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Profile of People Living with HIV after 24 Weeks of Treatment with Dolutegravir in Kinshasa

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Abstract

Introduction: The sixth month appointment (M6) is crucial because at the start of the ART course, it is an indicator of the prognosis of the evolution of care and decision-making on the continuity of treatment. **Objective:** The objective of this study is therefore to present the profile of People Living with HIV under treatment with Dolutegravir 6 months after starting ART in Kinshasa. **Methods:** The present study is a cross-sectional view at M6 of a prospective cohort to determine the profile of People Living with HIV (PLHIV) after 6 months of ARV Treatment (ART) in Kinshasa, DRC. During the M6 appointment, from April to August 2022, a sample of 5 ml of blood was taken for the various analyzes from all HIV patients included. The collection of sociodemographic data as well as biological and clinical data was carried out under the same conditions as at inclusion. The parameters recorded during M6 were: age, sex, and religion, level of study, marital status, profession, socio-economic level, height, weight, Body Mass Index (BMI), clinical profile, opportunistic infections as well as biochemical and molecular assessment. **Results:** In M6, 62 patients were registered including 38 women (61.3%), thus giving a sex ratio of 1.58 in favor of women. Fifty-seven (57) patients did not

respond to the appointment, representing a loss rate of 47.89%. The most common age group is between 36 and 45 years old with 16 patients (26.7%). The mean age was 42.4 ± 13.3 years. The mean weight was 60.5 ± 15.4 kg with a mean BMI of 22.6 ± 5.8 kg/m^2. Thirty-four (34) patients (61.82%) were in Clinical Stage 3. Thirty-six (36) patients (67.92%) had a normal clinical condition. The most common opportunistic infections among patients in M6 were: skin pruritus (25.8%), dermatitis (22.6%) and rash (21%). The mean values of biochemical parameters of patients in M6 were within normal ranges. The median VL value was 2.92 log10 RNA copies/ml with 17.75% of patients experiencing major failure of first-line treatment. Subtype A is dominant with 13 cases (20.98%); followed by CRF02_AG (16.13%) and C subtypes (14.52%). The mutations K65R (3 cases), T69P/N (6 cases), K70R (9 cases) and M184V (8 cases) were listed in M6. **Conclusion:** After 6 months of treatment, the majority of patients are in clinical stage 3 with a normal clinical state. Skin infections are the majority opportunistic infections. Certain biological parameters are considerably altered. A high virological failure rate with the presence of certain mutations associated with resistance to Lamivudine and Tenofovir.

Keywords

Profile, PLHIV, 6 Months of ART, Dolutegravir, Kinshasa

1. Introduction

After more than 40 years of the epidemic, the infection with the Human Immunodeficiency Virus (HIV) and Acquired Immunodeficiency Syndrome (AIDS) remains a public health problem across the world, according to reports from the United Nations Organization to Fight HIV/AIDS (UNAIDS), and particularly in Sub-Saharan Africa (SSA). In 2020, according to the UNAIDS report, 37.7 million [30.2 million - 45.1 million] people were living with HIV/AIDS and 1.5 million [1.0 million - 2.0 million] of these people were newly infected [1]. Sub-Saharan Africa was estimated to be the most affected region with 26 million People Living with HIV (PLHIV) according to the World Health Organization (WHO) and nearly 70% of all AIDS-related deaths in the world were recorded there [1].

The Democratic Republic of Congo (DRC) has a generalized HIV epidemic with a prevalence of 1.2% in the general population [2]. According to UNAIDS in 2020, the country had approximately 520,000 PLHIV [1]. Despite major advances in the fight against HIV, the epidemic continues to seriously harm public health in all regions.

The National Multisectoral Program for the Fight against HIV/AIDS (PNMLS) and the National Program for the Fight against HIV/AIDS and Sexually Transmitted Infections (PNLS) are the institutions that regulate the care of PLHIV at the national level [2] [3]. According to the published standards, the monitoring

schedule for PLHIV in the different centers, for the first year, recommends appointments, after initiation of Antiretroviral Treatment (ART), in the first month (M1), in the third month (M3), in the sixth month (M6), at the ninth month (M9) and at the twelfth month (M12) [2]. The M6 appointment is crucial because it is halfway through the ART course, it is an indicator of the prognosis of the evolution of care and decision-making on the continuity of treatment. However, there are no specific studies presenting the general M6 profile of PLHIV under Dolutegravir-based ART in Kinshasa.

Thus the objective of this study is to present the profile of People Living with HIV under treatment with Dolutegravir 6 months after initiating ART in Kinshasa.

2. Methods

2.1. Study Design, Patient Setting and Samples

The present study is a cross-sectional view of a prospective cohort to determine the profile of People Living with HIV (PLHIV) after 6 months of ARV Treatment (ART) based on Dolutegravir (DTG) in Outpatient Treatment Centers (CTA) held in Kinshasa, DRC. The period for the sixth month (M6) follow-up appointment was from April to August 2022.

During the M6 appointment, a sample of 5 ml of blood was taken for the various analyzes from all HIV patients included [4].

2.2. Study Population and Inclusion Criteria

The source population of this study was the patients included in the cohort who came for their M6 appointment in the period from April to August 2022, all having been previously received during the inclusion period (October 04, 2021, to February 15, 2022). The inclusion criteria for the cohort were: being at least 18 years old at inclusion, being confirmed HIV positive by RDT, being naïve to ART, consenting and having signed an informed consent.

2.3. Parameters of Interest

As on inclusion, the parameters recorded during M6 were: age, sex, and religion, level of study, marital status, occupation, socio-economic level, height, weight, Body Mass Index (BMI), clinical profile, opportunistic infections as well as biochemical and molecular assessment.

The collection of sociodemographic data as well as biological and clinical data was carried out under the same conditions as on inclusion, and all were recorded on the study worksheets.

2.4. Biochemical Analyzes

After collection, the samples were taken, under the same conditions as at inclusion (J0) to the Molecular Biology laboratory of the Department of Basic Sciences at the Faculty of Medicine of the University Of Kinshasa (UNIKIN) where they

were homogenized and separated into 2 tubes. The different analyzes were carried out under the same conditions in the same laboratories [5].

2.5. RNA Extraction and Viral Quantification

RNA was extracted from 140 µl of plasma at the Molecular Biology Laboratory using the QIAamp RNA Mini Kit QIAGEN® for RNA extraction [6]. After extraction, a Quantitative Real-Time PCR (qPCR) was carried out to determine the quantity of HIV RNA in the samples according to protocols previously described and validated [7] [8]. **Table 1** presents the conditions for the different amplifications.

2.6. Sequencing and Molecular Identification

A Reverse Transcription PCR (RT-PCR) and a Nested PCR were carried out on the extracted RNA to amplify the regions of interest for the Protease and for the Reverse Transcriptase (TR) for sequencing. The PCRs were carried out under the conditions previously described [6] [7].

Table 1. Primers and probes for amplification.

PCR Types	Primers	Sequences
Quantitative PCR (qPCR) for Viral Load on RNA		
qPCR	HIV1MG Forward	5'-GCCTCAATAAAGCTTGCCTTGA-3'
	HIV1MG Reverse	5'-GGCGCCACTGCTAGAGATTTT-3'
	HIV1MG Probe	FAM-5'-AAGTAGTGTGTGCCCGTCTGTTRTKTGACT-3'-BHQ1
PCR for Sequencing		
RT-PCR Prot	5' prot 1	5'-TAATTTTTTAGGGAAGATCTGGCCTTCC-3'
	3' prot 1	5'-GCAAATACTGGAGTATTGTATGGATTTTCAGG-3'
Nested PCR Prot	5' prot 2	5'-TCAGAGCAGACCAGAGCCAACAGCCCCA-3'
	3' prot 2	5'-AATGCTTTTATTTTTTCTTCTGTCAATGGC-3'
RT-PCR RT	MJ3	5'-AGTAGGACCTACACCTGTCA-3'
	MJ4	5'-CTGTTAGTGCTTTGGTTCCTCT-3'
Nested PCR RT	A (35)	5'-TTGGTTGCACTTTAAATTTTCCCATTAGTCCTATT-3'
	NE1 (35)	5'-CCTACTAACTTCTGTATGTCATTGACAGTCCAGCT-3'
RT-PCR Alt Prot	5' eprB	5'-AGAGCTTCAGGTTTGGGG-3'
	3' eprB	5'-GCCATCCATTCCTGGCTT-3'
Nested PCR Atl Prot	5' prB	5'-GAAGCAGGAGCCGATAGACA-3'
	3' prB	5'-ACTGGTACAGTTTCAATAGG-3'
RT-PCR Atl RT	RT1	5'-CCAAAAGTTAAACAATGGCCATTGACAGA-3'
	RT4	5'-AGTTCATAACCCATCCAAAG-3'
Nested PCR Alt RT	RT18	5'-GGAAACCAAAAATGATAGGGGGAATTGGAGG-3'
	RT21	5'-CTGTATTTCTGCTATTAAGTCTTTTGATGGG-3'

2.7. Ethical Consideration

The present study was approved by the research ethics committee of the School of Public Health, Faculty of Medicine, University of Kinshasa (ESP/CE/115/2021). Permission to access the centers was obtained from each competent authority. The samples in the centers were taken by the technical teams of the centers.

2.8. Statistical Analyzes

Analyzes were performed using SPSS software version 26. Only available data were analyzed; missing data were considered completely random. Continuous variables were presented as mean ± standard deviation and compared using Student's t-test. Proportions and their respective 95% confidence intervals (CIs) were calculated for categorical data.

2.9. Operational Definitions [5]

Clinical State: Clinical State was considered.

- Normal if the patient's vital functions are normal, he is able to do everything without assistance.
- Good if the patient's vital functions are almost normal, he is still able to walk, eat and take care of himself without assistance.
- Bad if the patient's vital functions have deteriorated, he is only able to walk, eat and take care of himself when he is assisted.
- Pre-moribund if the patient is in very poor condition, he is completely bedridden and has a clouded consciousness, but he is able to eat when assisted.
- Moribund if the patient is in very poor general condition, he is completely bedridden and is in an alert or deep coma.

Clinical Stage of Patients: According to the WHO, HIV infection is divided into 4 Clinical Stages.

- Stage 1: Asymptomatic patient, Persistent generalized lymphadenopathy;
- Stage 2: Weight loss < 10% of body weight, Shingles, Minor mucocutaneous manifestations, Recurrent upper airway infections;
- Stage 3: Weight loss greater than 10% of body weight, Unexplained chronic diarrhea > 1 month, Unexplained prolonged fever > 1 month, Persistent oral candidiasis (thrush), Oral hairy leukoplakia, Pulmonary tuberculosis during the previous year, Severe bacterial infection, Acute necrotizing ulcerative stomatitis, Persistent anemia (Hb < 8 g/dL)/Chronic neutropenia < 500/mm^3/ Chronic thrombocytopenia < 50,000/mm^3;
- Stage 4: Wasting syndrome due to HIV (>10% of body weight, associated with unexplained chronic diarrhea or chronic asthenia or unexplained prolonged fever), various and multiple opportunistic infections;

Body Mass Index (BMI): It is used to assess the weight status of an individual. It is calculated from the height and weight of the individual according to the formula BMI = weight in kg/height in meter square (kg/m^2).

- Underweight/Thinness: 15 < 18.5 kg/m^2

- Normal build: 18.5 < 24.9 kg/m^2
- Overweight: 25 < 29.9 kg/m^2
- Moderate obesity: 30 < 34.9 kg/m^2
- Severe obesity: 35 < 39.9 kg/m^2
- Morbid or massive obesity: >40 kg/m^2

Clinical Failure: Clinical failure is defined as the reappearance of Opportunistic Infections and/or progression to a higher clinical stage according to the World Health Organization classification, as well as weight loss of more than 10% after more than 6 months of ART.

Immunological Failure: Immunological failure is defined by the absence of an increase in the number of CD4 T lymphocytes despite ART after more than 6 months on ART.

Virological Failure: Virological failure is defined by a persistent Viral Load greater than 1000 RNA copies/ml (3.00 log10 RNA copies/ml) after 6 months of ART.

- Minimal failure (3.00 log10 < CV < 4.00 log10 RNA copies/ml),
- Moderate failure (4.00 log10 < CV < 5.00 log10 RNA copies/ml),
- Major or severe failure (CV > 5.00 log10 RNA copies/ml).

3. Results

During the M6 appointment, 62 patients were registered, including 38 women (61.3%) and 24 men (38.7%), thus giving a sex ratio of 1.58 in favor of women. Fifty-seven (57) patients from the main cohort did not respond to the M6 appointment, thus a loss rate of 47.89%. Table 2 presents the data on gender.

The age group most found is between 36 to 45 years old with 16 patients (26.7%), followed by the age group from 46 to 55 with 15 patients (25%), 26 to 35 years old with 11 patients (18.3%), follow-up from 56 to 65 years old with 10 patients (16.7%) and finally the age group from 18 to 25 years old with 8 patients (13.3%). The age range data mentioned above are described in Table 2. The

Table 2. Gender and age group of patients in M6.

Parameters	Values (M6) %
Sex	
Female	61.3
Male	38.7
Age range	
18 - 25	13.3
26 - 35	18.3
36 - 45	26.7
46 - 55	25.0
56 - 65	16.7
>65	0

average age was 42.4 ± 13.3 years with a range of 18 to 68 years. The average weight was 60.5 ± 15.4 kg with extremities of 40 to 108 kg; with a mean BMI of 22.6 ± 5.8 kg/m^2.

Thirty-four (34) patients (61.82%) were at Clinical Stage 3 according to the World Health Organization (WHO), 16 patients (29.09%) were at Stage 1 and 5 patients (9.09) was in Stage 2. Thirty-six (36) patients (67.92%) had a normal clinical condition, 15 patients (28.3%) had a good clinical condition and 2 patients (3.77%) had a good clinical condition. bad. Table 3 presents the above clinical data in an exhaustive manner.

The most common Opportunistic Infections found in patients at the sixth month of ART were: skin pruritus (25.4%), dermatitis and rash (22.2%), and malaria (11.1%). Table 4 presents the exhaustive list of OIs found.

The mean values of biochemical parameters of patients at the sixth month of ART were as follows: 35.9 ± 33.6 IU/L with ranges from 2.70 to 168.50 IU/L for ALT, mean amylase was 139.8 ± 117 IU/L with ranges from 5.40 to 455.50 IU/L, mean AST was 37.2 ± 27 IU/L with ranges from 0 to 152.90 IU/L, the mean Cholesterol was 129.7 ± 33.5 mg/dl with ranges from 74 to 212 mg/dl, the mean Creatinine was 1.3 ± 0.3 mg/dl with ranges from 0.5 to 2 mg/dl, mean Hemoglobin was 11.3 ± 1.5 g/dl with ranges from 6 to 14.60 g/dl, mean Total Protein was 4.7 ± 0.7 g/dl with extremities 3.20 to 6.70 g/dl, mean Triglyceride was 65.7 ± 29.2 g/dl with extremities 18 to 132 g/dl, mean Urea was 32.1 ± 42.6 mg/dl with endpoints of 7 to 302 g/dl. Table 5 presents the different biochemical parameters mentioned above.

The median VL value was 2.92 log10 RNA copies/ml (840 RNA copies/ml) with the lower and upper ends equal to 0.0 log10 and 5.99 log10 RNA copies/ml, respectively. With 17.75% of patients experiencing major first-line treatment failure. The results of the VLs mentioned above are presented in Table 6.

Table 3. Clinical aspects of patients in M6.

Patient clinic in M6	Frequency	Percentage
Clinical stage according to WHO (N = 62)		
Stage 1	18	29.03
Stage 2	7	11.29
Stage 3	37	59.68
Stage 4	0	0
Patient's Clinical Status (N = 62)		
Normal	42	67.74
Good	18	29.03
Bad	2	3.23
Pre-moribund	0	0
Moribund	0	0

Table 4. Opportunistic infections encountered in patients at M6.

Parameters	Patients (N = 62) %
Oral candidiasis	4.8
Vaginal mycosis	6.3
Vaginal pruritus	9.5
Skin pruritus	25.4
Shingles	3.2
Rash	22.2
Dermatitis	22.2
Diarrhea	3.2
Intestinal parasitosis	3.2
Rhinitis	9.5
Tuberculosis	6.3
Malaria	11.1
Urinary infection	9.5
Non-specific STI	0
Others	11.1

Table 5. Biochemical parameters of patients at M6.

Parameters	Normal Values	Averages of Patients in M6
ALAT (UI/L)	0 - 41	35.9
ASAT (UI/L)	0 - 31	37.2
Amylase (IU/L)	≤90	139.8
Total cholesterol (mg/dl)	110 - 200	129.7
Creatinine (mg/dl)	0.5 - 1.5	1.3
Blood glucose (mg/dl)	60 - 110	78.4
Hemoglobin (g/dl)	≥12	11.3
Total protein (g/dl)	6.6 - 8.2	4.7
Triglycerides (mg/dl)	35 - 185	65.7
Urea (mg/dl)	15 - 45	32.1

All 62 samples were successfully amplified. Subtype A is dominant with 13 cases (20.98%); followed by CRF02_AG (16.13%), C (14.52%), D (9.68%) and K (6.45%) subtypes as described in Table 6. No mutations were found for DTG after 6 months of ART. However, the mutations K65R (3 cases), T69P/N (6 cases), K70R (9 cases) and M184V (8 cases) were listed as existing mutations for Nucleotide Reverse Transcriptase Inhibitors (Table 7).

Good compliance was observed in 32 patients (64%) versus 18 patients (36%) who had not observed the treatment.

Table 6. Patient viral load values at M6.

Viral Load (RNA copies/ml)		
	Values	**Log_{10}**
Median	840	2.92
Lower limit	1	0.00
Upper Limit	971,874	5.99
Viral Load Intervals		
	Values	**Percentage**
Undetectable VL	25	40.32
VL < 3.0 log_{10}	12	19.35
3.0 log_{10} < VL < 5.0 log_{10}	14	22.58
VL > 5.0 log_{10}	11	17.75

Table 7. Molecular data of patients in M6.

Molecular data of patients in M6		
Prevalence of circulating subtypes		
Subtypes	**Number**	**Percent**
A	13	20.98
B	1	1.61
C	9	14.52
D	6	9.70
E	1	1.61
F	1	1.61
G	3	4.85
H	2	3.22
J	1	1.61
K	4	6.45
CRF01	3	4.85
CRF02	10	16.13
CRF05	1	1.61
CRF06	1	1.61
CRF11	1	1.61
CRF25	1	1.61
CRF45	1	1.61
CRF56	1	1.61
U	2	3.26
Total	**62**	**100.0**
Prevalence of mutations of interest		
NRTI	**Number**	**Percent**
K65R	3	4.84
T69P	6	9.68
K70E/R	9	14.52
M184V	8	12.90

4. Discussion

The total number of patients who respected the appointment of the sixth month of follow-up in the present work is 62 patients with a predominance of the female sex of the order of 38 women (61.3%) against 24 men (38.7%), thus giving a sex ratio of 1.58 in favor of women. The predominance of the female gender in the present study corroborates existing data. The majority of studies carried out in Kinshasa, in the Democratic Republic of Congo (DRC), or even in Central Africa mention this predominance of the female sex in the populations of PLHIV monitored [4] [5] [9].

Fifty-seven (57) patients did not respond to the M6 appointment, *i.e.* a loss rate of 47.89%. Similar loss rates have been found in various studies published across the DRC and other Central African countries [10]-[15]. These data present the difficulty of retaining and retaining PLHIV in treatment center cohorts. Patients are always looking for a better care center [16] [17] [18].

The most found age group is between 36 to 45 years with 16 cases (26.7%), followed by the age group from 46 to 55 with 15 cases (25%), 26 to 35 years with 11 cases (18.3%), followed by 56 to 65 years old with 10 cases (16.7%) and finally the age group 18 to 25 years old with 8 cases (13.3%). This general observation is reported by several authors in our field [4] [5] [9].

At M6, the average BMI was 22.6 ± 5.8 kg/m^2, which indicates a normal build with a state of thinness for the population. This state of thinness is surely due to the condition of the patients and the evolution of the infection and other associated factors. This state of thinness has also been documented in the literature because of the clinical condition of patients after 6 months of treatment [19].

Thirty-four (34) cases (61.82%) were in stage 3, 16 cases (29.09%) were in stage 1 and 5 cases (9.09) were in stage 2. Thirty-six (36) patients (67.92%) had a normal clinical condition, 15 patients (28.3%) had a good clinical condition and 2 patients (3.77%) had a poor clinical condition. After 6 months of ART, the clinical stage of patients in general seems to give hope for treatment; no patients were registered at WHO stage 4 and only 2 patients (3.77%) were registered in poor clinical condition. These data seem to support DTG as a preferential first-line ARV. The clinic seems to be favorable after 6 months of treatment in patients on DTG in Kinshasa.

The most common opportunistic infections among patients in the sixth month of ART are skin pruritus with 16 out of 62 patients (25.4%), dermatitis and rash (22.2%), and malaria (11.1%). It appears from these data that in M6, malaria (11.1%) and tuberculosis (6.3%), often dominant, had very low prevalence compared to dermatoses in general which had a higher prevalence. higher. This demonstrates the effectiveness of the treatment compared to certain recommendations. However, the high rates of dermatoses in general can also be linked to the living environment and personal hygiene of patients.

Although close to the limits (lower and upper), the average values of the biochemical parameters of the patients at M6 are for the most part within the ranges of normal values. The average value of AST (37.2 IU/L) is higher than the

normal value (Δ = 6.2 IU/L), Amylase (139.8 IU/L) is higher than the normal value (Δ = 49.8 IU/L), Hemoglobin (11.3 g/dl) is lower than the normal value (Δ = 0.7 g/dl), total protein (4.7 g/dl) is lower than the normal value (Δ = 1.9 g/dl). These values are markers of the progress of patient treatment. They are also markers of anemia [20], and other forms of deficiencies [21] [22] [23]. These disturbances are due to the progress of the infection, the appearance of opportunistic infections, the effectiveness of the treatment, and the general condition of the patient on ART [21] [22] [23]. Similar data have been presented in the literature for PLHIV followed in Kinshasa [21] [24].

After RNA amplification to determine plasma Viral Load (VL), the median VL value was 2.92 log10 RNA copies/ml (840 RNA copies/ml) with the lower and upper ends respectively equal to 0.0 log10 and 5.99 log10 RNA copies/ml. VL was undetectable for 25 patients, giving a rate of 40.32% of patients with undetected VL. With 19.5% of patients having a VL less than 3.00 log10 RNA copies/ml, the therapeutic success rate is 59.67%. The virological failure rate of first-line treatment according to the recommendations of the World Health Organization (WHO) is 40.33%, with 17.75% of patients experiencing major failure (greater than 5.0 log10). These data are justifiable by the fact that more than half of patients start ART with a poor prognosis of remission [5] [25] [26]. At M6 of this cohort, the failure rate of 40.33% is higher than those presented for the city of Kinshasa in previous years [27] [28]. This demonstrates the need for virological and molecular monitoring of patients on ART, and that this monitoring should continue to appear in treatment recommendations even for countries with limited resources.

After sequencing the different samples, 62 samples were successfully amplified. Subtype A is dominant with 13 cases (20.98%); followed by CRF02_AG (16.13%), subtypes C (14.52%), D (9.68%) and K (6.45%). No mutations were found for DTG after 6 months of ART. However, the mutations K65R (3 cases), T69P/N (6 cases), K70R (9 cases) and M184V (8 cases) were listed as existing mutations for Nucleotide Reverse Transcriptase Inhibitors. Even after the loss of patients, subtype A still remains dominant in the cohort of PLHIV on ART, followed by CRF02 and subtype C. This is the profile presented for Kinshasa through the various available literature [27] [29] [30]. After 6 months of ART, no mutation was found to induce resistance to DTG. This confirms the strong genetic barrier attributed to DTG in the various literature in the field [31] [32]. However, the mutations found are generally associated with resistance to Lamivudine-3TC (K65, T69, M184) and Tenofovir-TDF (K70) [27] [29] [33]. As a result, more than 14.52% of patients on ART, or 36% of patients with virological failure, are in a situation of failure because of the mutation which confers resistance to TDF. This invites a revision of the first-line formula based on the scientific evidence of local study.

5. Conclusion

After 6 months of treatment, the majority of patients are in clinical stage 3 with a

normal clinical condition. Skin infections (dermatoses) in general are the majority opportunistic infections. Certain biological parameters (ASAT, Amylase, Hemoglobin and Total Proteins) are considerably altered. A high virological failure rate with the presence of certain mutations associated with resistance to Lamivudine-3TC (K65, T69, M184) and Tenofovir-TDF (K70) calls into question the value of viral loads and molecular tests.

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Conflicts of Interest

The authors declare no conflict of interest.

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