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► To cite this version:

Josephine Brice, Mariam Sylla, Sophie Sayon, Fatoumata Telly, Djeneba Bocar-Fofana, et al.. Qualitative and quantitative HIV antibodies and viral reservoir size characterization in vertically infected children with virological suppression. *Journal of Antimicrobial Chemotherapy*, 2017, 72 (4), pp.1147-1151. 10.1093/jac/dkw537 . hal-01548242

HAL Id: hal-01548242

<https://hal.sorbonne-universite.fr/hal-01548242v1>

Submitted on 27 Jun 2017

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TITLE

Qualitative and quantitative HIV antibodies and viral reservoir size characterization in vertically infected children with virologic suppression

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SHORT TITLE

HIV antibodies and reservoir size characterization in children

22

SYNOPSIS

23

24 Background

25 The absence of detectable viremia after treatment cessation in some vertically HIV-infected (VHIV) children
26 suggests that early initiation of highly active combination of antiretroviral therapy (HAART) could lead to
27 functional cure.

28 Objectives

29 We described the factors associated with HIV antibody levels and the viral reservoir size in HAART-treated VHIV
30 children.

31 Patients and methods

32 This study included 97 VHIV children with virological suppression, in Bamako, Mali. The anti-gp41 antibody
33 activities and HIV serostatus were assessed. The viral reservoir size was measured by quantifying total cell
34 associated HIV DNA.

35 Results

36 Among the children studied, the median total HIV DNA level was 445 copies/10⁶ cells (IQR = 187 - 914), the
37 median anti-gp41 antibody activity was 0.29 OD (IQR = 0.18 - 0.75). Low activity of anti-gp41 antibodies was
38 associated with a younger age of HAART initiation ($p = 0.01$). Overall, eight HIV-1 seroreversions were
39 identified.

40 Conclusion

41 This study identified potential candidates with low viral reservoir and low antibody levels or activities for future
42 trials aiming to reduce HIV-1 reservoir in order to limit HAART duration.

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44

45 INTRODUCTION

46 Pediatric HIV infection remains a major public health issue despite large implementation of prevention
47 of mother-to-child transmission programmes. ¹ It requires early and lifelong HAART to control the viral
48 replication with a risk of accumulating toxicity and viral drug resistance. International guidelines are now
49 recommending initiation of HAART in all vertically HIV-1-infected (VHIV) children regardless of clinical and
50 immunological conditions ² as early treatment reduces mortality and improves immune recovery. ³

51 Proviral DNA HIV-1 reservoirs are established early during infection and represent a barrier to
52 functional cure. ⁴ A low HIV viral reservoir is associated with a lower risk of disease progression. Despite
53 prolonged HAART, HIV-1 persists as transcriptionally inactive proviruses in long half-life memory resting CD4 T
54 cells. ⁵ The lowest reservoir has been described in elite controllers (EC) in whom HIV-1 replication is controlled
55 without treatment. ⁶ However, in post-treatment controllers (PTC), HIV-1 remission and low reservoir are
56 observed after HAART interruption mainly when treatment was initiated during primary HIV infection (PHI). ⁷
57 The relation between the size of the viral reservoir, and the precocity and duration of HAART has been
58 previously described in children. ⁸⁻¹⁰ However, more studies are required to identify simple predictors of the
59 reservoir size in VHIV children, in real world conditions within high HIV prevalence settings, especially in VHIV
60 children.

61 The absence of detectable viremia 12 years after treatment cessation in one VHIV child has suggested
62 that early HAART initiation could lead to functional cure. ¹¹ HIV infected people first develop anti-gp41
63 antibodies and only several weeks later, anti-gp120 antibodies. ¹² The Berlin patient, who was cured of HIV
64 following a stem cell transplant, displayed a complete loss of anti-p24 antibodies and a low but still detectable
65 response to gp41. ¹³ Only few EC subpopulations show this HIV antibody profile. Thus, monitoring the response
66 to p24 and gp41 may be useful in HIV cure studies.

67 Several HIV-1 seroreversions have been observed in VHIV children who initiated HAART within the first
68 months of life. ^{14,15} By blocking viral replication, the early virostatic treatment might prevent the development
69 of the HIV-1 specific antibody response, either quantitatively (antibody level) or qualitatively (antibody

70 activity), thus leading to HIV seroreversion or substantially lower anti-HIV antibody levels. One analysis
71 demonstrated that age of HAART initiation and plasma viral load were strong predictors of serostatus, and both
72 were independently associated.¹⁶ Kuhn et al showed that the absence of HIV antibody response indicated a
73 smaller HIV-1 viral reservoir, and HAART initiated at 3 months of age was the upper limit to see the benefits of
74 early HAART.¹⁷ Only one study has demonstrated the utility of HIV serostatus as a surrogate marker of the
75 reservoir size.⁸

76 Here we describe the factors associated with HIV antibody activity or level and the viral reservoir size
77 in HAART-treated HIV children with heterogeneity of age, time of therapy, and duration of virological
78 suppression.

79 METHODS

80 This cross-sectional study conducted within a prospective cohort included HAART-treated HIV
81 children followed at Gabriel Touré Hospital (Bamako, Mali) with sustained virological suppression (HIV-1 RNA
82 plasma ≤ 50 copies/mL). All participants were known virologically suppressed at their previous visit (six months)
83 and confirmed during the study. Participants older than 20 years old, those with HIV-2 infection, treated for
84 less than three months, or without any data recorded were excluded from the study. After obtaining the
85 parent's written or oral informed consent from all participants, five millilitres of extra blood samples were
86 collected in EDTA tubes during the routine follow up visit. Assent was obtained from child participants
87 according to local institutional review board guidelines. The study was approved by the National AIDS program
88 at the Ministry Of Health in Mali (CSLS/MS) in collaboration with the Malian Institutional Ethics Committee at
89 the Faculty of Medicine, Pharmacy and Odontostomatology of health and life sciences in Bamako under the
90 reference number N°10-05-FMPOS.

91 In order to evaluate the size of the HIV-1 viral reservoir, total DNA was extracted from PBMC's derived
92 from whole blood using an automated technique (MagNA Pure, Roche, Mannheim, Germany). The cell-
93 associated HIV-1 DNA level was quantified using a real-time PCR method which amplifying a region in the LTR
94 gene, as previously described.¹⁸ Proviral burden was expressed as HIV DNA copies per 1 million cells
95 (quantification limit: 10 copies/PCR, i.e. 66 copies/ 10^6 cells considered as undetectable).

96 Dried serum spots (DSS) were used to evaluate the HIV-specific antibody response. The level of
97 antibodies targeting the gp41 immunodominant epitope (IDE) was measured following a previously described
98 protocol.¹⁹ An equimolar mixture of two 30 amino-acids oligopeptides was used, representing the IDE
99 consensus sequences of HIV-1 group M and subtype D, respectively. A low mixture concentration allowed the
100 binding of late antibodies that had acquired sufficient avidity and then semi-quantitative detection by
101 spectrophotometry. The result was expressed as an optical density (OD). The activity of anti-gp41 antibodies
102 was systematically tested in quadruplicate.

103 The 4th generation ARCHITECT HIV Ag/Ab Combo assay (Abbott Laboratories, Wiesbaden, Germany)
104 was performed as previously described to quantify the humoral response in DSS. The result was defined as
105 relative light units, then compared to a cut-off signal. Samples with signal-to-cut-off (S/CO) values ≥ 1.00 were
106 considered reactive and those < 1.00 non-reactive.

107 We used univariate association between HIV DNA level, anti-gp41 antibody activities or HIV-antibody
108 levels and variables defining current and past HIV disease (age, sex, WHO stage at study, HAART type at study,
109 CD4 cell count at study, HAART duration, maternal prophylaxis type, age at HAART initiation), with the
110 Spearman rank correlation coefficient for continuous variables and the Fisher's exact test for categorical
111 variables.

112 RESULTS

113 From August 2013 to April 2014, 97 VHIV children with virological suppression were enrolled. Their
114 median age was 9.8 years old at time of inclusion (IQR = 7 – 13.1), they started HAART at a median age of 3.3
115 years (IQR = 1.9 - 7), and were receiving HAART for a median 5.4 years (IQR = 3.5 - 7). Table 1 summarizes the
116 demographic, immunologic and virologic characteristics of the children.

117 The median anti-gp41 antibodies activity was 0.29 OD (IQR = 0.18 - 0.75). A low activity of anti-gp41
118 antibodies was associated with both a younger age at treatment initiation ($p = 0.01$; Figure 1) and with a lower
119 level of anti-HIV antibodies ($p = 0.0015$; Figure 1). Overall, eight seroreversions were identified (negative ELISA
120 Architect) in which 2 children had an HIV DNA under the threshold (1 detectable and 1 undetectable) and a low
121 anti-gp41 antibodies activity. All the seroreverted children started HAART before two years of age, at a median
122 age of 1.1 years, and were on HAART for the past 7.3 years in median.

123 The median level of total HIV DNA was 445 copies/10⁶ cells (IQR = 187 – 914). No correlation was
124 found between anti-gp41 antibodies activity or age at treatment initiation and HIV DNA ($p = 0.27$; Figure 1).
125 The 9 children with an HIV DNA level under the threshold tended to have a lower anti-gp41 antibodies activity
126 compared to children with an HIV DNA > 66 copies//10⁶ cells ($p = 0.11$).

127 DISCUSSION

128 This study indicates that a significant proportion of virologically suppressed VHIV children who
129 initiated HAART before the age of two years stopped to produce and/or progressively lost the HIV antibodies.
130 This is consistent with the idea that early HAART halts the antigenic stimulation which is necessary to sustain an
131 HIV-specific antibody response.^{8-10,20} In addition, some of these children with seroreversion had a very low HIV
132 reservoir, at least identified in the peripheral blood, and could therefore represent an ideal population for
133 studies investigating novel immunotherapeutic strategies aiming to achieve HAART-free remission.

134 Although current HAART can effectively control HIV replication to clinically undetectable levels for
135 years, existing strategies do not eradicate HIV-1 reservoirs in VHIV children.¹¹ One of the limitation of our
136 study was that our cohort did not include children who started HAART before five months of life, and therefore
137 we were not able to identify more seroreversions. Nonetheless, we found 50% of seroreversion in VHIV
138 children who initiated treatment before two or one years old (8/16 and 4/8 respectively), consistently with
139 other studies in occidental settings that showed 50% to 94% of seroreversion when treatment was initiated
140 before 3 months of life.^{14,15,20}

141 Children can acquire HIV-1 in utero, during delivery or breastfeeding.¹ In our study, the time of HIV
142 transmission was not known. But, we assumed that seroreversion probably occurred in children who had
143 treatment initiation soon after the HIV infection acquisition. Indeed, when using one of the most sensitive
144 assays available, we were able to find several seroreversions. In addition, we found an association between a
145 low anti-gp41 antibodies activity and a younger age at treatment initiation.

146 Early effective control of HIV replication has been associated with incomplete development of HIV-
147 specific immune responses in children.^{14,15,20} It would be of interest to study the initial development of anti-HIV
148 antibodies (like activity and quantification of anti-gp41 antibodies) in early treated infants to determine
149 whether the primary responses is affected or the influence occurs later on, leading to a decrease of responses

150 over time. The decrease of antibody production and/or their avidity against some epitopes should reflect the
151 absence of circulating antigenic viral particles, showing the absence of residual viral replication.

152 In conclusion, the results of this study show that HIV-1 seroreversion and low anti-gp41 activity in
153 VHIV children with early HAART initiation happened and should be considered as a proof-of-concept study to
154 evaluate strategies targeting HAART-free remission (i.e long-term undetectable viremia for an undefined
155 period, in the absence of HAART).²¹ This study identified potential candidates with low viral reservoir and low
156 antibody levels or activity. Defining the immunologic and virologic end-points after HAART in VHIV children and
157 identifying factors and biomarkers associated with limited proviral reservoir size are essential to define
158 therapeutic strategies, in order to achieve HIV remission or cure in this population.

159

160 ACKNOWLEDGEMENTS

161 Global Health Center, Department of Infectious Diseases, Northwestern University, Chicago IL, USA.

162

163 FUNDING

164 This study was funded by Agence Nationale de Recherche sur le Sida et les Hépatites virales.

165

166 TRANSPARENCY DECLARATION

167 None to declare.

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227 **Table 1** : Descriptive characteristics of HAART-treated VHIV children with virologic suppression

228 Abbreviations : cART, combination of antiretroviral therapy; HAART, highly active combination of antiretroviral
229 therapy; IQR, interquartile range; NNRTI, nonnucleoside reverse transcriptase inhibitor; NRTI, nucleoside
230 reverse transcriptase inhibitor; OD, optical density; PI, protease inhibitor; S/CO, signal-to-cutoff; WHO, World
231 Health Organization

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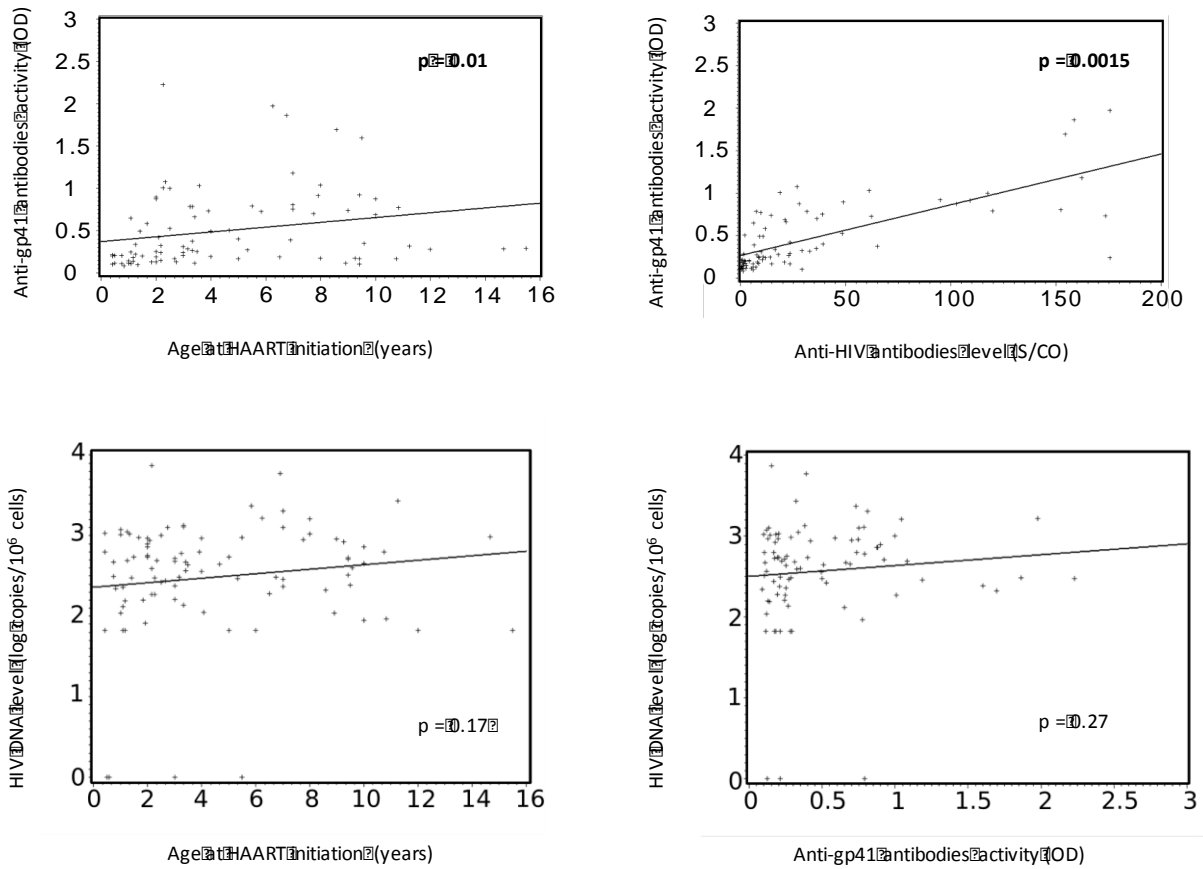
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Characteristics	n (%)	Median (IQR)	Global range
Age (years)	-	9.8 (7 - 13.1)	2.8 - 19
Sex :			
Female	38 (39)	-	-
Male	59 (61)	-	-
cART maternal prophylaxis type :			
2 NRTI + 1 NNRTI	60 (62)	-	-
2 NRTI + 1 PI	37 (38)	-	-
Age at HAART initiation (years)	-	3.3 (1.9 - 7)	0.41 - 16
Duration of HAART (years)	-	5.4 (3.5 - 7)	0.33 - 12.1
WHO stage at HAART initiation:			
1 or 2	36 (39)	-	-
3 or 4	52 (61)	-	-
Missing data : 9			
HAART type at study :			
2 NRTI + 1 NNRTI	61 (63)	-	-
2 NRTI + 1 PI	36 (37)	-	-
CD4 cell count at study (cells/mm ³)	-	820 (605 - 1120)	46 - 2000
Missing data : 1			
HIV DNA level at study (copies/10 ⁶ cells)	-	445 (87- 902)	0 - 7378
< 66 copies/10 ⁶ cells (i.e undetectable)	12 (13)	-	-
Missing data : 2			
Anti-gp41 antibodies activity at study (OD)	-	0.29 (0.18 - 0.75)	0.09 - 2.23
Missing data : 9			
Anti-HIV antibodies level at study (S/CO)	-	14.1 (4.1 - 39.3)	0.31 - 520.6
< 1.0 S/CO (i.e seroconversion)	8 (9)	-	-
Missing data : 10			

260 **Figure 1:** Distribution of anti-gp41 antibodies activity by age at HAART initiation (A) and by anti-HIV antibodies
261 level (B) using Spearman correlation. Distribution of HIV DNA level by age at HAART initiation (C) and by anti-
262 gp41 antibodies activity (D) using Spearman correlation.

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266 Abbreviations: HAART, highly active combination of antiretroviral therapy; OD, optical density; S/CO, signal-to-
267 cutoff

268 Anti-gp41 antibodies activity measured by manual immuno-enzymatic assay

269 Anti-HIV antibodies level measured by a 4th generation ARCHITECT HIV Ag/Ab Combo assay

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