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MAJOR ARTICLE

Characterization of Rare Spontaneous Human Immunodeficiency Virus Viral Controllers Attending a National United Kingdom Clinical Service Using a Combination of Serology and Molecular Diagnostic Assays

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Background. We report outcomes and novel characterization of a unique cohort of 42 individuals with persistently indeterminate human immunodeficiency virus (HIV) status, the majority of whom are HIV viral controllers.

Methods. Eligible individuals had indeterminate or positive HIV serology, but persistently undetectable HIV ribonucleic acid (RNA) by commercial assays and were not taking antiretroviral therapy (ART). Routine investigations included HIV Western blot, HIV viral load, qualitative HIV-1 deoxyribonucleic acid (DNA), coinfection screen, and T-cell quantification. Research assays included T-cell activation, ART measurement, single-copy assays detecting HIV-1 RNA and DNA, and plasma cytokine quantification. Human immunodeficiency virus seropositivity was defined as ≥3 bands on Western blot; molecular positivity was defined as detection of HIV RNA or DNA.

Results. Human immunodeficiency virus infection was excluded in 10 of 42 referrals, remained unconfirmed in 2 of 42, and was confirmed in 30 of 42, who were identified as HIV elite controllers (ECs), normal CD4 T-cell counts (median 820/mL, range 805–1336), and normal CD4/CD8 ratio (median 1.8, range 1.2–1.9). Elite controllers had a median duration of elite control of 6 years (interquartile range = 4–14). Antiretroviral therapy was undetected in all 23 subjects tested. Two distinct categories of ECs were identified: molecular positive (n = 20) and molecular negative (n = 10).

Conclusions. Human immunodeficiency virus status was resolved for 95% of referrals with the majority diagnosed as EC. The clinical significance of the 2 molecular categories among ECs requires further investigation.

Keywords. ART; HIV; HIV cure; HIV diagnostics; HIV elite control.

Accurate testing and diagnosis of human immunodeficiency virus (HIV) is essential for treatment, surveillance, and prevention, and it is crucial to achieve the UNAIDS 95-95-95 target towards zero new HIV infections [1, 2]. Testing for HIV-specific antibodies has been the backbone of HIV diagnostics since HIV immunoassays (enzyme immunoassays [EIA]) and Western blots came into use over 3 decades ago [3, 4]. The majority of clinical HIV testing algorithms use multiple markers including HIV-specific antibodies, viral p24 antigen, and HIV deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) to confirm status [5]. Testing algorithms recommended by the World Health Organization state that HIV-positive status must be based on the result of 2 or more HIV-specific antibody tests using assays with high sensitivity and specificity [6]. Although it is not universal, indeterminate HIV status is usually resolved through repeated testing [7–10], and it is impacted by the use of antiretroviral therapy (ART) for pre- and postexposure prophylaxis (PrEP and PEP) [11, 12]. Multiple factors may impact performance of HIV diagnostic tests, including autoimmune diseases, coinfection with leprosy, postmeasles virus infection, hepatitis B and C, [13] severe acute respiratory syndrome coronavirus 2 [14] elevated bilirubin, polyclonal gammopathies, and hemodialysis [15, 16].

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Although the majority of people with HIV require ART to fully suppress viral replication, approximately 1%–5% achieve this spontaneously; these people are referred to as "elite controllers" (ECs) [17]. However, there is no universally accepted definition of such a phenotype, and hence the literature describes a heterogeneous group, often with detectable but low-level viremia [17].

Multiple mechanisms underlie spontaneous viral control in EC cohorts, including host genetic factors, such as HLA-B alleles B*27, B*57, and B*14 [18, 19] and CCR5 delta 32 mutation [20]. A recent study reported that in comparison to fully suppressed individuals who are HIV positive and on ART, ECs have a distinctive reservoir in which intact HIV proviruses are predominantly located in nontranscribed locations of the host genome resulting in deep latency [21]. Immunologically mediated viral control has also been demonstrated [22, 23] through enhanced $CD8^+$ T-cell polyfunctionality, including highly effective degranulation perforin and granzyme B release as well as HIV antigen-specific responses as measured by the production of interferon-γ, tumor necrosis factor (TNF)-α, interleukin (IL)-2, and macrophage-inflammatory protein 1 (MIP-1)b [24–28]. Different patterns of HIV-specific antibody development in ECs have been demonstrated with more potent immunoglobulin (Ig)A responses to the HR1 domain of HIV envelope GP41, higher avidity gp41-specific IgA, and a higher number of IgA responses to HIV-1 gp160 in comparison to HIV progressors [25]. Lower plasma IL-18, C-reactive protein (CRP), and IP-10 concentrations in ECs have also been described in comparison to individuals who are HIV negative [26].

Clinical management of ECs remains unclear with (1) a lack of consensus on long-term clinical outcomes without ART [27] and (2) unknown risks versus benefits of ART. United States and United Kingdom guidelines currently recommend enrollment into clinical studies [29].

The aim of this service is to clarify diagnosis in a group of patients with indeterminate HIV status, many of whom have lived with this uncertainty for decades. In doing so, we have identified a group of ECs, using a rigorous definition, who have been further characterized to identify investigations that might help inform best management. The majority have elected to remain off ART but under review.

METHODS

Participants

A specialist national referral clinical service was established in 2017 between Imperial College London and the United Kingdom Health Security Agency (UKHSA), the Indeterminate Retrovirus Infection Service (IDRIS) after recognition of a discrete group of patients with longstanding, uncertain HIV status.

Forty-two individuals attending during 2017–2021 were recruited to the Imperial College Communicable Diseases Research Tissue Bank (CDRTB). Elite controllers were defined as individuals who maintain HIV viral load (HVL) <20 copies RNA/mL in the absence of ART on more than 1 occasion at least 6 months apart in the absence of ART. Molecular-positive status was based on the molecular assays used: any detection of HIV DNA or RNA is positive; no detection is negative.

Testing

The following routine diagnostic assays were performed through the local National Health Service Pathology services: (1) screening for anti-HIV antibodies (and/or p24 antigen) using the Alinity HIV Ag/Ab Combo assay (Abbott, Chicago, IL, USA) and confirmed with Bio-Rad Bioplex 2200 HIV Ag-Ab assay; HIV serotyping (types 1 or 2) using the Genius HIV 1/2 assay (Bio-Rad, Hercules, CA, USA); (2) HVL in plasma was measured using the COBAS HIV-1 assay (Roche, Basel, Switzerland), which quantifies HIV-1 RNA (20–10 000 000 copies/mL); (3) lymphocyte immunophenotyping (including quantification of $CD3^+$, $CD4^+$, and $CD8^+$ T lymphocytes and expression of CD25 and HLA-DR on T cells) performed by flow cytometry using the Navios system (Beckman Coulter, Brea, CA, USA); (4) hepatitis B surface antigen screening using the Abbott Alinity HBsAg assay and confirmed by LIAISON XL hepatitis B antigen assay (DiaSorin, Saluggia, Italy); (5) hepatitis C antibody detection using the Abbott Alinity i Anti-HCV assay; (6) anti- HTLV-1/2 antibodies tested using the Abbott Alinity HTLV-1/II assay; (7) cytomegalovirus (CMV) and Epstein-Barr virus (EBV) IgM and IgG serology performed using the respective Abbott Alinity assays; (8) syphilis serology using the Abbott Alinity Syphilis IgG/M EIA assay; and (9) autoantibody screen - antinuclear antibody (ANA) screening through the Phadia Immunocap 250 analyzer (Thermo Fisher Scientific) and confirmed with indirect immunofluorescence (Inova Hep2 slides); rheumatoid factor testing carried out using the Abbott Alinity assay; and antimitochondrial antibody screening carried out by indirect immunofluorescence using rodent liver, kidney, and stomach slides (Inova) and confirmed by immunoblot (Euroimmun, London, UK).

The following specialist assays were undertaken at the National HIV reference laboratory at UKHSA Colindale: (1) HIV Western blot - antibodies to HIV-1 gag, pol, and envelope proteins and HIV-2 were detected using the MP diagnostics HIV Blot 2.2 according to the manufacturer's instructions (MP Biomedicals, Santa Ana, CA, USA); (2) enzyme linked immunosorbent assay (ELISA) - ELISAs were performed with plasma using the MP diagnostics HIV Ag/Ab Combo ELISA 4.0 (MP Biomedicals, Santa Ana, CA, USA), Murex HIV Ag/Ab Combination (Abbott Diagnostics, Abbott Park), or Enzygnost Integral II HIV Ag/Ab Combination kit (Dade

Behring) according to the manufacturer's instructions; and (3) qualitative HIV-1 DNA and RNA assays - an in-house assay was used to detect HIV-1 RNA and proviral DNA simultaneously in 320 µL of whole blood by quantitative polymerase chain reaction (q-PCR) using TaqMan technology with long terminal repeat (LTR), pol, gag, and integrase primers; primer and probes details (Life Technologies, Bleiswijk, Netherlands) are in Supplementary Table 1 and PCR program are in Supplementary Table 2.

Research Assays Undertaken at Imperial College London

For sample processing, plasma and peripheral blood mononuclear cells (PBMCs) were harvested from whole blood using methods previously described [30]. For single-copy, seminested HIV-1 RNA qPCR, an HIV-1 ultrasensitive, seminested q-PCR targeting the integrase gene with a sensitivity of 1 HIV RNA copy/mL plasma was used to quantify HIV-1 in 10 mL of plasma using methods previously described [30]; for primers and probes see Supplementary Table 3, for PCR programs see Supplementary Table 4. For HIV-1 proviral DNA quantification, an HIV-1 ultrasensitive real-time PCR targeting the integrase gene with a sensitivity of 1 HIV DNA copy/200 000 PBMCs was used to detect and quantify HIV-1 by targeting the integrase gene. This was performed in triplicate; therefore, the input for each patient sample was 600 000 cells. The DNA was extracted from PBMCs using a QIAamp midi DNA kit (QIAGEN, Hilden, Germany), and an ACH-2 cell line [28] was used to prepare the standard curve. Details on primers, probes, and PCR programs are available in Supplementary Table 5. β-globin copy numbers were used as a housekeeping gene, and primers and probes details are in Supplementary Table 6. For plasma cytokine concentrations, the concentrations of IL-2, IL-6, CRP, TNF-α, and MIP-1β in plasma were measured using a multispot chemiluminescent assays (V-plex; Meso Scale Discovery [MSD]) as per the manufacturer's instructions. Cytokines were measured for 23 of 42 subjects due to limited plasma availability. Reference ranges were provided by manufacturer. For detection of antiretroviral compounds in plasma, to exclude undisclosed ART use, ultraperformance liquid chromatography (Waters; ACQUITY) (cut off 25 ng/mL) was used to detect tenofovir, emtricitabine, abacavir, and lamivudine in 23 of 30 ECs with available plasma using methods previously described [31].

Statistical Analysis

The Kruskal-Wallis test was used to test the variance between 3 groups of non-parametric data. The Fisher's exact test was used to determine whether there were nonrandom associations between categorical variable were assigned numbers to facilitate this. The Mann-Whitney *U* test was used to compare differences between 2 nonnormally distributed groups of numerical data. A *P* value of $\leq .05$ was considered to show evidence of statistical association.

Patient Consent Statement

Samples were donated by individuals who provided written informed consent via to the Imperial College CDRTB (National Research Ethics Service References 15/SC/0089 and 20/SC/0226). Tissues are stored under Human Tissue Authority License 12275. Ethical approval was obtained from CDRBT.

RESULTS

Initial Characterization

Forty-two individuals were assessed (Figure 1). Ten individuals had no bands on a Western blot and no detectable HIV RNA, DNA, or p24 antigen. They form the confirmed HIV-negative control group. Human immunodeficiency virus infection was

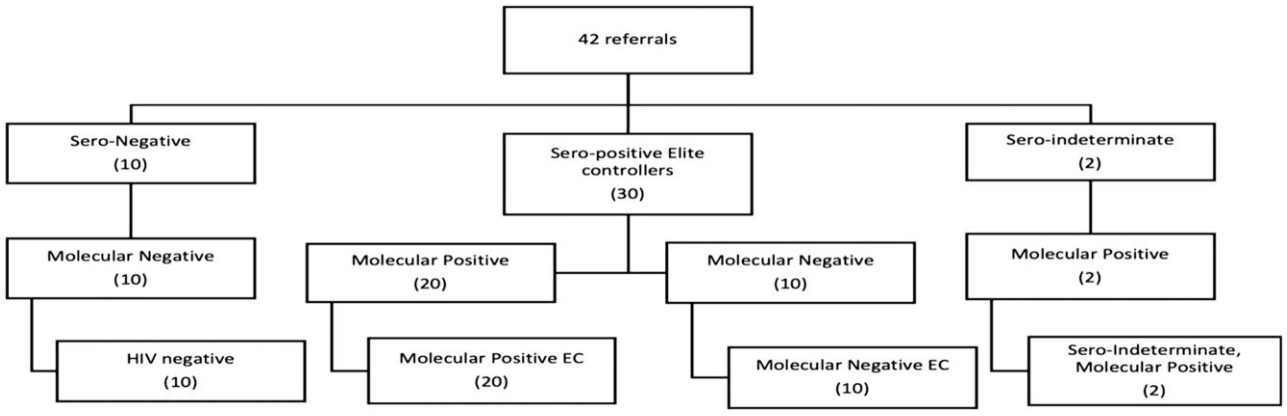

Figure 1. Forty-two referrals were assessed. Ten were found to be human immunodeficiency virus (HIV) negative, 30 were classified as elite controllers (ECs): 20 molecular-positive ECs and 10 molecular-negative ECs. Two remain indeterminate and are in follow up. Comparison of immune activation between molecular-positive ECs, molecular-negative ECs, and HIV-negative individuals.

confirmed in 30 individuals by presence of ≥3 bands, including 2 ENV (gp160/gp41 and gp120) and 1 GAG (p17, p24, p55) or POL (p31, p51, p66). Each met the criteria for EC, and among them 2 distinct categories of ECs were identified; 20 were categorized as "molecular positive" with HIV nucleic acids detected at first visit, and 10 were categorized as "molecular negative". Table 1 summarizes the characteristics of 40 of 42 participants for whom HIV status has been resolved.

The 2 remaining individuals have been diagnosed as "sero-indeterminate, molecular positive" based on HIV RNA and DNA detected on the ultrasensitive HIV-1 RNA and gag DNA assay, respectively, and continue to present with indeterminate HIV serology. The first has antibodies against p24 and gp160 on Western blot, a CD4 count of 1498 (normal range = 300–1400), and normal immune activation markers. The second has anti-p24 antibodies on Western blot and normal immune activation markers.

Clinical Characteristics of Referrals

There was no difference in terms of gender, ethnicity, age, and $CD4^+$ and $CD8^+$ T-cell counts or CD4:8 ratio among the HIV-negative, molecular-positive ECs, and molecular-negative ECs (Table 1). All ECs had CD4:8 ratio >1. Seven ECs (1 molecular negative, 6 molecular positive) had either a CD4 and/or CD8 count above the normal range (Supplementary Table 7).

T-Cell Activation Markers

T-cell activation markers were normal in all 10 subjects who were HIV negative but raised in 10 ECs (8 molecular positive, 2 molecular negative) (Supplementary Table 8). However, this did not reach statistical significance as determined by the Kruskal-Wallis test (Figures 2*A* and 3*E*).

Elite controllers with immune activation markers outside of normal range (n = 10) appear to have a lower mean CD4/CD8 ratio (1.6 in comparison to 1.8) and a lower median (1.4 [interquartile range {IQR} = 1.0–2.1] in comparison to 1.6 [IQR, 1.4–2.1]). However, when CD4/CD8 ratios between the 2 groups were analyzed using a Mann-Whitney *U* test, no significant difference was observed.

Inflammatory Cytokines

There was no evidence of inflammation as measured by IL-2, IL-6, CRP, TNF-α, and MIP-1β plasma concentrations in either EC groups or the HIV-negative groups ($P \geq .05$), with no differences between these 3 groups ($P \geq .05$) (Figure 3A–F).

Detection of Antiretroviral Compounds in Plasma

Tenofovir, emtricitabine, abacavir, or lamivudine (single time point analysis) was not detected in plasma samples from 23 of 23 ECs tested.

Coinfection and Autoimmunity Screen

Evidence of CMV and EBV infection was found in 50% and 75% of referrals, but there was no evidence of acute EBV or CMV infection, no evidence of HTLV-1/2 infection, nor of past or current infection with HCV or *Treponema pallidum* in 37 of 40 referrals tested. One individual who was HIV negative tested positive for ANA (Table 1).

Longitudinal Follow up of Elite Controllers

Clinical

The median interval from first reactive HIV test to first IDRIS assessment for the 30 individuals identified as EC was 6 years (IQR = 4–14 years), 6 years for molecular positive (IQR, 3–17 years), and 7 years for molecular negative (IQR, 5–15 years).

Two molecular-positive ECs progressed to overt HIV infection with HIV RNA detected at 7405 and 802 HIV RNA copies/mL, respectively, 2 months and 1 year postinitial IDRIS clinic visit, and started ART. Neither exhibited new coinfections or other clinical diagnoses associated with loss of viral control. CD4, CD8, and CD4/CD8 were all normal before viremia. Another 2 (1 molecular-positive EC and 1 molecular-negative EC) elected with their referring physician to commence ART. Twenty-six remain off ART and under follow up.

Evolution of Molecular Status

Twenty-one of twenty-six ECs maintained their status and attended follow-up appointments for up to 4 years since initial visit despite disturbance to clinic visits caused by the coronavirus disease 2019 pandemic during 2020 and 2021.

Longitudinal molecular sampling was available for 20 of 21 ECs over a 4-year period. Nine ECs retained the same molecular status (4 molecular positive and 5 molecular negative). Eleven ECs changed molecular status (Supplementary Table 9); 2 initially molecular-positive ECs on HIV-1 DNA assay targeting the LTR region of the HIV-1 gene were subsequently found to be molecular negative. In the remaining 9 molecular-negative individuals, molecular evidence of HIV infection has since been detected (Supplementary Table 9).

DISCUSSION

Our approach of using multiple research and diagnostic assays has allowed us (1) to provide a definitive HIV diagnosis for 40 of 42 referrals who were uncertain about their HIV status and (2) to characterize a cohort of HIV elite controllers who met a tight definition of elite control. Although many HIV EC cohorts have been reported, this is one of the first to detail the specific immune responses and molecular components, creating a distinction between seropositive elite controllers (<20 HIV RNA copies/mL plasma) with detectable HIV RNA and/or DNA (molecular positive) and those who remain molecular negative using validated clinical and research assays.

Table 1. Clinical Descriptions of 40 Subjects with Resolved HIV Status. Data on Two Individuals with Unresolved HIV Status are Excluded

	HIV Negative (n = 10)	Seropositive, Molecular Positive EC (n = 20)	Seropositive, Molecular Negative EC (n = 10)
Median age (range)	29 (30–44)	54 (38–59)	40 (35–48)
Sex (%)			
Male	7 (70%)	8 (40%)	6 (60%)
Female	3 (30%)	12 (60%)	4 (40%)
Ethnicity (%)			
White	3 (30%)	4 (20%)	2 (20%)
Black	6 (60%)	10 (50%)	7 (70%)
Other	1 (10%)	6 (30%)	1 (10%)
Region of Birth	Europe (8) South America (1) Guinea (1)	Europe (10) Western Africa (4) Central Africa (1) Southern Africa (2) East Africa (2) Caribbean (1)	Europe (7) Western Africa (1) East Africa (2)
Risk Exposure			
Heterosexual male partner	4	7	4
Heterosexual female partner	3	5	1
MSM	0	1	2
IVDU	0	0	2
None declared	3	7	1
Median Years Since Initial Reactive HIV Test (IQR)	3 (2–4)	6 (3–17)	7 (5–15)
Median CD4 (IQR)	881 (701–1493)	951 (805–1344)	736 (613–878)
Median CD8 (IQR)	538 (434–806)	634 (394–937)	481 (358–839)
Median CD4/CD8 (IQR)	1.6 (1.5–2)	1.7 (1–2)	1.5 (1.2–2)
Coinfection Screen (CMV, EBV, HTLV, *Treponema pallidum*, Hep C)	3/10 positive for CMV IgG 10/10 positive for EBV IgG EBNA 10/10 positive EBV IgG VCA 10/10 negative for EBV IgM VCA 10/10 negative for HTLV-1 Ab 10/10 negative for *T pallidum* 10/10 negative for Hep C Ab	8/20 positive for CMV IgG 11/20 positive for EBV IgG EBNA 11/20 positive EBV IgG VCA 15/20 negative for EBV IgM VCA (5 not tested) 18/20 negative for HTLV-1 Ab (2 not tested) 18/20 negative for *T pallidum* (2 not tested) 16/20 negative for Hep C Ab (4 not tested)	9/10 positive for CMV IgG (1 not tested) 9/10 positive for EBV IgG EBNA (1 not tested) 9/10 positive EBV IgG VCA (1 not tested) 9/10 negative for EBV IgM VCA (1 not tested) 9/10 negative for HTLV-1 Ab (1 not tested) 10/10 negative for *T pallidum* 9/10 negative for Hep C Ab (1 not tested)
Autoimmunity Screen			
(ANA, antidouble-stranded DNA, rheumatoid factor, antimitochondrial antibody)	1/10 positive for ANA	No evidence of nonspecific reactivity	No evidence of nonspecific reactivity
Number of bands on Western blot	0	≥3	≥3
Median HIV/CO Abbott	0.4 (0.2–0.8)	11 (8.4–15.1)	11.1 (7.3–14)

Abbreviations: Ab, antibody; ANA, antinuclear antibody; CMV, cytomegalovirus; CO, combination; DNA, deoxyribonucleic acid; EBNA, Epstein-Barr nuclear antigen; EBV, Epstein-Barr virus; EC, elite controller; Hep C, hepatitis C; HIV, human immunodeficiency virus; HTLV, human T-lymphotropic virus; Ig, immunoglobulin; IQR, interquartile range; IVDU, intravenous drug users; VCA, viral capsid.

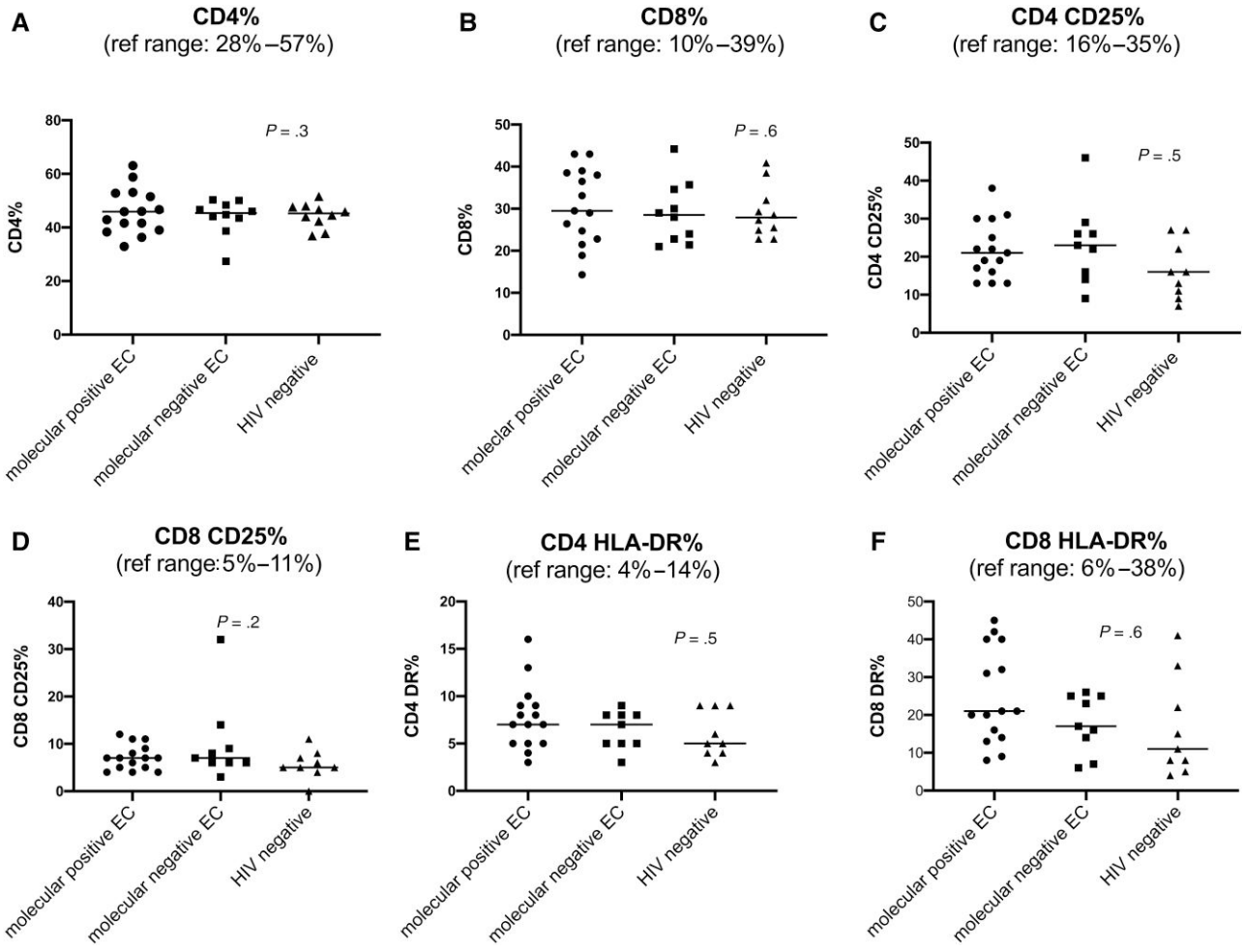

Figure 2. (*A–E*) Comparison of T-cell activation markers between 15 molecular-positive elite controllers (ECs), 10 molecular-negative ECs, and 10 human immunodeficiency virus (HIV)-negative individuals. Comparison of plasma inflammatory markers between molecular-positive ECs, molecular-negative ECs, and HIV-negative individuals.

The determination of molecular status clearly reflects the sensitivity and specificity of the assays used in this work. With fresh blood and larger volumes, detection of HIV DNA has been feasible, which has helped with confirmation of HIV status among all referrals and has been instrumental in determining HIV status for the 2 individuals who remain sero-indeterminate after 4 years of follow up. The recent description of unique viral integration sites for ECs compared with viral progressors may partly explain the differences we observe in these 2 distinct phenotypes [21]. An ongoing collaboration with the Harvard group will explore this further, and longitudinal sampling of this well characterized, strictly defined, EC cohort will be crucial in determining how ECs develop distinct viral integrations and how long they are maintained.

Further study of such rare cohorts has 2 key functions. The first is to inform clinical management, which to date remains uncertain. The rarity of such individuals means that powered, randomized trials to identify a risk-benefit of ART in this setting will not be feasible, and although studies such as START and TEMPRANO did include some participants with low-level viremia [32, 33], they were not powered to define the impact of ART in this context. The report of persistent immune activation and inverted CD4:8 ratio in some ECs and the observed increased risk of atherosclerosis in EC cohorts suggests that despite viral control, ECs [34–36] with detectable inflammation or immune activation long term may benefit from ART. Although we saw evidence of increased expression of markers of immune activation in 15% of molecular-negative ECs and 30% of molecular-positive ECs compared with HIV-negative controls, based on at least 1 T-cell activation marker and no evidence of inflammation as measured by plasma cytokine concentrations, this cohort is too small and the follow up is too short to inform clinical decision making regarding ART. However, it is notable that of the 30 ECs, only 2 elected to start ART once HIV diagnosis (which up to this point had been uncertain) was confirmed. It is also notable that 2 of 30

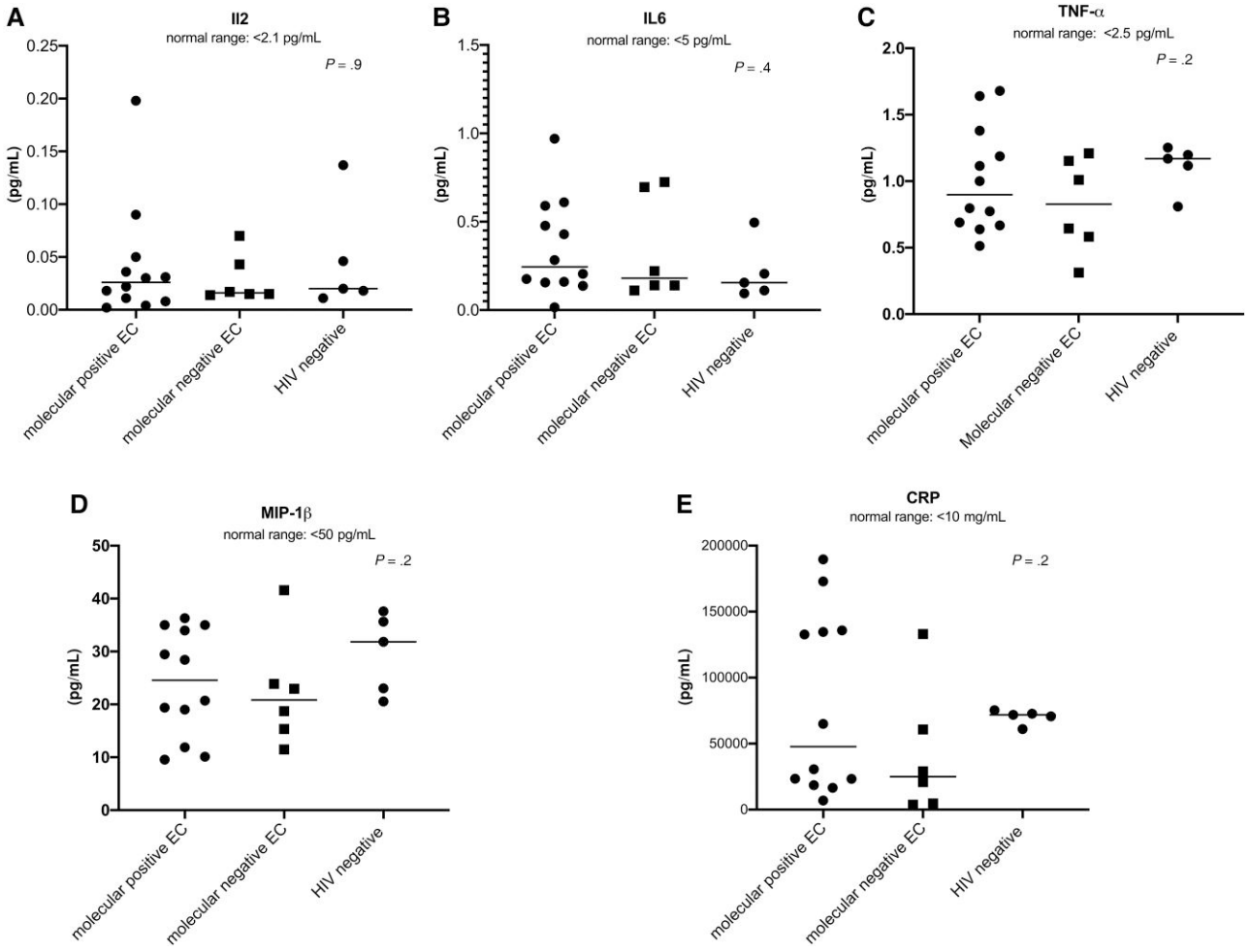

Figure 3. (*A–F*) Comaparison of inflammatory cytokine levels between 12 molecular-positive elite controllers (ECs), 6 molecular-negative ECs, and 5 human immunodeficiency virus (HIV)-negative individuals. CRP, C-reactive protein; IL, interleukin; MIP, macrophage-inflammatory protein; TNF, tumor necrosis factor.

developed detectable low-level viremia during follow up, emphasizing the importance of regular viral load monitoring. Long-term follow up and a larger cohort will allow us to determine whether increased immune activation can be linked to molecular status for ECs and whether this in turn predicts losing natural viral control, with subsequent implications for advice and recommendations for this cohort. Neither individual who developed viremia had evidence of increased immune activation before viremia.

The second key function is to better understand the mechanisms underlying spontaneous viral controls that are key to appreciating future HIV remission and cure interventions. Manipulation of HIV-specific immunity using therapeutic vaccines is a well explored [37, 38], largely unsuccessful route to enhance spontaneous viral control [39], although more recent data are encouraging [40].

The ability of some individuals to spontaneously control HIV replication to below the current limit of viral detection is well documented [17, 41, 42]. In the CASCADE trial, a longitudinal study designed to monitor CD4 T-cell dynamics in ECs after ART initiation, viral control was ultimately not sustained in 93.7% over a median of 14 years of follow up [41, 43]. However, we have seen viremia in 2 of 30 (6.7%) after 5 years and 6 years, respectively.

An important feature of this EC cohort is the exclusion of nondisclosed ART driving viral control by measuring 4 nucleoside reverse-transcriptase inhibitors (NRTI) agents. Although there is a limited possibility of nondisclosed usage of boosted protease inhibitors, integrase inhibitors, or non-NRTIs, these, unlike NRTIs, are not available for PEP and/or PreP in the United Kingdom. Future studies will also need to exclude the use of long-acting integrase strand transfer inhibitors.

In addition, although an attempt has been made to use ultrasensitive methods for categorizing ECs based on molecular status, the assays used in our investigation are dependent on cellular input. Several notable works have recently investigated

the size of the HIV-1 reservoir in ECs by measuring intact proviral-DNA measurements and near-full-length, individual, proviral sequencing using leukapheresis and described ECs with no evidence of viral replication as "exceptional elite controllers" [44–46]. These are integral in understanding HIV-1 elite control as a model for a sterilizing cure. However, our work consists of assays that are adaptations of routine clinical tools to inform the feasibility of such assays in clinical settings. These assays are better suited for understanding how molecular evidence of viral infection can impact clinical progression of HIV ECs.

As summarized on Table 1, 12 study participants originate from sub-Saharan Africa and are likely to have a non-B clade virus, which could impact detection of nucleic acid using the set of primers used in the quantitative single-copy assays. However, the primers used in the qualitative assays cover all major HIV-1 subtypes. Using multiple assays in conjunction provides reassurance in covering all HIV subtypes, but there remains a small possibility of missing subtypes circulating in sub-Saharan Africa. This cohort also remains to be investigated for genetic factors known to contribute to HIV control such as HLA type and CCR5 genotype.

Finally, in this work, we identified a gap in clinical pathways for individuals with natural viral control of HIV and highlighted the need for additional testing that we were able to use to provide a definitive diagnosis for 40 of 42 referrals. This has been instrumental in providing these individuals with certainty concerning their HIV status and has facilitated better clinical management and informed choice. Referrals to this clinic were via a clinician, although self-referral was also welcomed. Any individual with positive HIV serology, currently not taking ART, with a laboratory-confirmed HVL test of <20 copies HIV RNA/mL on more than 1 occasion at least 6 months apart and CD4 counts within normal range would meet our criteria of spontaneous viral control. We also suggest referral of individuals with uncertain HIV status, due to persistently indeterminate HIV test results in routine clinical settings, including those exposed to PrEP or PEP. This is done to resolve the HIV status for the particular individual as well as to gain insights into optimal clinical management of different groups with indeterminate HIV results.

Based on current UK HIV guidelines, we recommend the following list of clinical tests as essential to help guide treatment decisions: HIV viral load, CD4 count and CD4:8 ratio, and the coinfection screen outlined under Methods. Additional optional tests include autoimmune screen and T-cell activation markers for expression on CD4 and CD8 T cells of CD25 and HLA-DR.

We emphasize the potential impact of significant comorbidities and inflammation among people with HIV but controlling detectable viral replication [47], and we recommend clinical assessments of comorbidities and age with particular emphasis on cardiovascular disease, previous malignancy, and ongoing inflammatory or autoimmune conditions as factors integral to commence ART in this setting.

CONCLUSIONS

In conclusion, we present a highly characterized cohort of ECs, defined using a strict description of elite control, and demonstrate that with a rigorous and repeated multiassay approach, it is possible to further subdivide spontaneous viral control into molecular categories even when viral load is <20 HIV RNA copies/mL. Further exploratory and longitudinal work, including integration site analysis and CD8 T-cell responses, is ongoing.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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