Title

Discovery of False Elite Controllers: HIV Antibody-Positive RNA-Negative Blood Donors Found To Be on Antiretroviral Therapy

Journal

The Journal of Infectious Diseases, 220(4)

ISSN

0022-1899

Authors

Sykes, Wendy
Van den Berg, Karin
Jacobs, Genevieve
et al.

Publication Date

2019-07-19

DOI

10.1093/infdis/jiz145

Peer reviewed

Discovery of “false Elite Controllers”: HIV antibody-positive RNA-negative blood donors found to be on antiretroviral treatment

Wendy Sykes[1], Karin van den Berg[1], Genevieve Jacobs[1], Adam Jauregui[2], Nareg Roubinian[4], Lubbe Wiesner[3], Gary Maartens[3] Ronel Swanevelder[1], Brian Custer[2], Michael Busch[2], Ute Jentsch[1], Edward L. Murphy[4,3] and Marion Vermeulen[1] for the NHLBI Recipient Epidemiology and Donor Evaluation Study-III (REDS-III).

1. South African National Blood Service, Johannesburg, Durban and Port Elizabeth, South Africa
2. Vitalant Research Institute, San Francisco, CA
3. Division of Clinical Pharmacology, Department of Medicine, University of Cape Town, Cape Town, South Africa
4. University of California, San Francisco, CA

Corresponding author:
Wendy Sykes, B.Tech: Biomedical Technology
10 Eden Road, Pinetown, South Africa 3610
wendy.sykes@sanbs.org.za

Abstract 99 words

Text 1910 words

Abstract

Background: An increase in potential HIV elite controllers (EC) and anecdotal reports of antiretroviral therapy (ART) use among South African blood donors led us to verify EC status.

Methods: Stored plasma samples from potential EC were tested for ART drugs. Demographic and temporal associations were examined using multivariable logistic regression.

Results: 150 (66.4%) of 226 potential EC had detectable ART with increasing prevalence by year (OR=7.57 for 2016 vs. 2010, 95% CI 1.96-32.17).

Discussion: False presumptive EC status due to undisclosed ART represents a growing proportion of potential EC donors in South Africa coincident with the country's ART rollout.

Key words

HIV, Antiretroviral therapy, Elite Controller, South Africa, Blood donors,

Disclosure

Background

There are an estimated 7 million people living with HIV in South Africa with 3.7 million on antiretroviral therapy (ART) (1) making this the largest ART program in the world. To counteract this high HIV prevalence the South African National Blood Service (SANBS) uses the most sensitive testing technologies to screen donated blood for HIV antibodies (Ab) and HIV RNA.

An HIV elite controller (EC) is generally accepted to be an HIV infected person who maintains a viral load of <50 copies/ml for at least 2 years and who is not on ART (2). In the 11 years since the introduction of individual donation nucleic acid testing (ID-NAT) (2005 to 2016), 302 HIV Ab positive, RNA negative (i.e. Ab+/RNA-) donations have been detected as potential EC. As the 95% detection limit for the ID-NAT assay used is <20 copies/ml (3) and the pre-donation questionnaire excludes those with previously diagnosed HIV or on ART, the classification of Ab+/RNA- donors as potential EC seemed reasonable on the basis of previous analyses and publications albeit without two year follow up (4).

Failure to disclose HIV status and ART use has been confirmed in at least two settings namely a multinational clinical trial reported by Fogel *et al.* (5) and population studies in Kenya (6) and Uganda (7). This suggests that this phenomenon might be more widespread than previously thought and should be of concern in other blood donor populations or cohort studies of putative EC. In 2013 we observed an increase in the proportion of Ab+/RNA- donations relative to the number of HIV positive (Ab+/RNA+) donors; from 1.8% (2013) to 4.5% (2016) (Supplementary Figure 2). This is higher than the <1% proportion of EC among HIV positive persons reported in other settings (8) and at SANBS in 2005 (9). More recently, we received reports from field staff enrolling donors into an

EC cohort study of donors disclosing prior knowledge of HIV infection and ART use.

With South Africa moving into an era of “universal test and treat”(1) following the announcement in September 2016 that all HIV infected people would be eligible to receive treatment regardless of CD4 count, we can expect the number of people on ART to continue to increase. The study of EC is relevant to better understanding HIV pathogenesis, potential identification of host factors that could inform vaccine discovery, and mechanisms of HIV control. These research efforts could be jeopardized if potential false EC enroll into these studies and influence the findings.

We therefore performed a retrospective evaluation aimed at determining the prevalence of unadmitted ART use among presumptive ECs and examined whether this phenomenon was associated with the broader rollout of ART in South Africa.

Methods

Study design and population. We retrospectively tested stored plasma samples from HIV Ab+/RNA- blood donations between January 2010 and December 2016 for ART drugs. This study received ethics approval from the SANBS research ethics committee.

Pre-donation assessment. Per SANBS policy, a donor assessment, including a risk behavior and health questionnaire (including questions on previous HIV diagnosis and ART use) and a one-on-one interview by trained SANBS staff, was performed

prior to blood donation. Only those deemed to be at low risk of HIV were accepted as blood donors.

Viral marker testing. Blood donations were tested in parallel for HIV Ab and HIV RNA. The Abbott Prism HIV 1/2 (Abbott Diagnostics, Delkenheim, Germany) was used to screen for anti-HIV. Two different platforms, both supplied by Grifols Diagnostics (Barcelona, Spain), were used to screen for HIV RNA using ID-NAT. Procleix Ultrio and Ultrio Plus (Tigris platform) were used from October 2005 to April 2011 and May 2011 to December 2015, respectively, and Ultrio Elite (Panther platform) from January to December 2016. The sensitivity of HIV detection has been shown to be the same on all three assays (4) (3, 10). Duplicate repeat and discriminatory testing was performed on all initial reactives to determine which viral nucleic acid was detected by the triplex screening assay. An immunoblot assay was used to confirm the Ab result in Ab+/RNA- samples and only confirmed Ab+/RNA- donations were included as potential EC in the study (Supplementary Figure 3).

ART drug testing. Available stored Ab+/RNA- plasma samples were sent to the University of Cape Town for ART drug testing using validated assays for the detection of Nevirapine, Efavirenz, Lopinavir, Darunavir and Atazanavir on liquid chromatography tandem mass spectrometry (sensitivity 0.02µg/ml). This drug combination was selected to detect the majority of treatment options in use in South Africa during the period of the study.

Definitions. For the purposes of this study, we defined donors who tested HIV Ab+/RNA- and negative for ART drugs as true presumptive ECs, HIV Ab+/RNA- and positive for ART drugs as false presumptive ECs and HIV Ab+/RNA+ as HIV

positive donors. ART rollout statistics for the period 2007 to 2016 were published in the 2012 to 2016 report of the South African National Strategic Plan (1).

Statistical Analysis. We examined whether various donor characteristics were associated with false presumptive EC status. Chi-square tests were used to assess differences in the proportions of true/false presumptive EC by several factors (gender, population group, age, geographical area, donor incentives, clinic site and donation year), applying Yates' continuity correction when necessary and using a trend test of proportions on the variable donation year. For multivariable analysis, logistic regression was performed using R version 3.4.3. We considered the aforementioned factors for inclusion in the logistic regression model to predict false presumptive EC, evaluating for correlations and interactions between the predictor variables. A p value 0.05 was regarded as significant.

Results

SANBS collected 5,754,586 donations from January 2010 to December 2016, of which 12,705 (0.22%) were HIV positive and 270 (0.005%) were Ab+/RNA-. Only 1,486,202 (25.8%) collections were from Black donors but 11,449 (90%) of all HIV positives and 250 (93%) of the Ab+/RNA- donations were from this population group.

Of the 270 HIV Ab+/RNA- donations, 226 had sufficient plasma volume available for ART drug testing (Table 1). Of these 150 (66.4%) tested positive for the presence of ART drugs and were classified as false presumptive EC: 130 (87%) were positive for the presence of Efavirenz, 13 (8.7%) for Nevirapine and seven (4.7%) for Lopinavir. Darunavir and Atazanavir were not detected in any of the

specimens tested. Black donors made up 95% of donors found to be positive for ART drugs, while the small number of donors in other population groups had equal numbers of false and true presumptive EC (p=0.36; Supplementary Table 2). False presumptive EC prevalence was highest in donors 31 – 40 years of age and lowest in donors >50 years of age (p=0.36).

The proportion of presumptive EC's found to be positive for ART increased from 38.5% in 2010 to 76.1% in 2016 (Table 1). This correlated with year of donation and, by inference, with the progressive national rollout of ART and the number of people on ART in South Africa (Figure 1).

In the multivariable analysis, the odds of a donor testing positive for ART drugs increased significantly by year from 2010 to 2016 (OR: 7.57; 95% CI: 1.96 - 32.17) and was less than one for the Eastern Cape geographical area. There were borderline associations with male gender and at mobile versus fixed blood collection clinics.

Discussion

In our study, we found that two thirds of donors who tested Ab+/RNA- had evidence of ART drugs in their donation plasma and therefore designated as false presumptive EC. We found a significant association between year of donation and the number of presumptive EC found to be on ART drugs coinciding with the massive ART rollout in South Africa. In addition to having blood safety implications, these results are important because nondisclosure of ART use has implications for other studies of EC, HIV clinical trials as well as diagnostic testing and HIV incidence assays.

We hypothesize that the blood collection service is likely experiencing a spillover effect from the ART rollout with the estimated number of people on ART increasing from 933,621 in 2010 to 3.7 million in 2016 (1). As most HIV infected people in South Africa are treated in the public sector where access to monitoring tests is limited, it is possible that some are seeking HIV-related testing that may be perceived to be of a better quality in the blood collection service.

Unreported ART use has previously been detected in community surveys and HIV clinical trials (5-7). Our finding that the phenomenon occurs in prospective blood donors with the EC phenotype suggests that other cohorts of EC may wish to incorporate ART testing into their enrollment criteria. In addition, there are other settings where undisclosed ART use may influence outcomes including health estimates of ART coverage and clinical trials of HIV prevention and vaccines. Finally EC may be misclassified as recent infections in Recent Infection Testing Algorithms, with such “false recents” causing overestimation of HIV incidence (11).

ART use by potential blood donors poses a risk to blood safety for countries that, like South Africa, have high HIV prevalence and good ART coverage. Although initiation of ART shortly after diagnosis may limit the size of the viral reservoir and provide a better prognosis for persons with HIV it may also affect laboratory test results if HIV antibodies fail to develop or decline after ART initiation (12-14). We previously modelled that a potential EC (presumed not on ART) had a 2% and 15% chance of causing transmission related to transfusion of a single unit of red blood cells and fresh frozen plasma component, respectively, when a minimum infectious dose of 316 virions is used (4).

The findings of this study may also have relevance to the recent introduction of Pre-exposure prophylaxis (PrEP) for sex workers, MSM and at-risk young women in South Africa. Donnell *et al*. describe delayed HIV detection and prolonged HIV seroconversion in individuals on PrEP who become infected with HIV (15). One can hypothesize that the same would apply to blood donors who may fail to seroconvert, have delayed seroconversion or sero-revert (loss of previously detectable antibodies), hindering laboratory diagnosis and counselling of HIV-infected donors or, in a worst case scenario, a missed HIV positive donation.

This study had some limitations. We were not able to assess 16% of Ab+/RNA- donations for ART drug testing in which samples were not available. Nor did we test samples from concordant HIV Ab+/RNA+ donations. This group may include donors with low levels of viremia who may also be on ART with incomplete viral suppression (3). Given the short half-life of the drugs tested, it is also possible that we are underestimating ART use (6). Heterogeneity in individual drug metabolism may have resulted in donors who failed to take their ART in the days prior to donating being incorrectly classified as true presumptive EC.

In conclusion, we have demonstrated that unadmitted ART use occurs in prospective blood donors in addition to previously reported clinical trial settings. The failure to exclude such individuals brings into question the effectiveness of current pre-donation assessment procedures and has implications for other HIV studies. Although it represents a small, but serious risk to blood transfusion recipients, any perceived risk of HIV transmission would result in a lack of public trust in the blood service. The reasons for non-disclosure of HIV status and ART drug use by donors and what proportion is intentional or unintentional is

currently unknown and requires further investigation to evaluate the extent of this phenomenon and to determine motivations for this behavior.

232**Table 1**: Anti-retroviral treatment (ART) drug prevalence among HIV Ab+/RNA-
233blood donations sent for ART drug testing between 2010 and 2016 and
234multivariable odds ratios for associations with donor and donation
235characteristics.

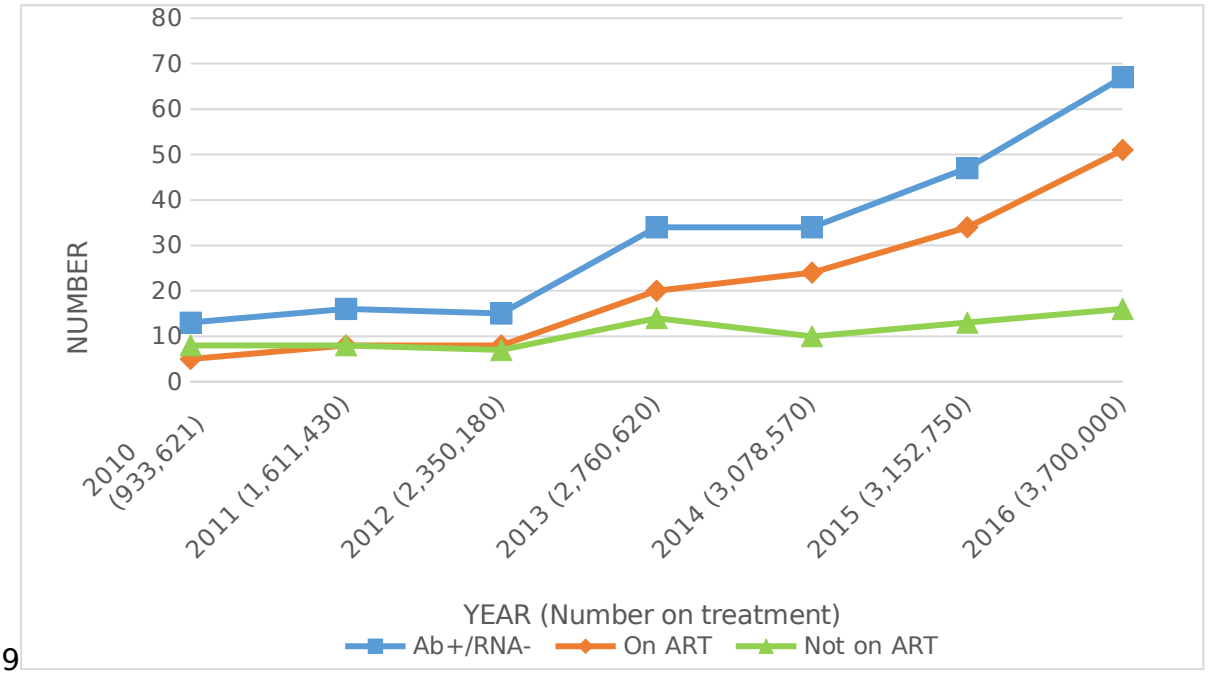
Donor Demographics	HIV Ab+/RNA- samples sent for ART testing	False presumptive EC - positive for ART Drugs		Odds Ratio (95% Confidence Interval)	
	N	N	%	OR	95% CI
Total	226	150	66.4		
Gender					
Male	51	38	74.5	1	----
Female	175	112	64.0	2.15	0.97-5.10
Population Group					
Non-Black*	14	1	7.1	1	----
Black	212	143	67.5	2.46	0.72-8.53
Age (Years)					
<20	22	15	68.2	1	----
20-30	61	38	62.3	1.06	0.33-3.26
31-40	91	66	72.5	1.78	0.55-5.47
41-50	41	26	63.4	0.98	0.26-3.54
>50	11	5	45.5	0.46	0.08-2.43
Geographic Area					
Eastern Cape	12	4	33.3	1	----
Egoli (Johannesburg)	42	29	69.0	0.20	0.04-0.90
Free State/North Cape	9	6	66.7	1.15	0.20-7.73
KwaZulu Natal	52	38	73.1	1.22	0.43-3.42
Mpumalanga	38	26	68.4	1.05	0.36-3.08
Northern	43	29	67.4	0.94	0.33-2.66
Vaal	30	18	60.0	0.73	0.24-2.20
Donor Incentives**					
No Incentives offered	193	125	64.8	1	----
Incentives offered	33	25	75.8	1.03	0.36-3.15
Clinic Site					
Fixed Site Clinic	29	16	55.2	1	----
Mobile Clinic	197	134	68.0	2.46	0.98-6.22
Year					
2010	13	5	38.5	1	----
2011	16	8	50.0	2.05	0.41-10.92
2012	15	8	53.3	2.86	0.53-16.6

2013	34	20	58.8	2.97	0.71-13.3
2014	34	24	70.6	5.41	1.24-26.0
2015	47	34	72.3	7.16	1.55-36.3
2016	67	51	76.1	7.57	1.96-32.2

* "Non-black" includes Asian, White, Coloured (local term for mixed race), and unknown

** Incentive periods were calculated according to date ranges provided by SANBS.

Figure 1 The number of SANBS blood donations found to be potential HIV elite controllers (EC) (Ab+/RNA-; squares), false presumptive EC (On ART; diamonds) and true presumptive EC (Not on ART; triangles) between 2010 and 2016. The estimated number of persons on ART in the general South African population is given beneath the X-axis [1].



253References

2541. Department of Health. Reflections on NSP 2012-2016 and moving forward 255to NSP 2017-2022. 2016.
2562. Deeks SG, Walker BD. Human immunodeficiency virus controllers: 257mechanisms of durable virus control in the absence of antiretroviral therapy. 258Immunity. 2007;27(3):406-16. Epub 2007/09/26. doi: 25910.1016/j.immuni.2007.08.010. PubMed PMID: 17892849.
2603. Grabarczyk P, van Drimmelen H, Kopacz A, Gdowska J, Liszewski G, 261Piotrowski D, et al. Head-to-head comparison of two transcription-mediated 262amplification assay versions for detection of hepatitis B virus, hepatitis C virus, 263and human immunodeficiency virus Type 1 in blood donors. Transfusion. 2642013;53(10 Pt 2):2512-24. Epub 2013/04/18. doi: 10.1111/trf.12190. PubMed 265PMID: 23590145.
2664. Vermeulen M, Coleman C, Mitchel J, Reddy R, van Drimmelen H, Fickett T, 267et al. Comparison of human immunodeficiency virus assays in window phase and 268elite controller samples: viral load distribution and implications for transmission 269risk. Transfusion. 2013;53(10 Pt 2):2384-98. Epub 2013/03/01. doi: 27010.1111/trf.12117. PubMed PMID: 23445273; PubMed Central PMCID: 271PMCPMC5061040.
2725. Fogel JM, Wang L, Parsons TL, Ou SS, Piwowar-Manning E, Chen Y, et al. 273Undisclosed antiretroviral drug use in a multinational clinical trial (HIV Prevention 274Trials Network 052). J Infect Dis. 2013;208(10):1624-8. Epub 2013/08/03. doi: 27510.1093/infdis/jit390. PubMed PMID: 23908493; PubMed Central PMCID: 276PMCPMC3805242.
2776. Kim AA, Mukui I, Young PW, Mirjahangir J, Mwanyumba S, Wamicwe J, et al. 278Undisclosed HIV infection and antiretroviral therapy use in the Kenya AIDS 279indicator survey 2012: relevance to national targets for HIV diagnosis and 280treatment. AIDS. 2016;30(17):2685-95. Epub 2016/10/27. doi: 28110.1097/QAD.0000000000001227. PubMed PMID: 27782965.
2827. Grabowski MK, Reynolds SJ, Kagaayi J, Gray RH, Clarke W, Chang LW, et al. 283The validity of self-reported antiretroviral use in persons living with HIV: a 284population-based study. AIDS. 2018;32(3):363-9. Epub 2017/12/02. doi: 28510.1097/QAD.0000000000001706. PubMed PMID: 29194115; PubMed Central 286PMCID: PMCPMC6171354.
2878. Okulicz JF, Lambotte O. Epidemiology and clinical characteristics of elite 288controllers. Curr Opin HIV AIDS. 2011;6(3):163-8. Epub 2011/04/20. doi: 28910.1097/COH.0b013e328344f35e. PubMed PMID: 21502920.
2909. Vermeulen M, Lelie N, Sykes W, Crookes R, Swanevelder J, Gaggia L, et al. 291Impact of individual-donation nucleic acid testing on risk of human 292immunodeficiency virus, hepatitis B virus, and hepatitis C virus transmission by 293blood transfusion in South Africa. Transfusion. 2009;49(6):1115-25. Epub 2942009/03/25. doi: 10.1111/j.1537-2995.2009.02110.x. PubMed PMID: 19309474.
29510. Grabarczyk P, Koppelman M, Boland F, Sauleda S, Fabra C, Cambie G, et 296al. Inclusion of human immunodeficiency virus Type 2 (HIV-2) in a multiplex 297transcription-mediated amplification assay does not affect detection of HIV-1 and 298hepatitis B and C virus genotypes: a multicenter performance evaluation study. 299Transfusion. 2015;55(9):2246-55. Epub 2015/06/24. doi: 10.1111/trf.13193. 300PubMed PMID: 26103564.
30111. Curtis KA, Hanson DL, Kennedy MS, Owen SM. Evaluation of a multiplex 302assay for estimation of HIV-1 incidence. PLoS One. 2013;8(5):e64201. Epub 3032013/05/30. doi: 10.1371/journal.pone.0064201. PubMed PMID: 23717568; 304PubMed Central PMCID: PMCPMC3661489.

12. Bruner KM, Murray AJ, Pollack RA, Soliman MG, Laskey SB, Capoferri AA, et al. Defective proviruses rapidly accumulate during acute HIV-1 infection. Nat Med. 2016;22(9):1043-9. doi: 10.1038/nm.4156. PubMed PMID: 27500724; PubMed Central PMCID: PMCPMC5014606.
13. Lee SA, Deeks SG. The Benefits of Early Antiretroviral Therapy for HIV Infection: How Early is Early Enough? EBioMedicine. 2016;11:7-8. Epub 2016/08/16. doi: 10.1016/j.ebiom.2016.08.009. PubMed PMID: 27515690; PubMed Central PMCID: PMCPMC5049928.
14. de Souza MS, Pinyakorn S, Akapirat S, Pattanachaiwit S, Fletcher JL, Chomchey N, et al. Initiation of Antiretroviral Therapy During Acute HIV-1 Infection Leads to a High Rate of Nonreactive HIV Serology. Clin Infect Dis. 2016;63(4):555-61. doi: 10.1093/cid/ciw365. PubMed PMID: 27317797.
15. Donnell D, Ramos E, Celum C, Baeten J, Dragavon J, Tappero J, et al. The effect of oral preexposure prophylaxis on the progression of HIV-1 seroconversion. AIDS. 2017;31(14):2007-16. Epub 2017/07/12. doi: 10.1097/QAD.0000000000001577. PubMed PMID: 28692542; PubMed Central PMCID: PMCPMC5578893.

Footnote page:

1. Conflict of interest statement

No conflicts of interest noted

2. Funding statement

This work was supported by the National Heart, Lung and Blood Institute (NHLBI) (Recipient Epidemiology and Donor Evaluation Study-III contract HHSN268201100009I) and the National Institute of Allergy and Infectious Diseases (NIAID) of the National Institutes of Health (Award nos. UM1 AI068634, UM1 AI068636 and UM1 AI106701) of the National Institutes of Health, Bethesda, MD, USA. In addition, we wish to acknowledge the support from the University of California, San Francisco's International Traineeships in AIDS Prevention Studies (ITAPS), U.S. NIMH, R25MH064712 and a NIH Fogarty HIV Research Training Grant D43-TW010345 to Dr. Murphy. The content is solely the responsibility of the authors and does not necessarily represent the official views of the sponsors.

3. Meetings where information has previously been presented
    a. IPFA, May 2017, Zagreb, Croatia
    b. ISBT, June 2017, Copenhagen, Denmark abstract code (3B-S07-04)

4. Corresponding author contact information

Wendy Sykes, B.Tech: Biomedical Technology
10 Eden Road, Pinetown, South Africa 3610
wendy.sykes@sanbs.org.za

5. Alternate corresponding author

Marion Vermeulen
South African National Blood Service
marion.vermeulen@sanbs.org.za

Acknowledgements

We would like to thank the Operations Testing staff of the South African National Blood Service for assistance with specimen preparation and Dr. Steven Deeks of the University of California San Francisco for scientific input.