High rates of non-reactive HIV serology after antiretroviral treatment initiated in acute HIV infection

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ABSTRACT

Background
Non-reactive HIV serology may be a marker of low HIV viral burden. We examined the evolution of HIV antibody in a cohort of individuals treated during acute HIV infection (AHI).

Methods
Between April 2009 and May 2015, adults attending voluntary HIV testing in Bangkok, Thailand, were screened for AHI, by either pooled nucleic acid testing (NAT) of 4th generation immunoassay (4G IA) non-reactive samples or by 3rd (3G) or 2nd generation (2G) enzyme immunoassay (EIA) of 4thG IA reactive samples.
Immediate antiretroviral therapy (ART) was offered. Western blot and p24 quantification were performed for Fiebig staging. HIV serology at baseline, weeks 12 and 24 were performed.

Results
271 Thai adults were enrolled from 139,397 samples screened; 3 individuals who did not initiate ART and 6 non-Thai participants were excluded from analysis. The median age of the volunteers was 27 years and 96% were male. Median time from history of HIV exposure to enrollment was 18 days and median time from enrollment to ART initiation was 1 day.
Of 234 baseline 2G EIA non-reactive subjects, results were available for 202 at week 12 and 188 at week 24. At week 12, 30% were non-reactive by 2G, 3% by 3G and 16% by 4G IA; at week 24, 33% were non-reactive by 2G, 4% by 3G and 17% by 4G.
Baseline HIV RNA<5 $\log_{10}$ copies/ml (p=0.001), CD4 count ≥350 cells/μL (p=0.03) and Fiebig stage 1 or 2 (p=0.005) were predictive of non-reactive 2G EIA at week 24. Lower $AUC_{0\text{-}24wk}$ for HIV RNA was also associated with non-reactive 2G EIA at week 24 (p=<0.0001).
Seroreversion was uncommon. 1 of 28 individuals with reactive 2G EIA at baseline was non-reactive at week 12, but not week 24; 12 of 234 demonstrated transient 2G EIA reactivity at week 12 only.

Conclusions
Approximately one third of individuals who initiated treatment in AHI maintained non-reactivity to 2G EIA after 24 weeks of ART. Rapid ART initiation and HIV RNA decline as well as low HIV RNA and high CD4 at baseline predicted subsequent serological nonreactivity. HIV serologic non-reactivity is likely due to low viral burden, further supporting the benefits of early initiation of ART.

BACKGROUND

- Recent revisions to HIV treatment guidelines favour the initiation of combination antiretroviral therapy (cART) for all HIV-infected individuals regardless of CD4+ T cell count.
- Treatment with cART from the earliest stages of HIV infection may result in:
  - preservation the $CD4^+$ T cell population
  - restriction of seeding of the viral reservoir
  - curtailment of opportunity for viral evolution
- Patients with rapid suppression of HIV viraemia may represent attractive candidates for future HIV cure research.
- Incomplete maturation of serological responses may be a marker of low HIV viral burden.
- We, therefore, examined the evolution of serological responses in a cohort of Thai adults treated with cART during acute HIV infection (AHI).

METHODS

- Ongoing prospective cohort study launched in April 2009 at a large voluntary HIV counseling and testing (VCT) centre in central Bangkok, Thailand.
- Individuals presenting for VCT were considered eligible for the study if found to have AHI, defined as either one of:
  - 4th generation immunoassay reactive and 3rd/2nd generation enzyme immunoassay non-reactive
  - 4th generation immunoassay non-reactive with detectable nucleic acid testing
- All study participants were offered immediate initiation of one of two suppressive cART regimens (namely, tenofovir (TDF), emtricitabine (FTC)/lamivudine (3TC) and efavirenz (EFV) or the same regimen with the addition of maraviroc (MVC) and raltegravir (RAL)).
- Volunteers underwent clinical interview and blood draw for HIV-specific and pre-cART assays.

Assay	Period used	Generation	Env	Gag	Pol	Antibody
Genetic Systems rLAV EIA (Bio-Rad)	Apr '09 – Mar '13	2	gp41 +viral lysate			NA
Avioq HIV-1 Microelisa (Avioq)	Aug '13 – May '15	2	viral lysate			NA
Genscreen HIV 1/2 (Bio-Rad)	Throughout	3	gp160	p24	–	NA
AxSYM HIV antigen/antibody Combo (Abbott)	Jun '09 – Dec '10	4	gp41	–	–	p24
HIV Combi Assay (Roche)	Jan '11 – Jun '11	4	gp41	–	–	p24
ARCHITECT HIV antigen/antibody Combo (Abbott)	Jul '11 – May '15	4	gp41	–	–	p24

Table 1: Assays used throughout the study to define HIV-specific serology

- p24 quantification and Western blot (WB) were also performed for Fiebig staging purposes.
- HIV serology was performed at baseline, week 12 and week 24.

RESULTS

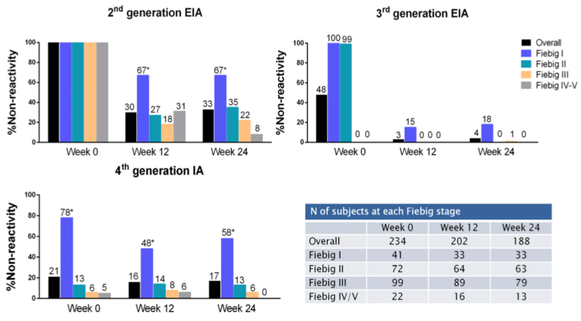
- 271 subjects were enrolled during April 2009 to May 2015 from 139,397 samples screened
- 9 subjects were excluded from analysis as they did not start cART (n=3) or were not Thai (n=6)

Characteristics	N = 262
Age (Years), Median (IQR)	27 (23 – 32)
Sex, n(%)	
Male	251 (96)
Female	11 (4)
Behavior risk, n(%)	
Heterosexual female	11 (4)
Heterosexual male	6 (2)
MSM	245 (94)
Education, Bachelor degree or higher, n(%)	160 (61)
Financial income (USD/Month), Median (IQR)	1,061 (606 – 2,121)
Infection duration (days), Median (IQR)	18 (14 – 25)
Time form HIV Exposure to ARV initiation (days), Median (IQR)	19 (14 – 25)
Fiebig stage, n(%)	
I (RNA+, p24 antigen-, HIV IgM-)	41 (15.7)
II (RNA+, p24 antigen+, HIV IgM-)	72 (27.5)
III (HIV IgM+/HIV IgG-)	106 (40.5)
IV (HIV IgG+/WB indeterminate)	26 (9.9)
V (HIV WB+ without p31)	17 (6.5)
Treatment, n(%)	
HAART (TDF, FTC/3TC, EFV)	177 (68)
Mega-HAART (TDF, FTC/3TC, EFV, MVC, RAL)	85 (32)
HIV subtype: CRF01_AE, n(%)(N=207)	168 (81)
CD4 T cells (cells/μL), Median (IQR)	380 (273 – 505)
HIV RNA ($\log_{10}$copies/mL), Median (IQR)	5.78 (5.18 – 6.61)

Table 2: Demographic characteristics of study participants

RESULTS

- Of the 234 subjects non-reactive by 2nd generation immunoassay at week 0, baseline Fiebig 1 subjects demonstrated significantly higher rates of non-reactivity to 2nd and 4th generation HIV immunoassays during follow up than those enrolled at later Fiebig stages (Figure 1).



N of subjects at each Fiebig stage	Week 0	Week 12	Week 24
Overall	234	202	188
Fiebig I	41	33	33
Fiebig II	72	64	63
Fiebig III	99	89	79
Fiebig IV/V	22	16	13

Figure 1: The percentage non-reactivity to second- (top left), third- (top right) and fourth-generation (bottom left) HIV immunoassays over 24 weeks of follow-up is shown with stratification by baseline Fiebig stage. Asterisks (*) indicate that percentage non-reactivity is significantly higher in Fiebig 1 than other Fiebig stages, p = <0.05

- Baseline $CD4^+$ T cell count of ≥350 cells/μL (p = <0.03), HIV RNA <5 $\log_{10}$ copies/ml (p = 0.001) and Fiebig stage 1 or 2 (p = 0.005) were found to predict non-reactivity by 2nd generation EIA following 24 weeks of cART in these subjects.
- Lower area under the curve for HIV RNA (Figure 2) was found to associate with persistence of non-reactivity by both 2nd generation and 4th generation immunoassays at 24 weeks after cART initiation, but was not predictive in either case.

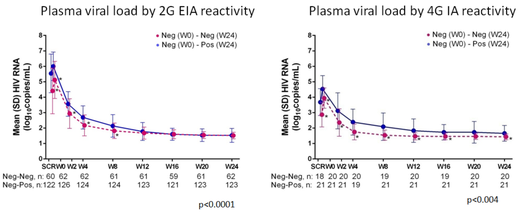


Figure 2: The area under the curve of HIV RNA in subjects treated with cART during acute HIV infection was found to be significantly associated with non-reactive 2nd generation (left) and 4th generation (right) HIV immunoassay results at week 24

- Baseline $CD4^+$ T cell count of ≥350 cells/μL (p = 0.10) and HIV RNA <5 $\log_{10}$ copies/ml (p = 0.13) were found to be associated, but not predictive, of non-reactivity by 4th generation immunoassay following 24 weeks of cART in the 234 subjects non-reactive by 2nd generation immunoassay at week 0..
- Among all 262 subjects analysed, 1 subject had seroreverted at week 12 by 2nd generation EIA, but was reactive again at week 24. 17 subjects had seroreverted by 4th generation IA at week 12; of these, 9 were reactive again at week 24.
- At week 24, 0, 1 and 4 subjects were found to be non-reactive by 2nd, 3rd and 4th generation HIV immunoassay respectively after reactive results at weeks 0 and 12. 2 of subjects non-reactive by 4th generation IA had not attended the week 12 visit.
- 12, 1 and 4 subjects were seen to be transiently reactive at week 12 only by 2nd, 3rd and 4th generation HIV immunoassay respectively.

INTERPRETATION AND CONCLUSIONS

- 33% of subjects who initiated combination antiretroviral therapy during acute HIV infection maintained non-reactivity to 2nd generation HIV enzyme immunoassay after 24 weeks of follow up.
- Approximately 20% of subjects also maintain non-reactivity to 4th generation HIV immunoassay at 24 weeks of follow up.
- Low baseline HIV viral load and high $CD4^+$ T cell count predict non-reactivity to 2nd generation enzyme immunoassay.
- Incomplete maturation of serologic responses likely results from low viral burden, providing further support for the early initiation of antiretroviral therapy .
- Individuals who remain serologically non-reactive following early treatment may represent an attractive cohort for further exploration of potential HIV cure strategies.

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