Anti-Gp41 Antibody Levels Reflect HIV Viral Suppression and Cellular Reservoir in Long-Term Antiretroviral-Treated Trial Participants

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Synopsis

Background

A major challenge to HIV cure strategies is the quantification of persistent reactivation-prone virus in people living with HIV.

Objectives

Our aim was to determine whether anti-gp41 antibody levels correlate with viral suppression and HIV-1 DNA levels on antiretroviral therapy.

Patients and methods

Participants with plasma HIV-1 RNA below 50 copies/ml for >12 months were included from 3 ANRS cohorts (COPANA, MONOI and APROCO). Antibody levels to gp41 were measured by a low-sentivity enzyme-linked immunoassay. Correlations with individual and viral characteristics, plasma HIV-1 RNA (standard and ultrasensitive) and cell-associated HIV-1 DNA were assessed.

Results

Seventy seven percent of the 683 participants were men. Median age was 41, median CD4+ T-cell count was 582/µL and median viral suppression duration was 6.6 years (IQR, 2.0-9.5). The overall median anti-gp41 antibody titer was 1.3 (0.6-1.9); median (IQR) HIV-1 DNA level was 2.6 (2.1-3.0) $\log_{10}$ copies/10^6 cells; ultrasensitive HIV-1 RNA tested below 1 copy/mL in 56% of samples. A lower titer of anti-gp41 antibodies was correlated to male gender, longer viral suppression and lower HIV-1 DNA burden. Consistent HIV-1 RNA <1copy/mL was associated with lower gp41 levels (median: 1.1 (0.5-1.6) *vs* 1.4 (0.7-1.9), p=0.009).

Conclusions

Anti-gp41 levels decreased with the duration of antiviral suppression on ART. Lower titers were associated with lower HIV-1 DNA levels and maximal viral suppression, reflecting minimal antigen stimulation. Anti-gp41 antibody titration may be a useful biomarker reflecting long-term HIV-1 suppression on ART.

Introduction

Combination antiretroviral therapy (ART) suppresses human immunodeficiency virus type 1 (HIV-1) replication but does not cure the infection, due to the persistence of long-lived latent HIV-1 reservoirs.[1] Reactivation-prone HIV-1 DNA persists in infected CD4+ T cells and macrophages, thus preventing viral elimination in spite of long-term ART.

ART leads to the suppression of plasma HIV-1 RNA viral load below the limit of quantification of standard clinical assays (20-50 copies/mL). Using an ultrasensitive "single copy" assay with a limit of quantification of 1 copy/mL, Palmer *et al* demonstrated that low-level viremia persists for at least 7 years in many patients on suppressive antiretroviral therapy.[2]

The origin of this residual viremia remains elusive. Low-level replication may persist in tissue sanctuaries due to limited drug penetration, and/or the production of HIV-1 RNA may arise from the sporadic reactivation of infected T cells. In addition, the majority of integrated HIV-1 DNA sequences are defective and a fraction only of the 10% viral genomes preserved from deletions and hypermutation will produce infectious viral particles following *in vitro* activation of infected CD4+ T cells.[3] Nonetheless, defective proviruses are able to produce protein-encoding RNA species in patients on ART.[4] Regardless of the origin of the residual viremia, current tools based on PCR amplification to detect plasma HIV-1 RNA fall short of an assay that would detect the production of actual infectious particles.

A major challenge to HIV cure strategies is the quantification of persistent replication-competent HIV. Current methods to quantify the HIV reservoir in blood and tissues in individuals on ART are continually challenged. The quantification of total cell-associated HIV-1 DNA overestimates the actual reservoir size, while the viral outgrowth assay

measuring the production of infectious virus from resting CD4+ T cells underestimates the number of reactivation-prone cells and requires large volumes of blood, emphasizing the need for new approaches.[5] HIV-1 infection elicits a strong adaptive immune response involving both cellular and humoral immunity. Measuring the specific immune responses to HIV may be an alternate way to assess HIV persistence and replication in blood and tissue.

Interestingly, immunoassays for recent HIV infection that were developed to estimate HIV incidence are based on the maturation of HIV-specific antibody responses. They mostly measure antibody levels or antibody avidity towards major antigenic epitopes of HIV-1[6,7]. Previous studies demonstrated the impact of ART on the proportion of HIV-specific IgG, and that patients who were falsely classified as recently infected (low titers or/and low avidity) had been treated for a longer period and with longer viral suppression than those correctly classified[8,9]. The prevailing hypothesis is that the suppression of viral replication is likely to lessen antigen presentation to immune cells, leading to a decrease in anti-HIV antibody production. Several groups have since studied anti-HIV antibodies as potential surrogate markers of HIV reservoir. These studies included various types of participants (untreated, ART-treated at the early or the chronic stage, elite controllers) in limited numbers[10,11] and described declining antibody levels during ART. Our aim was to determine whether anti-gp41 antibody levels are correlated with HIV-1 reservoir size and residual viremia and could be a valuable marker of this reservoir in a large homogenous group of successfully ART-treated participants from three ANRS cohorts, who initiated ART at the chronic stage and had prolonged viral suppression.

Patients and methods

Participants

We analyzed a large number of participants in a cross-sectional design, accounting for a wide range of ART durations. Adult participants with ART-treated chronic HIV infection suppressed below 50 copies/mL were included from three French ANRS cohorts. Participant characteristics, clinical and biological data were obtained from the ANRS cohort databases.

We obtained samples from the final evaluation of ANRS-EP11-APROCO (Anti-PROtease COhort) treatment-controlled participants followed for a median 11 years (IQR 10-12)[12,13]. We included on-ART samples from participants with sustained viral suppression below 50 copies/mL for at least 12 months in the ANRS-C09-COPANA prospective cohort of HIV-1-infected adults initiating therapy.[14] Baseline pre-intervention samples from the ANRS-136 MONOI trial (NCT00412551),[15] which included ART-treated participants with plasma HIV-1 RNA suppressed below 400 copies/mL for at least 18 months and below 50 copies/mL at the screening visit were also included.

Prospectively stored (-80°C) serum and whole blood samples were obtained from the ANRS Biobank.

Ethics

All participants to the 3 studies provided written informed consent. Research protocols were conducted in accordance with the Declaration of Helsinki and with national and institutional standards and were approved by the relevant local interventional review boards (Cochin-Tarnier, Paris-Cochin). Personal information and samples were de-identified and analyzed anonymously.

Detection of antibodies to the immunodominant epitope of the gp41 viral protein: EIA-RI

The level of antibody to the immunodominant epitope (IDE) of gp41 was estimated following a procedure already described [6,8,18]. We used an equimolar mixture of two 30 amino-acids oligopeptides, representing the consensus sequences of the IDE of HIV-1 group M and subtype D, respectively. A low concentration of this mixture (0.05 µg/mL each) allows the binding of antibodies that are present at sufficient level or sufficient avidity rendering feasible the semi-quantitative detection by spectrophotometry. The antibody levels were expressed as absorbance values (OD values).

Cell-associated HIV-1 DNA quantification

HIV-1 DNA data from the ANRS-MONOI trial were described elsewhere.[16] For COPANA samples, total cellular DNA was extracted from blood using the DSP DNA Mini Kit with a QiaSymphony instrument (Qiagen, Courtaboeuf, France). The number of extracted genomes in DNA samples was quantified by a qPCR of the human albumin gene. Total HIV-1 DNA load was assayed using the qPCR Generic HIV-1 DNA Cell® kit (Biocentric, Bandol, France) and normalized by the albumin quantification.

Ultrasensitive HIV-1 RNA viral load (usVL)

As previously described[16] MONOI participants were tested twice for usVL: at the screening visit (week -10) and at inclusion (week 0: sample included in our study). Participants with undetectable usVL (<1copy/mL) at both sampling times were considered negative and participants with one or both positive results were considered positive for usVL. Plasma samples from COPANA participants were assayed using the Cobas Ampliprep/Cobas Taqman HIV-1 Test, version 2.0 (Roche Diagnostics). The published limit of detection for this system is 20 copies/mL of plasma. The actual copy

number of <20 HIV RNA copies/mL was determined by extrapolating Ct values as described by Chun *et al*[17]. UsVL data was not available for APROCO.

Statistical analysis

Patient characteristics are presented as medians and interquartile ranges for continuous variables and percentages for categorical variables. In the univariate analysis, the association between anti-gp41 levels and the following variables was tested using Pearson correlation coefficients or the Kruskall-Wallis test: age, sex, time since HIV diagnosis (years), ART duration (years), viral suppression duration (years), CD4 and CD8 counts (cell/µL), nadir CD4 (cell/µL), CD4/CD8 ratio, total cell-associated HIV-1 DNA (log_{10}copies/10^6 cells), usVL (positive or negative as described above) and anti-gp41 levels (OD). A series of univariate models were fitted to the data and all variables providing $p < 0.05$ were retained into the final multivariate models. Each multivariate model included the retained variables and either the duration of viral suppression or HIV-1 DNA quantification. Final models were selected using a stepwise procedure selecting variables with a $p<0.05$. Statistical analyses were performed using SAS software.

Results

We quantified anti-gp41 antibody levels in 683 chronically HIV-1-infected participants from ANRS-APROCO (n=354), COPANA (n=119) and MONOI (n=210) studies . Individual and immuno-virological characteristics at the time of anti-gp41 testing are detailed in **Table 1**. Anti-gp41 antibody levels (OD) ranged from 0.0 to 3.5, the median value was 1.3 (IQR 0.6-1.9). 77.5% of the participants were male, with a median age of 41 years. The median time from HIV-1 diagnosis was 13 years. All participants were treated with ART, spanning a wide range of treatment durations from 1 to 18 years (median: 10.7 years) and virally suppressed below 50 copies/mL. The overall duration of viral suppression was a median 6.6 years (IQR, 2-9.5). usVL data was available for 328 participants, amongst whom 56% (n=184) tested negative (<1 copy/ml). The overall median cell-associated HIV-1 DNA level was 2.6 (IQR, 2.1-3.0) $\log_{10}$copies/10^6 cells. HIV-1 DNA level decreased with the number of years on ART (p<0.0001).

Associations between anti-gp41 antibody levels and participants' clinical, virological and immunological characteristics were assessed in a univariate analysis (**Table 2**). Anti-gp41 level did not correlate with the overall duration since HIV-1 diagnosis. Alternatively, it decreased with the duration of ART (Pearson r=-0.26, p<0.0001) and most markedly with the duration of effective viral suppression <50 copies/mL (r=-0.46, p<0.0001) (**Figure 1**). When taking into account the 328 available results for usVL, there was no association with anti-gp41 antibodies (negative usVL, 1.4 OD (0.7-2.1) *vs* positive usVL, 1.5 OD (0.8-1.9), Kruskall-Wallis p=0.77). However, in the MONOI participants, who were tested for usVL both at week -10 and at inclusion, anti-gp41 levels were significantly lower when usVL tested consistently negative (negative usVL, 1.1 OD (0.5-1.6) *vs* 1 or 2 positive usVL, 1.4 OD (0.7-1.9), p=0.01) (**Figure 2**). Anti-gp41

206 levels were positively correlated to cell-associated HIV-1 DNA quantification (r= 0.23,
207 p<0.0001). In addition, a lower titer of anti-gp41 antibodies was observed in
208 participants with non-detectable HIV DNA compared to those with quantified HIV DNA
209 (0.9 (0.5-1.6) *vs* 1.5 (0.8-2.1), p=0.02). Finally, antibody levels were higher in women
210 than in men (1.5 (0.8-2.1) vs 1.2 (0.6-1.8), p=0.0002).
211 In multivariate models, anti-gp41 levels remained significantly correlated with the
212 duration of ART (p=0.03), the duration of viral suppression (<0.0001) and the levels of
213 cell-associated HIV-1 DNA (p=0.0002), as well as with gender (p=0.008).
214
215

Discussion

We studied the anti-gp41 antibody levels in long-term ART participants with suppressed HIV-1 viremia below the clinical threshold of 50 copies/mL. Participants initiated ART at the chronic phase of HIV-1 infection and were treated for a median of 10.7 years (1 to 18 years). Our results confirm the hypothesis that a decrease in anti-gp41 antibodies mirrors the loss of virus production and antigenic stimulation. Anti-gp41 levels were inversely correlated with the duration of viral suppression below 50 copies/mL, and were positively correlated with HIV-1 DNA quantification, residual viremia and female gender. Thus, there was an association between specific HIV antibody levels and both the size of the total cell-associated HIV-1 DNA burden and the presence of a residual HIV-1 RNA viremia.

These results confirm previous studies in smaller cohorts which described a decrease in anti-HIV antibodies along time on ART, in both chronically infected individuals and primary HIV-1 infection participants, as well as in perinatally infected children[8,10,11,18,19]. Similarly to our results, Chaillon *et al*. using the same assay also reported higher antibody levels in ART-treated women compared to men[8]. Gender differences in HIV pathophysiology remain debated, with conflicted reports of stronger immune activation and antiviral responses in HIV-infected women[20].

Previous reports however vary regarding the correlation between anti-gp41 levels and the viral reservoir size. Brice and colleagues did not find an association between both variables in a cross-sectional study of children who initiated ART at a median age of 3 years.[18] Anti-gp41 levels, although estimated through the same immunoassay as we used in the current study, were markedly lower than the numbers we observed. The use of Dried Serum Spot samples may explain this discrepancy, along with the differences in

240 immune responses between childhood and adult age. Lee *et al.* used a luciferase
241 immunoprecipitation assay to quantify anti-HIV-1 antibodies[10,19] and did not observe an
242 association with integrated HIV-1 DNA levels. Technical variations between that study
243 and ours may explain the differences in results.
244 Alternatively, our results back up the study from Keating and colleagues in recently
245 infected participants, reporting a correlation between anti-gp41 levels by the gp41
246 limiting antigen avidity assay (LAg, described elsewhere[7]) and PBMC-associated HIV-1
247 DNA for up to six years of viral suppression.[11] We observed a similar correlation
248 between anti-gp41 EIA-RI and CD4+ T-cell-associated HIV-1 DNA for 12 years (r=0.25).
249 These results suggest the maintenance of a higher production of anti-gp41 antibodies
250 by the immune system when HIV-1 DNA burden is high. Total cell-associated HIV-1 DNA
251 has the advantage of taking virtually all viral species into account, given that full-length
252 intact viral DNA is not mandatory to expose viral antigens to the immune system. On the
253 whole, the general reduction in anti-gp41 antibodies we observed on sustained and
254 prolonged viral suppression on ART may arise concurrently from the reduction of the
255 overall viral reservoir over time and/or from its enrichment in defective proviruses,
256 leading to an impaired production of viral particles and/or viral antigens.[3,21]
257 In addition, we provide new data on usVL clinical significance. We observed an
258 association between lower anti-gp41 levels and a repeatedly negative residual viremia,
259 suggesting that systemic exposure to low-level viremia contributes to the maintenance
260 of anti-gp41 humoral responses and that the time dimension of antigenic exposure is
261 critical. Interestingly, such a time component is the basis of the “viremia copy-years”
262 biomarker, a time-updated measure of cumulative HIV-1 exposure akin to cigarette
263 pack-years in smoking exposure. Cumulative viremia copy-years is a calculation of the
264 area under the plasma viral load curve along follow-up and was described to associate

with clinical and immunological outcomes of HIV infection[22–24]. To our knowledge, such a marker has not been described regarding cumulative single-copy viremia, and would be tremendously demanding technically. Interestingly, our results point at the potential of anti-gp41 antibody activity as a surrogate marker of residual viral exposure on long-term ART, warranting further longitudinal studies.

As expected and in accordance with the previously published data, we observed heterogeneous anti-gp41 levels in the participants to our study. Individual humoral responses are driven by a variety of individual and viral factors and not all participants initiate ART with the same baseline antibody level[11,19]. At least by current laboratory assays, defining a "significance threshold" seems elusive. Accordingly, the potential of anti-gp41 titration as a biomarker for the reactivation-prone viral reservoir may be further evaluated timewise for individual follow-up, or at the population level.

Our study has several limitations. The retrospective and cross-sectional design did not allow a longitudinal follow-up of paired values of viremia and anti-gp41 antibodies, although the cross-sectional design allowed the inclusion of a wide range of ART durations. As the 3 different ANRS cohorts used for this study were run independently, some retrospective data were missing and sufficient sample material was not always available to complete HIV-1 DNA or usVL testing.

A major challenge to HIV cure strategies is the quantification of persistent replication-competent HIV. A variety of laboratory assays coexist and none is fully satisfactory. In addition, transient low-level viremia or viral blips cannot be always detected because of sparse viral load testing performed as part of the standard care for HIV-1 infection. Further longitudinal studies will be necessary to closely explore the kinetics of anti-gp41 levels and their variations in relation to low-level viremia and individual factors. A biomarker for the persistence of replication-competent HIV would be invaluable to the

evaluation of long-term adherence and efficacy of ART, to the development of new antiretroviral strategies as well as to HIV cure studies.

In conclusion, assessing HIV specific immune response through the measurement of anti-gp41 antibody levels could reflect the size of the viral reservoir and residual replication during prolonged viral suppression on long-term ART. Lower titers of anti-gp41 might reflect low antigen stimulation and better control of chronic HIV infection.

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Transparency declarations

All authors: none to declare.

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Tables

Table 1: Viral and immunological features at the time of anti-gp41 assay

Participants: n=683	**ANRS COPANA (n=119)**		**ANRS MONOI (n=210)**		**ANRS APROCO (n=354)**	
	n	median [IQR]	n	median [IQR]	n	median [IQR]
Individual characteristics						
Age (years)	119	39.5 [33.8-48.7]	210	45.7 [40.3-53]	354	37.9 [33.3-43.7]
Gender: Male	119	71.4%	210	75.2%	354	80.8%
Time from HIV-1 diagnosis (years)	79	4.6 [3.6-6.2]	209	10.7 [4.9-15.6]	354	16.1 [12.3-19.8]
Time on ART (years)	119	2.0 [1.6-2.4]	207	8.4 [3.8-11.3]	352	11.3 [10.8-11.9]
HIV-1 RNA <50cp/mL (years)	119	1.6 [1.3-1.9]	NA	…	351	8.3 [5.6-10.2}
Immunology						
Nadir CD4+ T cells(cells/µL)	119	264 [205-325]	210	220 [147-298]	354	207 [104-320]
Current CD4+ T cells (cells/µL)	119	497 [367-655]	189	583 [445-772]	350	618 [442-785]
Current CD4/CD8 ratio	119	0.9 [0.6-1]	181	0.8 [0.6-1.1]	350	0.8 [0.6-1.1]
Biomarker measures						
usVL HIV-1 RNA <1cp/mL (%)	119	72%	210	46%	NA	…
HIV-1 DNA (log_{10} cp/10^6cells)	119	2.9 [2.3-3.3]	186	2.4 [2-2.7]	NA	…
Anti-gp41 antibody (OD value)	119	1.7 [1-2.3]	210	1.3 [0.7-1.8]	354	1.1 [0.6-1.7]

n: number with data; IQR: interquartile range; cp: copy ; NA : not available

Table 2: Association between anti-gp41 levels and quantitative characteristics (univariate analysis)

Variables	Participants (n)	Pearson coefficient	Significance (2-tailed)
Age	683	-0.03	0.41
HIV-1 infection (years)	642	0.02	0.55
ART (years)	678	**-0.26**	**<0.0001**
Viral suppression (years)	470	**-0.46**	**<0.0001**
Nadir CD4+ T-cell count	683	-0.01	0.88
Current CD4+ T-cell count	658	**-0.08**	**0.02**
CD4+/CD8+ ratio	650	**-0.10**	**0.04**
Cell-associated HIV-1 DNA	305	**0.23**	**<0.0001**
Variables	**Participants (n)**	**Kruskall-Wallis test**	**Significance**
Sex	683	Male *vs* Female	**0.0002**
usVL HIV-1 RNA	328	Negative *vs* Positive	0.77
Cell-associated HIV-1 DNA below limit of detection	305	Negative *vs* Positive	**0.02**

Figure Legends

Figure 1: Anti-gp41 titers and the duration of viral suppression

Association between anti-gp41 antibody titers (OD) and duration of viral suppression below 50 copies/mL (cross-sectional analysis of 470 participants).

Figure 2: Anti-gp41 titers according to residual viremia

Anti-gp41 antibody levels according to the usVL status in MONOI participants. Undetectable usVL: negative assay at both week -10 and the inclusion visit. Positive usVL: ≥1 copy/mL usVL at one or both visits.

Figures

Figure 1

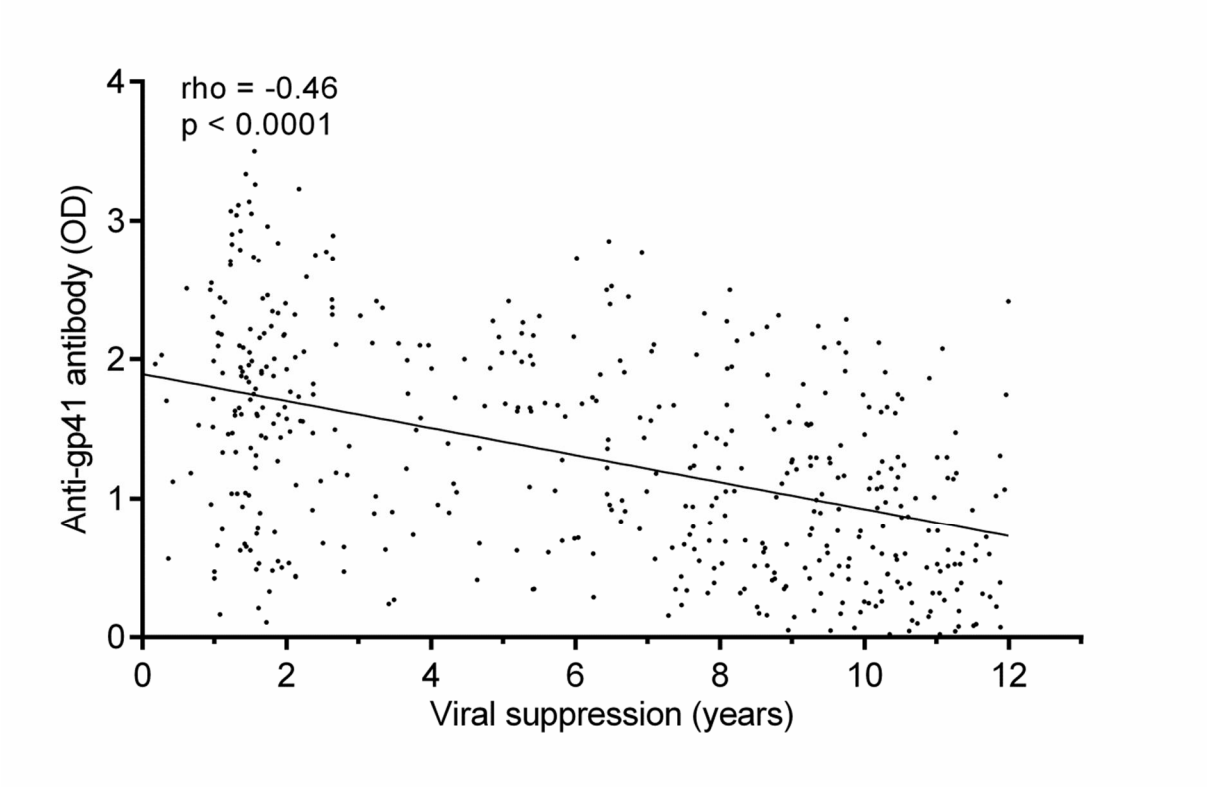
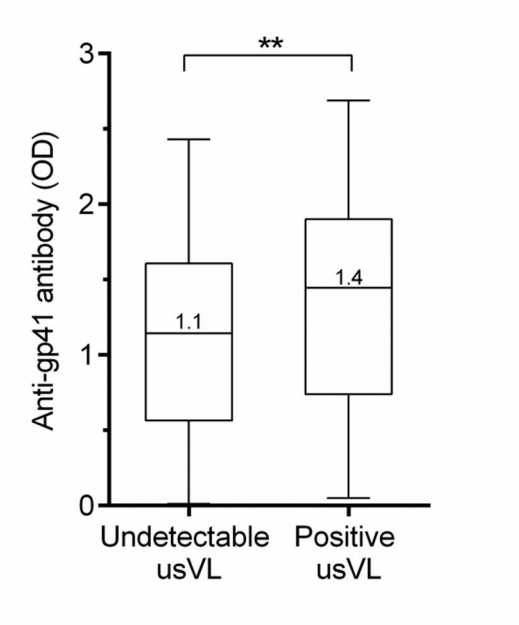


Figure 2


**
3
2
1
0
Anti-gp41 antibody (OD)
1.1
1.4
Undetectable usVL
Positive usVL