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Loss of HIV Serological Markers Following Early Treatment of Acute HIV Infection

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BACKGROUND

Initiation of highly active antiretroviral therapy (HAART) during acute HIV infection (AHI) may alter or delay the emergence of serological markers of infection and progression of infection. Typical HIV infection dynamics present as detectable HIV RNA followed immediately by HIV-1 p24 Antigen, then emergence of antibody markers approximately 2-3 weeks post infection. The fourth Generation immunoassays detect both HIV-1 Ag and Ab, allowing for earlier detection of infection than third Generation assays which detect only antibody. HAART interrupts viral fusion, integration, and/or replication leading to a rapid decline in plasma viral load which declines to undetectable levels within a few weeks post initiation of therapy. Previous studies have shown that early therapy, prior to appearance of antibody prevents or reverses accumulation of viral DNA in PBMC, and may interrupt establishment of stable infection. We examined the effect of initiation of HAART prior to emergence of antibody immune response on the subsequent appearance of HIV p24 antigen (Ag) and Antibody (Ab) markers as measured by current serological screening tests. The rate of marker appearance was compared to that of a similar population of untreated Acute HIV infected patients.

MATERIALS AND METHODS

Study Design Volunteers seeking HIV counseling and testing at the Thai Red Cross AIDS Research Center in Bangkok, Thailand were enrolled into a IRB approved cohort study (RV254) of acute HIV infection. Plasma samples were screened for HIV infection by a 4th Generation immunoassay capable of detecting both HIV antibody and p24 antigen. Non-reactive samples were retested by either pooled or individual HIV-1 qualitative nucleic acid test (Hologic/Gen-Probe Aptima). A total of 33 participants identified at very early stages of HIV infection were placed on HAART and screened for presence of serological markers of HIV. Plasma samples collected at Weeks 0, 2, and 12 were tested by p24 Ag (RUO), HIV-1/2 Ab/Ag Combo (4th Gen), EIA 1/2 Plus O (3rd Gen), HIV-1 Western blot (WB), and Multispot assays (all from Bio-Rad Laboratories, Inc; Redmond, WA). The results of the 3rd and 4th Gen EIAs were recorded as signal to cut-off ratio (s/co) where any signal above 1.0 s/co considered reactive. Acute HIV infection was classified by Fiebig stage based on the results of RNA, Ag, Ab, and WB assays.

RESULTS

The rate of appearance of serological markers was evaluated from samples collected from populations at high risk for HIV infection (RV217, Early Capture HIV Cohort (ECHO) Study). The very sensitive HIV-1 RNA Aptima (Hologic/Gen-Probe, Inc) assay was used to identify time of infection from fingerstick samples collected at 3-4 day intervals. Sequential appearance of serological markers in 29 participants post RNA detection is shown in Fig. 1. HIV-1 RNA and p24 Antigen are detectable at very early time points with p24 antigen rapidly decreasing to undetectable levels post evolution of Anti-HIV antibodies. Diagnostic markers of HIV infection become positive on average by Day 7, 16, 20 and 22 as detected by the 4th Gen EIA, 3rd Gen EIA, Multispot and Western blot assays, respectively. All, but one participant were positive by all assays by day 27 and remained positive thereafter. One patient with a delayed antibody response was positive by MS by Day 33 and WB by Day 40.

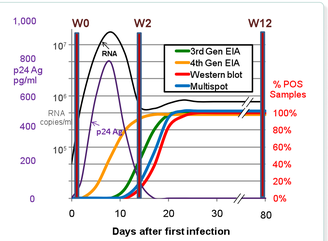
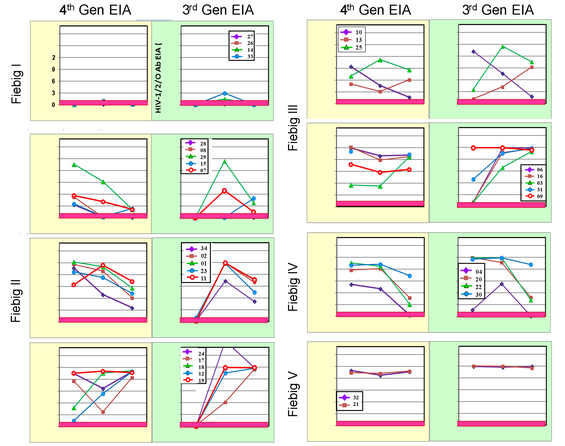


Figure 1. Rate of appearance of serological markers in untreated HIV participants after infection detection.

	PID	Day	RNA cps/ml	p24 Ag pg/ml	4th Gen Ag/Ab	3rd Gen 1/2/O	Multi spot	Western Blot
Fiebig I	27	0	2.5E+04	7	0.1	0.0	N	NR
		14	3.8E+03	0	0.1	0.2	N	NR
		82	5.0E+01	0	0.1	0.1	N	IND
	26	0	5.5E+03	0	0.1	0.0	N	NR
		13	2.0E+04	3	1.1	0.9	N	NR
		81	5.0E+01	0	0.1	0.1	N	IND
	14	0	2.3E+03	0	0.1	0.0	N	NR
		14	5.0E+01	0	0.9	1.5	N	NR
		89	5.0E+01	0	0.3	0.1	N	NR
	33	0	3.9E+03	0	0.1	0.0	N	NR
		21	5.0E+01	0	0.9	2.9	N	NR
		79	5.0E+01	0	0.1	0.0	N	NR
Fiebig II	28	0	1.7E+05	72	3.3	0.0	N	IND
		12	9.2E+03	0	0.2	0.3	N	IND
		74	5.0E+01	0	0.1	0.1	N	NR
	8	0	2.9E+05	108	5.2	0.0	N	NR
		15	4.7E+02	0	0.1	0.0	N	NR
		82	5.0E+01	0	0.3	0.3	N	IND
	29	0	1.2E+05	1,000	13.5	0.0	N	NR
		13	1.9E+04	2	9.2	14.3	H1R	R
		81	5.0E+01	0	2.1	3.8	H1R	R
	15	0	7.1E+04	57	3.5	0.0	N	IND
		13	2.9E+03	0	0.2	0.1	N	IND
		88	5.0E+01	0	2.3	5.0	N	IND
	7	0	4.8E+05	76	5.6	0.0	N	NR
		14	7.3E+02	0	4.1	6.9	N	NR
		83	5.0E+01	0	2.1	1.5	N	IND
	34	0	4.1E+06	1,502	13.6	0.1	N	IND
		13	5.2E+04	14	6.9	10.3	H1R	IND
		83	6.7E+01	0	3.5	5.1	N	R
	2	0	3.2E+06	1,744	14.6	0.0	N	NR
		13	1.7E+04	0	12.9	14.9	N	IND
		83	1.0E+02	0	6.1	10.0	N	R
	1	0	7.4E+06	3,419	15.2	0.2	N	NR
		14	4.2E+03	1	14.0	14.9	H1R	R
		82	5.0E+01	0	8.6	10.7	N	R
	23	0	3.1E+07	757	12.7	1.0	N	NR
		13	3.3E+03	0	11.3	14.7	N	R
		88	6.2E+01	0	7.2	7.4	H1R	R
	11	0	2.4E+06	348	9.4	0.2	N	IND
		14	1.0E+04	0	14.4	14.9	N	R
		81	5.0E+01	0	10.2	10.6	N	R
	24	0	3.3E+05	416	13.4	0.1	N	NR
		18	7.3E+02	0	9.6	21.6	H1R	R
		87	5.0E+01	0	13.9	14.9	H1R	R
	17	0	3.6E+05	449	11.5	0.2	N	NR
		13	5.4E+02	0	3.7	6.1	N	IND
		84	5.0E+01	0	12.4	14.4	H1R	R
	18	0	1.3E+05	103	4.8	0.0	N	NR
		16	6.1E+04	33	13.6	14.9	N	R
		93	8.8E+04	3	14.1	14.8	H1R	R
	12	0	2.8E+05	16	1.5	0.0	N	NR
		15	8.4E+01	0	8.3	13.5	N	IND
		85	5.0E+01	0	13.9	14.7	N	R
	19	0	3.7E+07	858	13.5	0.0	N	IND
		17	5.3E+04	33	14.0	14.9	H1R	R
		93	1.4E+04	0	13.7	14.8	H1R	R
Fiebig III	13	0	9.1E+05	160	5.0	1.1	N	NR
		14	2.5E+02	0	3.1	4.2	N	IND
		90	5.0E+01	0	6.1	9.2	N	IND
	25	0	3.2E+05	241	7.0	3.5	N	NR
		14	4.3E+02	0	11.1	14.5	N	R
		81	5.0E+01	0	8.6	10.6	N	R
	10	0	4.8E+04	3	9.4	13.2	N	NR
		17	5.5E+01	0	4.5	7.6	N	IND
		81	5.0E+01	0	1.6	1.8	N	IND
	6	0	5.5E+06	2,103	14.9	1.0	N	NR
		16	1.4E+03	0	12.9	14.8	H1R	R
		86	1.1E+02	0	13.1	14.9	H1R	R
	16	0	3.9E+05	3,134	15.1	1.1	N	NR
		14	2.8E+04	5	11.9	13.7	N	IND
		86	4.2E+02	0	12.7	14.6	N	R
	3	0	3.0E+05	105	5.5	1.1	N	NR
		15	2.9E+03	0	5.2	9.9	N	IND
		84	6.7E+01	0	12.7	13.9	H1R	R
	31	0	4.9E+06	1,523	14.1	6.9	N	NR
		80	5.0E+01	0	13.3	14.7	N	R
	9	0	8.2E+04	4	10.7	14.9	N	NR
		15	5.0E+01	0	8.7	14.9	N	IND
		85	5.0E+01	0	9.4	14.3	N	R
Fiebig IV	4	0	5.7E+05	223	8.1	1.7	N	IND
		14	2.3E+02	0	7.0	8.3	N	IND
		81	5.0E+01	0	0.3	0.1	N	IND
	20	0	7.7E+05	19	11.7	14.9	H1R	IND
		13	1.3E+03	0	12.1	13.7	H1R	R
		81	5.4E+01	0	4.7	4.8	N	R
	22	0	3.3E+05	137	13.6	14.9	N	IND
		12	5.6E+02	0	12.7	14.9	H1R	R
		86	5.0E+01	0	3.0	4.2	N	IND
	30	0	4.1E+05	233	13.0	14.5	N	IND
		15	1.5E+03	0	13.2	14.9	H1R	R
		89	5.0E+01	0	10.3	13.2	H1R	R
Fiebig V	32	0	1.3E+06	352	13.9	14.9	H1R	R
		19	1.2E+03	0	12.7	14.7	H1R	R
		83	5.0E+01	0	13.6	14.9	H1R	R
	21	0	5.1E+05	302	13.4	15.0	N	R
		15	1.7E+02	0	13.2	14.9	H1R	R
		83	5.0E+01	0	13.8	14.5	N	R

Negative	<50	<10	<1.0	<1.0		
Weak Positive	<10,000	<100	<7.0	<7.0		IND
Strong Positive	>10,000	>100	>7.0	>7.0	H1R	R

Table 1. Summary of data for weeks (W) 0, 2 and 12. RNA, Antigen, 3rd and 4th Gen EIA as well as Western Blot and Multispot results are shown sorted by the Fiebig Stage at which treatment was initiated. Two of the Fiebig II patients failed to respond to therapy and retained High HIV RNA levels even by W12. The colors and shading indicate positive signals with shading differentiating between high and low signals.



Fiebig Stage I (RNA detected). Of 4 participants receiving HAART, two showed a transient low reactive response by 3rd Gen assay at W2. All were non reactive by W12.

Fiebig Stage II (RNA and Antigen detected). Of 15 participants all were initially reactive by 4th Gen EIA, and non reactive by 3rd Gen EIA. By W2, 4th Gen signal to cut off (s/co) declined in all but 4 participants, reverted to non-reactive in 2, and remained low in 7 by W12. 3rd Gen Ab reactivity failed to evolve in 2 participants, and was greatly delayed in another. In six participants s/co increased through W2, then declined to lower levels by W12.

Fiebig Stage III (RNA, Ag, and Ab (EIA) detected, WB NEG). All 8 Fiebig III participants were initially reactive by both 3rd and 4th Gen EIA assays. The 4th Gen signal declined in one participant and remained low in two others through 12W. 3rd Gen s/co also declined in one participant.

Fiebig Stage IV (RNA, Ag, Ab (EIA) detected, WB IND). All 4 Fiebig IV participants, were initially reactive by both 3rd and 4th Gen EIAs, then showed a decline in 3rd and 4th GEN EIA s/co by W12. One participant actually screened non-reactive by both assays at W12.

Fiebig Stage V (RNA, Ab (EIA) detected, WB POS). Both participants initiating HAART at Stage V retained high s/co at W0, W2 and W12.

Figure 2. Time line of reactivity of the 4th Gen and 3rd Gen EIA assay results sorted by Fiebig Stage at which treatment was initiated.

	4th Gen Ag/Ab		3rd Gen 1/2/O		Multispot		Western Blot	
	NR	Low	NR	Low	Neg	Rev	Neg	IND
Fiebig I N = 4	4 100%		4 100%		4 100%		4 100%	
Fiebig II N = 15	2 13%	4 27%	2 13%	5 33%	9 60%	2 13%	3 20%	1 7%
Fiebig III N = 8		2 25%		1 13%	6 75%			2 25%
Fiebig IV N = 4	1 25%	2 50%	1 25%	2 50%	3 75%	2 50%	2 50%	1 25%
Fiebig V N = 2					1 50%	1 50%		
Total N = 33	7 21%	8 24%	7 21%	8 24%	23 70%	5 15%	9 27%	4 12%

Table 2. Summary of rates of inhibition, delay or loss of serological marker intensity (s/co) when treatment is initiated at early Fiebig stages. Samples that remained Neg or with s/co below 8 (Low) by W12 are shown for each Fiebig stage. In addition, the number of samples that had Neg Multispot or reverted (REV) from Pos at W2 to Neg at W12, and Western blot that remained Neg or IND by W12 are shown.

The opinions or assertions contained herein are the private views of the authors and do not reflect the views of the U.S. Army or the Department of Defense.

CONCLUSIONS

Initiation of antiviral therapy at very early stages of infection can prevent, delay or even reverse the emergence of anti-HIV antibody with decrease or absence of HIV-1 markers in plasma even after 12 Weeks post first detection of infection.

Evolution of anti-HIV antibody was completely inhibited in 4 of 4 patients treated at Fiebig I and 2 of 15 patients treated at Fiebig II.

Serological reactivity was reduced or delayed in 25-50% of patients treated at Fiebig II to IV, with one stage IV patient becoming negative on both the 3rd and 4th Gen assays by Week 12.

Early therapy delayed evolution of serological markers as detected by WB and Multispot, such that 54% of patients remained Multispot negative and 36 Western blot negative by as late as W12 (ave. 80 days post first HIV-1 detection).

Evidence of seroreversion was observed in five patients who were initially Multispot positive at W2, but became negative by W12. One WB patient went from Western blot POS to IND. ?

Additional studies investigating initiation of HAART early in AHI on the course of infection and impact on the evolution of serological response is warranted.

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