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HIV diagnosis in Equatorial Guinea. Keys to reduce the diagnostic and therapeutic delay

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ABSTRACT

Background: In Equatorial Guinea, only 54 % of people living with HIV know their HIV status. There are no confirmatory or molecular diagnostic techniques for early diagnosis or monitoring of infection in the country. Rapid diagnostic tests can induce false-positive diagnoses if used as a confirmatory technique. Our study aimed to identify the challenges of early HIV diagnosis in Equatorial Guinea by analyzing the rate of false positive diagnoses, diagnostic and therapeutic delays, and treatment failures among those on antiretroviral therapy.
Methods: From 2019–2022, dried blood from 341 children, adolescents and adults diagnosed in Equatorial Guinea as HIV-positive by rapid diagnostic testing, and from 54 HIV-exposed infants were collected in Bata and sent to Madrid to confirm HIV-infection by molecular (Xpert HIV-1Qual, Cepheid) and/or serological confirmatory assays (Geenius-HIV-1/2, BioRad). HIV diagnostic delay (CD4 <350cells/mm^3), advanced disease at diagnosis (CD4 <200cells/mm^3) and antiretroviral treatment delay and failure (viraemia $>1{,}000$RNA-HIV-1-copies/ml) were also studied after viral quantification (XpertVL HIV-1, Cepheid).
Results: False-positive diagnoses were identified in 5 % of analysed samples. HIV infection was confirmed in 90.5 % of previously diagnosed patients in Equatorial Guinea and 3.7 % of HIV-exposed children undiagnosed in the field. Two-thirds of each new HIV patient had delayed diagnosis, and one-third had advanced disease. Treatment delay occurred in 28.3 % of patients, being around four times more likely in adolescents/adults than children. More than half (56 %) of 232 treated patients presented treatment failure, being significantly higher in children/adolescents than in adults (82.9 %/90 % *vs.* 45.6 %, $p < 0.001$).
Conclusion: We identified some challenges of early HIV diagnosis in Equatorial Guinea, revealing a high rate of false positive diagnoses, diagnostic/treatment delays, and treatment failures that need to be addressed. The implementation of more accurate rapid diagnostic techniques and confirmatory tests, along with

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improving access to care, treatment, awareness, and screening, would contribute to controlling the spread of HIV in the country.

Introduction

HIV is currently one of the biggest public health problems worldwide, with 39 million people affected in 2022 and 1.3 million new infections. Fifty per cent of these new infections occur in sub-Saharan African countries [1]. We know that early diagnosis that allows access to early treatment is essential to control the epidemic, prevent transmission, avoid new infections, and reduce the morbidity and mortality associated with the infection [2]. The UNAIDS 95–95-95 targets propose that by 2030, 95 % of all people living with HIV should know their HIV status, 95 % of those diagnosed should be on antiretroviral therapy (ART), and 95 % should have suppressed viral load [3].

Equatorial Guinea (EG) has an HIV rate of 6.6 %, which is three points higher than the average for the West-Central African region to which it belongs [4]. Despite government efforts to bring diagnosis and treatment closer to the population, in Guinea only 54 % of people with HIV know their HIV status, 77 % of them are on treatment and the number of people with suppressed viral load is unknown [5].

Molecular diagnostic tests are currently the most sensitive techniques on the market, capable of diagnosing HIV around the tenth day post-infection. They are confirmatory and recommended for diagnosing HIV in children under 18 months of age [6]. However, their price and the need for specialized equipment and trained personnel limit their use in low-income countries, such as Equatorial Guinea. In contrast, serological techniques diagnose HIV in later stages (around three weeks post-infection) by detecting anti-HIV antibodies, and include screening rapid serological tests (RDTs), which are cheaper than molecular diagnostic assays.

Equatorial Guinea's diagnostic scheme relies solely on the performance of two RDTs (according to WHO recommendations, as it is a high HIV prevalence country, > 5 %) [7]. RDTs allow for HIV surveillance and implementation of control programmes in resource-limited settings, helping to reduce mortality. However, diagnostic algorithms based solely on RDTs can cause false-positive diagnoses [8–10], which have a strong impact on the patient's mental health and expose the patient to unnecessary ARV-derived toxicity [11]. Any HIV diagnosis must be confirmed by additional highly specific diagnostic tests [12].

Without molecular diagnostic techniques, it is not possible to make a reliable early diagnosis in HIV-exposed infants under 18 months of age, as they retain maternal antibodies for several months [13]. According to WHO guidelines, molecular testing of infants born to HIV-positive mothers should be performed at birth, or before six weeks of age if this is not possible [14]. Early diagnosis in this vulnerable population is critical to avoid the high mortality observed during the first two years of life [15] due to a weakened immune system and increased opportunistic diseases as a result of delayed ART.

In countries with fragile health systems and insufficient infrastructure, conflict zones or emergencies, dried blood spots (DBS) are a good alternative to traditional serum or plasma samples for HIV diagnosis, viral load (VL) quantification or resistance monitoring [16]. They are easy to collect, store and transport as they do not require a cold chain or trained personnel.

Since inadequate HIV diagnosis can affect HIV infection progression and delay early treatment initiation, impacting virus control, identifying the challenges of early HIV diagnosis in EG is crucial to propose improvements. Thus, our study aimed to identify limitations in early HIV diagnosis by analyzing the rate of false positive diagnoses, diagnostic and therapeutic delays, and treatment failures among those on antiretroviral therapy in that country.

Methods

Selected patients and sample collection

We included all 341 subjects with a positive HIV-diagnosis and all 54 HIV-exposed infants under 18 months old born to HIV-infected mothers who were under clinical follow-up in Bata Regional Hospital and María Gay Health Centre (Bata, EG) during 2019–2022. All HIV-positive subjects had been previously diagnosed in EG with RDTs, following national guidelines [17], or by serologic tests (ELISA assay). The 54 HIV-exposed infants had no previous HIV diagnosis in EG since HIV confirmation in infants under 18 months requires molecular testing, which is unavailable in EG. Demographic and clinical data from clinical records were also collected in all 395 patients under study.

DBS were prepared by adding 70 μL of venous blood collected by venipuncture on a Whatman 903 Protein Saver Card (Schleicher & Schuell). They were dried overnight at room temperature, stored in a hermetically sealed bag with desiccant bags, and kept at − 20ºC until transport on dry ice to Madrid (Spain), where the samples were stored at − 80ºC until further analysis.

HIV diagnosis in EG and confirmation in Spain

Fig. 1 shows the HIV diagnostic flowchart in the study sample set. Seventeen samples were excluded due to loss during shipping. The HIV diagnosis of the remaining 341 subjects in EG was conducted by 1, 2, or 3 RDTs: Determine™ HIV-1/2 Ag/Ab (Alere), Bioline HIV-1/2v3.0 (Standard Diagnostics) and Uni-Gold™HIV (Trinity Biotech), following the national guidelines' algorithm [17], or by serology (ELISA).

In Madrid HIV molecular diagnosis was performed on one DBS dot from the 54 exposed infants using the point-of-care (POC) Xpert®HIV-1 Qual Assay (Cepheid), which utilizes real-time PCR-based molecular diagnostic technology to amplify and detect HIV genetic material within 90 min, providing a binary result of "detected"/"not detected"[18].

HIV-1 VL quantification in positive subjects was conducted using the POC Xpert® HIV-1 Viral Load Assay (Cepheid) [19], also based on real-time PCR amplification of viral genetic material. For both Cepheid tests, the DBS-dot was eluted in 1 mL of Xpert Qualitative buffer for 15 min at 56 °C. We determined the number of HIV-1 RNA copies per plasma milliliter in one dot adjusting for patient hematocrit levels, assuming 33 % for children < 5 years-old, 39 % for children > 5 years-old, 31.7 % for pregnant women, 42 % for non-pregnant women, and 47 % for men, based on previous studies [20]. These hematocrits led to plasma volumes of 46.9 μL, 42.7 μL, 47.8 μL, 40.6 μL and 37.1 μL, respectively, in 70 μL blood collected per dot.

In samples with undetected viraemia (< 40 cp/ml), HIV-1/2 infection was confirmed with the Geenius HIV-1/2 Confirmatory Assay

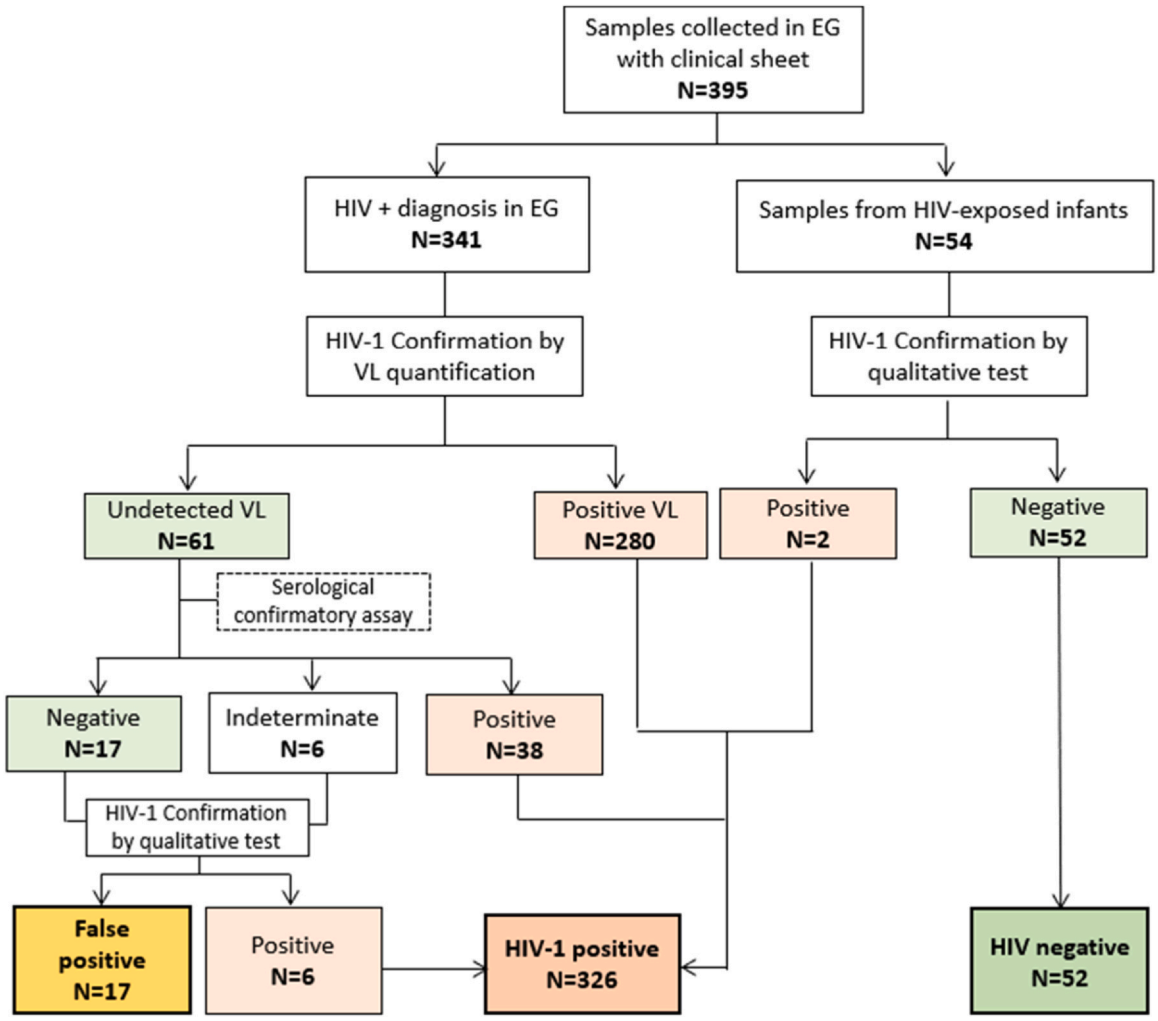

Fig. 1. HIV diagnostic flowchart in the study sample set from EG.

(BioRad), a single-use immunochromatographic test that confirms and differentiates individual antibodies to HIV-1 and HIV-2, using one DBS dot per patient eluted in 150 μL Nuclisens EasyMag elution buffer (BioMérieux) for 1 h at room temperature with gentle rotation, as previously published by our group [21].

False positives in EG were detected using the Xpert HIV-1 Qual (Cepheid), and the Determine HIV Early Detect (Abbott), both conducted with one eluted DBS dot in 1 mL of Xpert Qualitative buffer for 15 min at 56 °C.

EG, Equatorial Guinea; RDT, rapid diagnosis test; DBS, dried blood specimens; VL, HIV-1 viral load. Serological confirmatory assay: Geenius HIV 1/2 Confirmatory Assay (BioRad); VL assay: Xpert HIV-1 Viral Load Assay (Cepheid); Qualitative test: Xpert HIV-1 Qual Assay (Cepheid). Confirmation by other tests: Xpert HIV-1 Qual (Cepheid) and Determine HIV Early Detect (Abbott).

Diagnostic delay and advanced disease among naive subjects

We analysed the rate of diagnostic delay and advanced disease at diagnosis in subjects with known CD4 counts at HIV diagnosis according to clinical records. The CD4 count was performed at the Bata Regional Hospital using the FACSCount (BD) automated flow cytometry system. We considered diagnostic delay when a patient had < 350 CD4 cells/mm^3 at HIV diagnosis, and advanced disease when they had < 200 CD4 cells/mm^3.

ART delay and therapeutic failure among treated subjects

We considered treatment to be immediate if ART started during the first month after HIV diagnosis. Treatment delay (time between definitive diagnosis and ART start) was classified into three categories: from 1 month to < 1 year, from 1 to 3 years, and up to 3 years. We identified treated subjects with therapeutic failure when their HIV-1 viraemia levels were ≥ 1000 cp/ml, clinical treatment failure threshold using DBS [22,23].

Statistical analysis

Medians were assessed for non-normally distributed data. Statistical analyses were performed using the software GraphPad Prismv8.0.1. Statistical significance between each population group was calculated using the Fisher exact test or Chi-square for categorical variables and the Mann-Whitney test for continuous variables. Two-sided p-values < 0.05 were considered statistically significant. The association between therapeutic delay and patients' age and sex was assessed by multivariate logistic regression analysis using Stata 16.0 statistical software.

Ethics

This research was conducted in accordance with the Declaration of Helsinki and received approval from the local Ethics Committee for Clinical Investigation at Hospital Universitario Ramón y Cajal,

Madrid, Spain (ACTA 369, 24/06/2019) and by the Ministry of Health and Social Welfare of EG. Signed informed consent was obtained from all adults and the parents or legal guardians of children included in the study cohort. Upon receipt, each sample was assigned a unique code to ensure patient anonymity.

Results

A total of 395 DBS were collected from adult and paediatric populations in EG, of which 341 had a previous positive HIV serological diagnosis and 54 were undiagnosed HIV-exposed infants. Diagnostic test information was only documented in 144 (42.2 %) of the 341 subjects with positive HIV diagnosis mainly by RDTs (81.2 %), ELISA (8.3 %) or both (0.7 %). Of the 117 RDTs-diagnosed patients, 11 had been tested with one test, 89 with two different rapid tests, and 29 with three. The number of performed RDTs was unknown for two patients.

Among the 341 samples under study, 280 (82.1 %) had a detectable HIV-1 VL (80–3349,754 copies/ml), and in the remaining 61 (17.9 %) HIV-1 was not detected (Fig. 1). To determine the HIV status of these 61 patients, a serological confirmatory POC assay (Geenius HIV-1/2) was performed, resulting in 38 HIV-positive, 17 HIV-negative, and 6 indeterminate. Negative and indeterminate results by Geenius were confirmed by other POC tests, such as Xpert HIV-1 Qual (Cepheid) and Determine HIV Early Detect (Abbott), identifying 17 false positives and 6 HIV-positive. Among the 54 HIV-exposed infants, we detected two HIV-positives and 52 HIV-negative by Xpert HIV-1 Qual (Cepheid). No HIV-2 infection was found in the whole study cohort (Fig. 1).

Thus, HIV-1 molecular/serological confirmatory POC testing confirmed the infection in 95 % (324/341) of individuals with previous HIV-positive diagnoses in EG (92 ART-naive/232 ART-experienced at sampling) and in 3.7 % (2/54) of HIV-exposed infants.

HIV-1 infected cohort with a confirmed diagnosis

Table 1 displays the epidemiological and virological characteristics of the 326 individuals with confirmed HIV infection in Madrid, including 324 subjects (41 children, 20 adolescents, 263 adults) already diagnosed in EG and two newly diagnosed HIV-exposed infants. All subjects were under clinical follow-up in the Regional Hospital of Bata and María Gay Health Center (EG), being 80 % females. The median age at sampling of HIV-infected children/adolescents/adults was 6/13.5/32 years old. The last available median CD4 levels were lower in adults than in adolescents and children (294 *vs.* 460 *vs.* 869) and comorbidities were more frequent in adults. Only half of the study cohort had known information on the route of transmission, being mainly sexual (98.5 %) in adults and

Table 1
Epidemiological and virological features of the 326 individuals from EG with confirmed HIV-1 diagnosis in Madrid.

	1	2	3	4	P value		
Epidemiological and clinical features	**Children (< 12 y) No. (%)**	**Adolescents (12 y – 17 y) No. (%)**	**Adults (≥18 y) No. (%)**	**Total cohort No. (%)**	**1 *vs.* 2**	**1 *vs.* 3**	**2 *vs.* 3**
No. (%)	43 (13.2)	20 (6.1)	263 (80.7)	326 (100)			
Female, No. (%)	27 (62.8)	10 (50)	224 (85.2)	261 (80.1)	0.413	**0.001**	**0.0005**
Median age, years [range]	6 [0.6 – 11]	13.5 [12–17]	32 [18–64]	30 [0.6 – 64]			
Transmission route, No. (%)							
Vertical	24 (55.8)	7 (35)	0	31 (9.5)	0.581	**< 0.0001**	**< 0.0001**
Sexual	0	0	129 (49)	129 (39.6)	> 0.999	**< 0.0001**	**< 0.0001**
Transfusion	3 (7)	2 (10)	2 (0.8)	7 (2.1)	0.581	**0.035**	**0.020**
Unknown	16 (37.2)	11 (55)	132 (50.2)	159 (48.8)			
ARV exposure, No. (%)					> 0.999	**< 0.0001**	**0.0003**
Exposed/naive	2 (4.7)	0	92 (35)	94 (28.8)			
Under ART	41 (95.3)	20 (100)	171 (65)	232 (71.2)			
Median CD4 levels, cells/mm3 [range]	869 [5-1839]	460 [2-1188]	294 [2-1116]	338 [2-1839]	**0.0005**	**< 0.0001**	0.070
Comorbidities, No. (%)							
0	11 (25.6)	1 (5)	15 (5.7)	27 (8.3)	0.052	**< 0.0001**	0.316
1	2 (4.6)	3 (15)	90 (34.2)	95 (29.1)	0.052	**0.018**	0.622
2	0	0	52 (19.8)	52 (16)	> 0.999	**0.021**	0.3231
3	0	0	13 (4.9)	13 (4)	> 0.999	0.604	> 0.999
4	0	0	4 (1.5)	4 (1.2)	> 0.999	> 0.999	> 0.999
Unknown	30 (69.8)	16 (80)	89 (33.9)	135 (41.4)			
HIV-1 viral load in ART-treated subjects. No.	41	20	171	232	0.7044	**< 0.0001**	**0.0002**
< 1000 cp/ml	7 (17.1)	2 (10)	93 (54.4)	102 (43.9)			
> 1000 cp/ml	34 (82.9)	18 (90)	78 (45.6)	130 (56)			
ART-naive subjects with CD4 data at diagnosis	0	0	60	60			
HIV diagnosis delay (< 350 CD4 cells/mm^3)	––	––	39 (65)	––			
Advanced HIV disease at diagnosis (< 200 CD4 cells/mm^3)	––	––	19 (31.6)	––			
No. of treated	41	20	171	232			
Immediate ART[a]	35 (85.4)	12 (60)	62 (36.3)	109 (47)	**0.041**	**0.018**	0.794
On ART Delay	0	2 (10)	14 (8.2)	16 (6.9)	0.099	**0.010**	> 0.999
1 month- < 1 year							
1-3 years	4 (9.8)	3 (15)	10 (5.8)	17 (7.3)	0.670	> 0.999	0.460
> 3 years	1 (2.4)	2 (10)	7 (4.1)	10 (4.3)	0.240	0.434	0.647
Unknown	1 (2.4)	1 (5)	78 (45.6)	80 (34.5)			

No, number; %, percentage; y, years; ART, antiretroviral treatment; cp/ml, copies of HIV-1 RNA per millilitre; cells/mm3, CD4 cells per cubic millimetre of blood; In bold, significant p values ($p < 0.05$) comparing children-adolescents *vs.* adults: HIV diagnostic delay and advanced HIV disease at diagnosis only could be analysed in 95 ART-naive adults since they had available related CD4 data at HIV diagnosis.

[a] less than a month. The variable "unknown" in the Transmission route refers to instances where the physician could not record the information about the route of transmission. This could be either because the patient either did not remember or did not know how they contracted the infection or because they did not want to disclose this information to the physician. Comorbidities included AIDS-defining diseases such as tuberculosis, toxoplasmosis, Kaposi's sarcoma, pneumonia, oral candidiasis, urinary tract infections, sexually transmitted infections, as well as malaria, filariasis, and coinfections with hepatitis B or hepatitis C viruses.

Table 2
Seventeen false positives of HIV diagnosis in EG found in the study cohort.

							TEST CONDUCTED IN EG		TEST CONDUCTED IN THE RAMÓN Y CAJAL HOSPITAL (MADRID, SPAIN)				
ID	**DATE OF HIV DIAGNOSIS**	**AGE AT SAMPLING**	**GENDER**	**CD4 (cel/mm^3)**	**ART AT SAMPLING**	**TIME ON ART**	Diagnostic Technique	HIV Diagnosis	Geenius (confirmatory test)	Determine HIV-Early Ag/Ab	Xpert HIV viral load	Xpert Qual (confirmatory test)	**DEFINITIVE HIV DIAGNOSIS**
1	2019	59 years	Female	815	NO	––	2 RDT	POSITIVE	NEG	Ab band	NOT DETECTED	NOT DETECTED	**NEG (FALSE POSITIVE)**
2	2020	28 years	Female	393	NO	––	2 RDT	POSITIVE	NEG	Ab band	NOT DETECTED	NOT DETECTED	**NEG (FALSE POSITIVE)**
3	2019	29 years	Female*	nd	NO	––	2 RDT	POSITIVE	NEG	NEG	NOT DETECTED	NOT DETECTED	**NEG (FALSE POSITIVE)**
4	2020	18 years	Female*	nd	NO	––	2 RDT	POSITIVE	NEG	NEG	NOT DETECTED	NOT DETECTED	**NEG (FALSE POSITIVE)**
5	2021	39 years	Female*	nd	NO	––	2 RDT	POSITIVE	NEG	NEG	NOT DETECTED	NOT DETECTED	**NEG (FALSE POSITIVE)**
6	2016	57 years	Female	1109	NO	––	ELISA	POSITIVE	NEG	NEG	NOT DETECTED	NOT DETECTED	**NEG (FALSE POSITIVE)**
7	2018	50 years	Female	nd	NO	––	nd	POSITIVE	NEG	NEG	NOT DETECTED	NOT DETECTED	**NEG (FALSE POSITIVE)**
8	2020	15 months	Male	nd	YES	1 year	nd	POSITIVE	NEG	NEG	NOT DETECTED	NOT DETECTED	**NEG (FALSE POSITIVE)**
9	2020	14 moths	Female	nd	YES	2 years	nd	POSITIVE	NEG	NEG	NOT DETECTED	NOT DETECTED	**NEG (FALSE POSITIVE)**
10	2015	4 years	Male	2552	YES	5 years	nd	POSITIVE	NEG	NEG	NOT DETECTED	NOT DETECTED	**NEG (FALSE POSITIVE)**
11	nd	29 years	Female*	nd	NO	––	2 RDT	POSITIVE	INDET	NEG	NOT DETECTED	NOT DETECTED	**NEG (FALSE POSITIVE)**
12	2020	50 years	Female	659	NO	––	ELISA	POSITIVE	INDET	NEG	NOT DETECTED	NOT DETECTED	**NEG (FALSE POSITIVE)**
13	2022	18 years	Female*	nd	NO	––	1 RDT	POSITIVE	INDET	NEG	NOT DETECTED	NOT DETECTED	**NEG (FALSE POSITIVE)**
14	nd	20 years	Female*	nd	YES	3 years	2 RDT	POSITIVE	INDET	NEG	NOT DETECTED	NOT DETECTED	**NEG (FALSE POSITIVE)**
15	2021	26 years	Female*	nd	YES	nd	2 RDT	POSITIVE	INDET	NEG	NOT DETECTED	NOT DETECTED	**NEG (FALSE POSITIVE)**
16	2019	23 years	Female	nd	YES	nd	nd	POSITIVE	INDET	Ab band	NOT DETECTED	NOT DETECTED	**NEG (FALSE POSITIVE)**
17	2021	4 months	Male	nd	YES	1 month	nd	POSITIVE	POSITIVE	Ab band	NOT DETECTED	NOT DETECTED	**NEG (FALSE POSITIVE)**

ID, sample identification; EG, Equatorial Guinea; ART, antiretroviral treatment; Years on ART until the final HIV confirmatory diagnosis in Madrid; RDTs, rapid diagnostic tests (Determine HIV-1/2, Hexagon HIV); Geenius, Geenius HIV 1/2 Confirmatory Assay; nd, no information recorded in clinical files; pos, positive; neg, negative; indet, indeterminate; no detected; Ag, p24 Antigen; Ab, antibodies; *pregnant women.

vertical in children and adolescents (88.9 % and 77.8 %, respectively). HIV acquisition by HIV-contaminated blood was found in 3 adolescents (22.2 %), 2 children (10 %) and 2 adults (1.5 %) with related data in EG. Regarding treatment, 232 (71.1 %) were ART-experienced at sampling.

HIV diagnostic delay and advanced HIV disease at diagnosis in ART-naive adults

Among the 92 ART-naive subjects with confirmed HIV diagnostic included in the study (2 children/90 adults), only 60 (65.2 %) had available CD4 data at diagnosis, all of them adults. Among them, two-thirds (65 %) presented diagnostic delay (< 350 T CD4 +) and one-third (31.6 %) advanced HIV disease (< 200 T CD4 +) at HIV diagnosis.

High rate of false positive HIV diagnosis in EG

By using molecular and serological confirmatory assays in Madrid we detected 17 (5 %) individuals with false HIV-positive diagnoses among the 341 individuals diagnosed as HIV-positive in EG (Table 2). Among them, 7 were pregnant women, 6 were non-pregnant women and 4 were children, with 3 of them being infants under 18 months of age. None of the 4 children had received maternal breastfeeding. Seven patients had been on unnecessary ART for 1 month-5 years (3 adults, 4 children). Among the 11 false positives with available data, 8 had been tested in EG with two RDTs, 1 with one RDT, and, surprisingly, 2 only with non-rapid serological assay (ELISA). Results were communicated to clinicians in EG to inform these 17 patients of their definitive HIV status and to stop ART in those being treated.

High ART failures and ART-delay among HIV-1 infected and treated population in EG

More than half (56 %) of the 232 patients with HIV-confirmed diagnosis and under ART in EG presented treatment failure at sampling, with VL ≥ 1000 cp/ml (Table 1). ART failure was significantly higher in children and adolescents than in adults (82.9 % and 90 % *vs.* 45.6 %, p < 0.001).

Regarding ART delay, among the 152 individuals with known ART initiation dates of the first HIV diagnosis in EG (40 children/19 adolescents/93 adults), 109 (71.7 %) started ART immediately (during the first month after HIV diagnosis) in EG (Table 1, Supplementary Table 1). The median age at diagnosis was 9, 13, and 41 years old for children, adolescents and adults, respectively (Supplementary Table 1). Prompt treatment initiation was significantly higher in children than in adolescents/adults (87.5 % *vs.* 63.2 %/66.7 %, p < 0.05) (Table 1, Fig. 2). Thus, ART delay occurred in a third of patients. In more detail, 43 (28.3 %) patients with data presented ≥ 1 month of ART delay, being similar in adolescents and adults (36.8 %/33.3 %), but lower in children (12.5 %). The ART delay ≥ 1 year was higher in adolescents (26.3 %) than in adults (20.4 %) and children (12.5 %) (Fig. 2, Supplementary Table 1). Four patients had long delays, starting treatment at 8, 10, 11 and 16 years after HIV diagnosis, respectively.

The age group significantly influenced the likelihood of treatment delay after HIV diagnosis (Table 3). In our study population, adolescents were 4 times and adults 3.5 times more likely to experience treatment delay than children, and this association was statistically significant (p < 0.05). Gender did not significantly influence the likelihood of treatment delay.

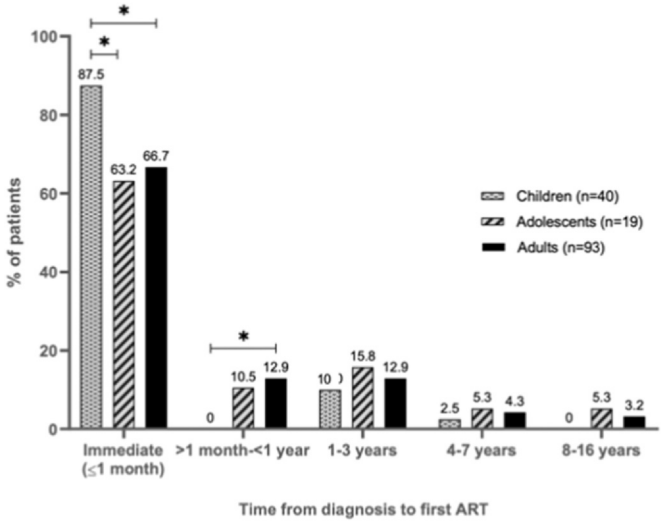

Fig. 2. Treatment delay in HIV-infected children, adolescents and adults of EG under ART with available data.

Table 3
Relationship between the probability of suffering therapeutic delay as a function of age and gender.

Variable	Odds Ratio	p-value	95 % Confiance Interval
Group 2 *vs* 1	4.08	0.037	1.088-15.311
Group 3 *vs* 1	3.5	0.017	1.247-9.818
Gender	0.51	0.125	0.215-1.205

Group 1, children; Group 2, adolescents; Group 3, adults.

Discussion

Early HIV diagnosis, particularly in children, is a WHO priority for initiating ART early and reducing nervous and immune system damage, viral reservoirs, and HIV transmission. Correct diagnosis is crucial, as false diagnoses lead to unnecessary treatment, psychological stress, stigma, and loss of trust in healthcare. The use of molecular POC tests can enhance the clinical management of people living with HIV by reducing diagnostic delays, accelerating ART access, and identifying treatment failures.

Current EG guidelines recommend molecular testing for early infant diagnosis within 72 h and repeat testing after 6 weeks, but these techniques are unavailable in the country. Instead, an algorithm based on repeat RDTs is used for both children over 18 months and adults. This study analyzed the current diagnostic strategy in EG, examining HIV diagnostic and treatment delays, and treatment failure rates in a cohort of HIV-infected individuals from 2019 to 2022. It also assessed the clinical impact of lacking routine HIV molecular testing for early infant diagnosis and confirmatory serological techniques for older children and adults.

Our study found HIV misdiagnosis in 1 out of every 20 cases, likely due to RDT use in EG [17], leading to false positive diagnoses [8–10]. Using HIV-1 molecular and serological confirmatory POC tests, we identified 17 (5 %) HIV-negative individuals with previous positive diagnoses in EG. Seven of them under unnecessary ART for 1–5 years, including 2 pregnant women and 4 children. The 4-month-old infant was misdiagnosed due to maternal antibody transmission. This misdiagnosis and that of the other 2 children under 18 months could be avoided by exclusively using molecular assays as recommended. In 14 false positives in older individuals, test interpretation errors might have occurred. Additionally, many clinical files omitted the number of RDTs performed or showed that only one RDT was done instead of the recommended two. This

underscores the need for continuous diagnostic training for healthcare professionals and implementing molecular and confirmatory serological POC techniques in national guidelines for improved accuracy. We also recommend integrating medical records into a nationwide accessible electronic registry.

HIV diagnosis delays and subsequent ART initiation delays are common in resource-limited settings due to limited healthcare access, stigma, and lack of HIV education. Our study provides the first data on HIV diagnostic delays in EG, showing two-thirds of naïve adults with baseline CD4 information experienced delays and one-third were diagnosed with advanced disease, which increases the risk of death even after ART initiation [24]. Median CD4 levels were lower in adults than in adolescents and children (294 *vs.* 460 *vs*. 869 cells/mm^3), suggesting fewer opportunistic infections in younger populations.

New HIV infections and AIDS-related deaths are rising in Equatorial Guinea [1,25,26]. Our study indicates the absence of early infant HIV diagnosis may increase infant mortality. Two infants under 18 months, born to HIV-infected women and undiagnosed in EG, died before receiving a confirmatory diagnosis. This underscores the urgent need for molecular testing to enable early diagnosis, early ART initiation, and reduce HIV-related infant mortality.

Regardless of WHO clinical stage or CD4 count, ART should ideally begin immediately upon diagnosis, if feasible, to enhance clinical outcomes and reduce HIV transmission [27]. In our study, 7 out of 10 HIV-infected individuals started ART within the first month post-diagnosis. Children experienced less ART delay than adults and adolescents, with no significant gender differences observed. Notably, adolescents exhibited the highest treatment delay (36.8 %) in our cohort. Delayed ART initiation up to one year was more prevalent in adolescents (1 in 4) compared to adults (1 in 5), consistent with previous studies in sub-Saharan Africa [28]. This underscores the critical need for HIV diagnosis, ART management, and adherence support in adolescents, who are increasingly affected by HIV globally and are at high risk of infection [29]. In contrast, the majority of children (87.5 %) promptly initiated treatment, indicating relatively better control of the pediatric population despite healthcare system constraints.

Treatment failure, stemming from drug resistance mutations or poor adherence, can compromise treatment effectiveness and options. In our study, over half of the patients experienced treatment failure, evident by a VL ≥ 1000 cp/m at sampling. This rate surpasses those reported in Gabon (41.3 %) [30], Cameroon (22.2 %) [31], Ethiopia (16.6 %) [32], or Tanzania (10.59 %) [33]. Children and adolescents were twice as likely as adults to experience ART failure, underscoring their heightened vulnerability. Young HIV-infected individuals often undergo multiple treatment regimens due to treatment chronicity and resistance emergence, increasing their risk of ART failure [34,35]. Although we couldn't confirm the cause of treatment failure in this study, previous research by our group found a high resistance rate (63.8 %) among HIV-infected individuals experiencing ART failure in EG. Without data on the number of HIV ART-treated patients achieving viral suppression in EG, there's an urgent need to implement molecular techniques for VL quantification and drug resistance testing to guide personalized rescue therapy and achieve undetectable viremia.

Although sexual transmission was the primary route among adults and vertical transmission among adolescents/children, 2.1 % enrolled patients contracted HIV through blood transfusions. With a high HIV prevalence (7.8 %) among blood donors in EG [36], rigorous screening of donors and establishing infectious disease screening protocols for safe transfusions is crucial. Five of the seven cases were children or adolescents, indicating current inadequate blood product control. Molecular testing confirmed HIV in 3.7 % of exposed children, slightly higher than the 2.95 % rate reported during 2012–2013 [37]. However, the 2022 vertical transmission rate was 24.1 %. This disparity may result from our study focusing solely on perinatal transmission, excluding breastfeeding. Efforts to prevent mother-to-child transmission must be intensified through National Protocols and ensuring maternal ART compliance.

This study has several limitations. First, the absence of CD4 data at HIV diagnosis in children, adolescents, and treated adults hampers assessing diagnostic delays and advanced disease in these groups. Unfortunately, we could not estimate the sensitivity and specificity of the rapid diagnostic tests conducted in Equatorial Guinea, as no HIV-negative patients were included in this study for screening. It would be worthwhile to incorporate an HIV-uninfected group in future similar studies. Additionally, incomplete medical records regarding transmission routes, comorbidities, and antiretroviral regimens limit comprehensive cohort analysis. Incomplete or absent information related to performed RDTs was found in 57.8 % of subjects with positive HIV diagnoses. Limited HIV-exposed infant samples hinder establishing vertical transmission rates in EG during the study period. New studies would benefit from sampling across more centers in EG and including a higher number of HIV-infected children and adolescents.

Future HIV research in EG could focus on key areas to enhance diagnosis, care, and management. Firstly, evaluating the clinical impact of the implementation of new molecular and serological POC techniques for HIV diagnosis through pilot studies. These should be user-friendly, with rapid response times and accuracy comparable to reference methods [38]. Secondly, understanding barriers adolescents face in accessing diagnosis and treatment and maintaining adherence. Thirdly, monitoring antiretroviral resistance in treated individuals through periodic surveillance studies. Fourthly, investigating causes of high vertical transmission and evaluating national prevention programs' effectiveness. Lastly, assessing the benefits of an electronic registry system in pilot health centers for patient management to improve care. These areas offer opportunities to enhance HIV care in Equatorial Guinea based on our study findings.

This study reveals critical health concerns in Equatorial Guinea due to delayed HIV diagnosis, treatment initiation, and ART failure, necessitating urgent government action to improve individual and public health. Early infant HIV diagnosis and blood donor screening remain pressing issues in the country. Key recommendations include implementing accurate rapid diagnostic and confirmatory POC tests, increasing screening programs for key populations and adolescents, reducing delays between diagnosis and treatment, implementing VL quantification POC assays for early identification of therapy failures, and reinforcing diagnostic training. These measures would significantly improve HIV management in Equatorial Guinea, reducing transmission and associated morbimortality and contributing to controlling HIV infection.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jiph.2024.102476.

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