explanatory logistic regression (All sample)	4 th Qrt.	3 rd Qrt.		2 nd Qrt.		1 st Qrt.		OR per Qrt. Decrease		CD4/CD8 ratio <0.4	
		OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
#Cases/Controls	11/91	19/83		31/71		48/53		109/298		109/298	
Unadjusted	1.0	1.89 (0.85, 4.21)		3.61 (1.69, 7.68)		7.49 (3.58, 15.66)		1.96 (1.57, 2.45)		4.79 (2.91, 7.90)	
Adjusted*	1.0	2.57 (0.63, 10.54)		10.61 (2.78, 40.50)		15.77 (3.65, 68.18)		2.56 (1.66, 3.94)		5.11 (2.31, 12.42)	
#Cases/Controls in subjects with nadir <200	4/24	13/35		19/39		34/41		70/139		70/139	
Unadjusted	1.0	2.23 (0.65, 7.66)		2.92 (0.89, 9.62)		4.98 (1.57, 15.74)		1.61 (1.19, 2.17)		2.91 (1.59, 5.35)	
Adjusted*	1.0	1.45 (0.14, 15.09)		6.82 (0.75, 62.30)		15.49 (1.53, 156.16)		2.77 (1.41, 5.42)		7.90 (2.37, 26.38)	
#Cases/Controls in subjects with CD4>350	10/86	15/75		17/64		17/18		59/243		59/243	
Unadjusted	1.0	1.72 (0.73, 4.05)		2.28 (0.98, 5.32)		8.12 (3.19, 20.62)		1.88 (1.40, 2.53)		7.16 (3.24, 15.83)	
Adjusted*	1.0	1.48 (0.14, 15.55)		8.40 (0.92, 76.06)		15.03 (1.49, 150.75)		2.67 (1.38, 5.16)		6.57 (2.04, 21.14)	
Conditional logistic regression (Matched analysis) [†]	4 th Quartile	3 rd Quartile		2 nd Quartile		1 st Quartile		OR per Quartile Decrease		CD4/CD8 ratio <0.4	
		OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
#Cases/Controls	17/33	25/25		24/26		34/16		100/100		100/100	
Primary	1.0	2.45 (1.02, 5.86)		2.28 (0.92, 5.67)		11.46 (3.20, 40.97)		1.95 (1.32, 2.73)		3.43 (1.48, 7.96)	
Adjusted [§]	1.0	3.04 (0.45, 20.43)		3.56 (0.83, 32.57)		31.99 (2.70, 378.85)		2.89 (1.32, 6.17)		5.43 (1.89, 26.99)	

Seven subjects from the 407 individuals were not included in the multivariate analysis due to missing data on CD4 nadir/cumulative ART exposure.

*Multivariate analysis adjusted by age, gender, nadir CD4+ cell count, date of ART initiation and cumulative ART exposure.

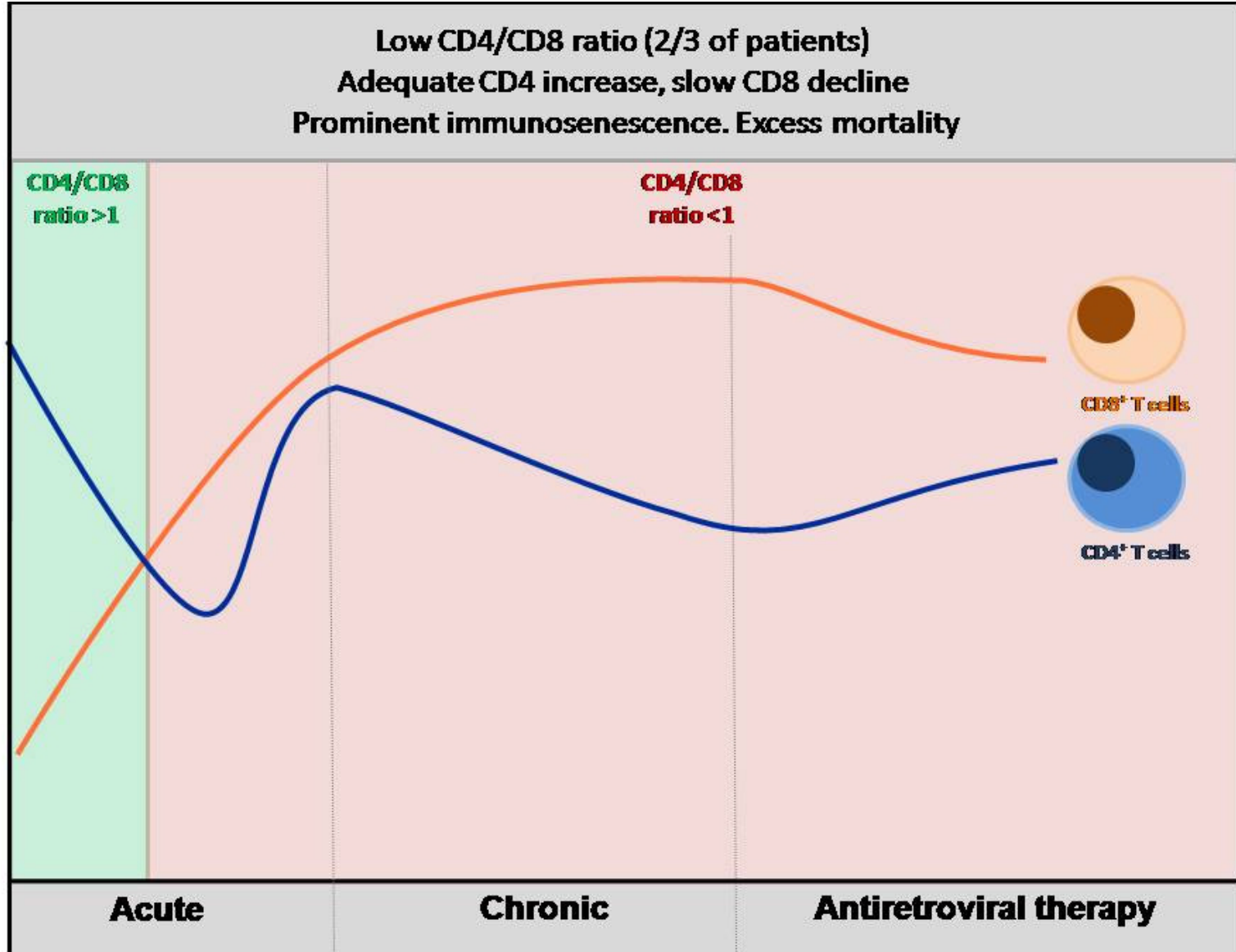
[†]Case-control substudy matched by age, gender, nadir CD4+ cell count and proximal CD4+ cell count.

[§]Conditional logistic regression analysis adjusted for date of ART initiation and cumulative ART exposure.

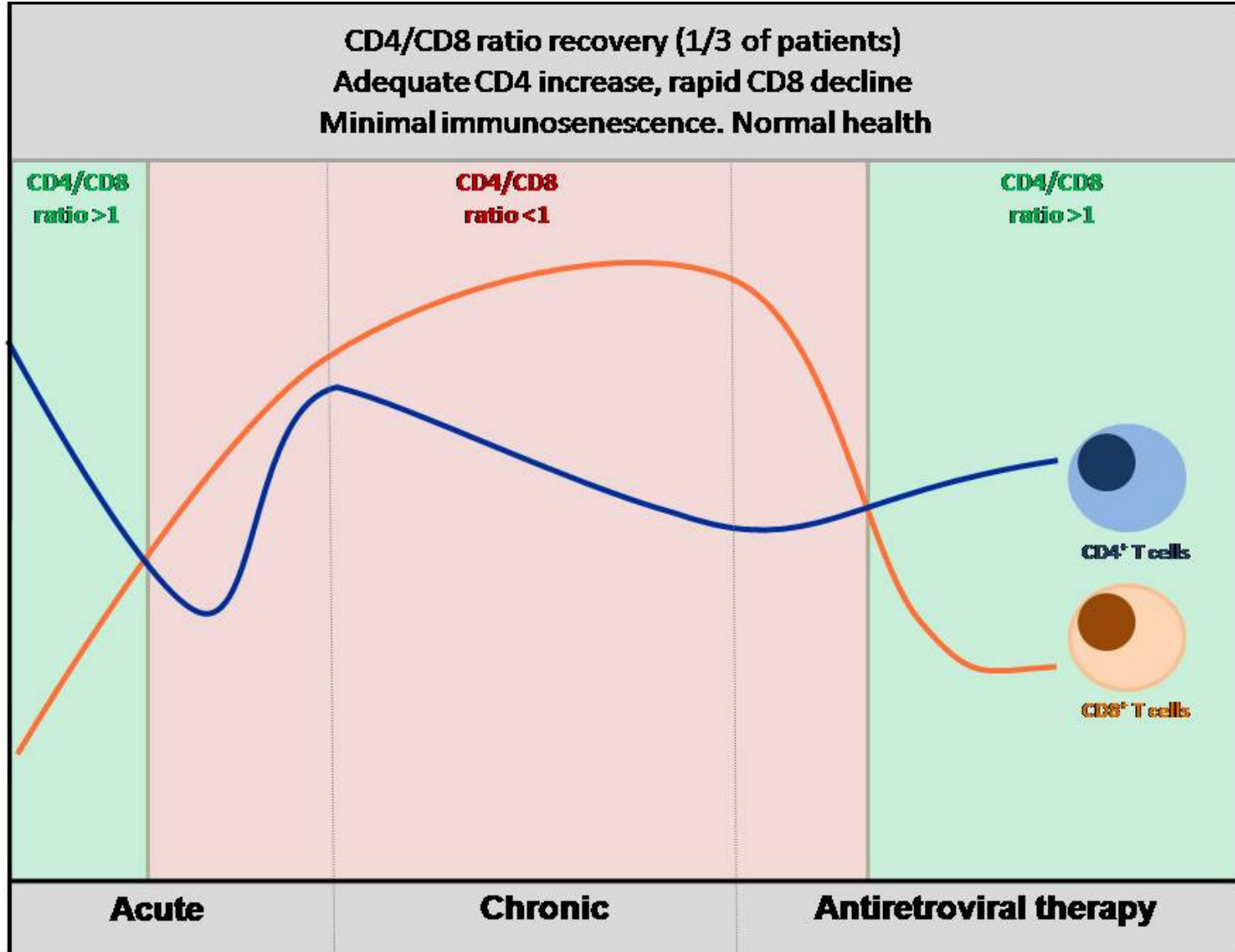
doi:10.1371/journal.pone.0085798.t003

Serrano-Villar S, Pérez-Elías MJ, Dronda F, Casado JL, Moreno A, et al. (2014) Increased Risk of Serious Non-AIDS-Related Events in HIV-Infected Subjects on Antiretroviral Therapy Associated with a Low CD4/CD8 Ratio. PLoS ONE 9(1): e85798. doi:10.1371/journal.pone.0085798

<http://journals.plos.org/plosone/article?id=info:doi/10.1371/journal.pone.0085798>



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Resumen Métodos

- Modelos lineales mixtos
 - para calculo de asociaciones entre variables con medidas repetidas
 - comparar pendientes en dos grupos entre diferentes puntos.
- Comparación de coeficientes de variación
- Curvas ROC (mejor punto de corte)
- Procedimiento Mahapick (emparejar casos/controles)
- Regresión logística, modelo estimativo
- Regresión logística condicional (estimar el efecto de una exposición sobre un desenlace en un estudio casos/controles)

¡Muchas gracias!



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