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Evaluating Second-line ART Efficacy in Pediatric HIV1 Infections: A Study at Albert Royer Children's Hospital

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

Introduction: In developing countries, around 15% of children infected with the Human Immunodeficiency Virus (HIV) have access to early diagnosis, and 28% are eligible for antiretroviral treatment (ART). The evaluation studies carried out in Senegal have been limited to children on first-line ART. The main objective of our study was to evaluate the efficacy of second-line ART.
Methodology: Data were collected at 3 different sites. This was a retrospective, longitudinal and analytical study, conducted from March 4 to August 28, 2015. The study population consisted of 65 HIV1-infected children. BD FACSCountTM Flow Cytometer for TCD4 lymphocyte counting. For genotyping, 2 sets of PCRs were required. Data were entered and analysed using Word/Excel 2013 and Epi-info7 software.
Results: We had 43 boys and 22 girls, giving a sex ratio of 1.95. The extreme ages were 5 and 15 years, and the mean age was 11.43 years. The majority belonged to stage 4 and 2 of the WHO classification. The clinical evolution was favourable in 86.15%, unfavourable in 3.07% and unspecified in 10.76%. We found 9 genotypes and recombinant forms; 80% of the strains were sequenced.
Discussion: The general aim of ART is the same as in adults. Clinical stages were not specified in 15.38% of cases. In total, 58.46% had an excellent immunovirological response. We conclude from this that a good IR doesn't mean a good immunovirological evolution. Initiation of ART should be guided by genotyping.
Conclusion-Recommendations: ART reduced morbidity and mortality. Our study showed a virological failure rate of 21.53% and an IR rate of 86.15%.

Keywords: Human immunodeficiency virus; acquired immunodeficiency syndrome; viral ribonucleic acid; immuno-virological evaluation.

1. INTRODUCTION

Described in 1981, Acquired Immunodeficiency Syndrome (AIDS) is the advanced stage of infection by the Human Immunodeficiency Virus (HIV). According to the December 2014 UNAIDS/WHO report, there were 35 million people living with HIV (PLHIV) worldwide, 1.5 million of whom died during the year. Sub-Saharan Africa alone accounted for 24.7 million PLHIV and 1.1 million deaths [1]. In 2010, around 3.4 million children under the age of 15 were infected, nearly 90% of whom lived in sub-Saharan Africa [2]. In developing countries (DCs), only 15% of exposed children have access to early diagnosis and 28% are eligible for antiretroviral treatment (ART) [3].

Diagnosis of HIV infection before the age of 18 months requires the detection of viral genetic material using tests such as the Polymerase Chain Reaction (PCR). This is used to detect proviral deoxyribonucleic acid (DNA) and/or viral ribonucleic acid (RNA). Diagnosis in children in this age group began in Senegal in 2000. The management of HIV infection is therefore based on maintaining the circulating viral load (VL) below the detection threshold, which is the only guarantee of long-term virological, immunological and clinical efficacy [4]. Immunovirological monitoring of ART will therefore be based routinely on the measurement of VL and the quantification of CD4 T lymphocytes (TCD4).

ART came into effect in Senegal in 1998, and progress has been made in making paediatric forms of antiretrovirals (ARVs) available. These are classified as 1st and 2nd line drugs, and are evaluated fairly regularly in DCs. Immuno-virological evaluation studies conducted in Senegal in children have been limited to those on 1st-line ART [5].

The main objective of our study was to evaluate the efficacy of 2nd-line ART in children treated at the Albert Royer Children's Hospital (HEAR) in Dakar. It is the logical continuation of work carried out in 2013, and entitled "Paediatric HIV1 infection in Senegal: diagnosis and resistance to ARVs".

The specific objectives were to:

- Determine the number of cases of virological failure due to VL
- To determine the number of cases of immunological failure using CD4 counts
- Assess regression of viral multiplication with VL
- Evaluate IR using CD4 counts

- Determine the efficacy of different 2nd-line therapeutic regimens
- Identify resistance genes through genotyping

2. METHODOLOGY

Clinical and biological data were collected in 3 different sites, all located in Dakar. This was a retrospective, longitudinal and analytical study conducted from March 4 to August 28, 2015. The study population consisted of 65 HIV1-infected children aged 0 to 15 years, followed at HEAR and all on 2nd-line ART.

Inclusion criteria were as follows:

- Be infected with HIV1
- Be on 2nd-line ART
- Be no more than 15 years old
- Of either sex
- Have usable clinical records
- Have usable laboratory results
- Be followed at HEAR during the study period For the study material, we had

The population studied: All children on 2nd-line ART from the database at the "Pavillon des Mères".

The data carriers used: That is to say the forms that we had written, tested and validated beforehand. They contained various items including anthropological, clinical and biological information.

Other media were used: Patients' hospitalisation records, data from the Medical Information Service (SIM), laboratory registers, databases available at the 3 sites, etc.

Blood samples analysed:

- For the VL assay, we had used blood plasma obtained by centrifugation of venous whole blood (taken from the elbow crease on EDTA tube). The plasma was transferred to Nunc tubes and stored at -80°C.
- For the TCD4 lymphocyte count, we used whole venous blood collected in an EDTA tube and stored at room temperature (+20 to +25°C) for a maximum of 24 hours.
- For genotyping: same as collection for VL assay
- At the Regional Center for Research and Training in Clinical Management (CRCF) for PLHIV, we also searched the database for the results of CD4 count determination
- The information contained in the database of the Molecular Biology Unit of the Virology Laboratory was used. We looked for the results of VL determination and genotyping.

Laboratory equipment and consumables:

- These included the basic equipment and reagents required for molecular biology, as well as the specific equipment and their reagents essential for performing VL, CD4 count and genotyping.
- The test performed by m2000rt HIV-1 v2.0 consisted of amplification of plasma viral RNA combined with a low-temperature amplification-detection step using real-time PCR. This operation uses plasma as the starting material. Viral RNA is extracted using m2000sp's automated platform.
- The basic equipment used for VL determination in the HALD Molecular Biology Unit is an Abbott ABI 7500 real-time PCR. The reagents used are from the same company and are specific to the device
- The BD FACSCountTM Flow Cytometer was used to count TCD4 lymphocytes
- For genotyping, it was necessary to amplify the protease and RT genes separately and therefore to carry out 2 different PCR series from the same samples (prepare 2 different mixes); we used 2 techniques:

RT-PCR: the Firm's Thermocycler (m2000rt HIV-1 v2.0) was used to achieve this. The primers used are listed in Table 1.

Table 1. External primer sequence

Gene to amplify	External Primers	External Primer Sequence
RT	MJ3 (sense)	5'-AGTAGGACCTACACCTGTCA-3'
	MJ4 (antisense)	5'-CTGTTAGTGCTTTGGTTCCTCT-3'
Protease	5' prot 1 (sense)	5'-TAATTTTTTAGGGAAGATCTGGCCTTCC-3'
	3' prot 1 (antisense)	5'-GCAAATACTGGAGTATTGTATGGATTTTCAGG-3'

Table 2. Nested PCR: Here too, the list of primers used is presented in

Genes to amplify	Internal primers	Internal primer sequence
RT	A (35) (sense)	5'-TTGGTTGCACTTTAAATTTTCCCATTAGTCCTATT-3'
	NE1(35) (antisense)	5'-CCTACTAACTTCTGTATGTCATTGACAGTCCAGCT-3'
Protease	5' prot 2 (sense)	5'-TCAGAGCAGACCAGAGCCAACAGCCCCA-3'
	3' prot 2 (antisense)	5'C-AATGCTTTTATTTTTTCTTCTGTCAATGGC-3'

The data were processed, entered and analysed using Word 2013, Excel 2013 and Epi-info7 software. The Chi-2 test was used to determine the relationship between the variables, and the significance threshold was set at p=0.05.

3. RESULTS

Data were collected from 65 children, including 43 boys (66%) and 22 girls, giving a sex ratio of 1.95.

The extreme ages were 5 and 15 years respectively; the mean age was 11.43 years. Grouped by age group gave:

- 0-5 years]: 1
- 5-10 years]: 16
- 10-15 years]: 48

The maximum number of children were put on 2nd-line ARVs in 2010 and 2013; the start-up was slow, with a single case in 2008 (Fig. 1).

Using the WHO classification, 10 children could not be classified. The majority of the remaining 55 (26) belonged to stage 4, followed by stage 2; stages 3 and 1 accounted for the minority (Fig. 2).

The most commonly prescribed combinations were: ABC+3TC+LPV/r, AZT+3TC+LPV/r and TDF+3TC+LPV/r (Table 3).

All the children were infected with HIV1, but there was one case of co-infection with HIV2. The clinical course is shown in 'Table 4'. One case of opportunistic infection was noted: Tuberculosis in a 10-year-old child.

Therapeutic failure was observed in 14 of our 65 patients; their VL was above the threshold (VL >1000 copies/ml) used as a criterion for failure of ART in children. IR (TCD4 >500 cells/mm3) was achieved in 56 of the 65 children.

The treatment regimens with the best responses were ABC-3TC-ATZ/r, Combivir-LPV/r, ABC-3TC-LPV/r and AZT-3TC-LPV/r (reported in Table 5).

Genotyping revealed the existence of 9 genotypes and recombinant forms. The CRFO2-AG recombinant form is the most common (Fig. 3). These genotypes are usually derived from mutational drifts in the parental HIV1 genome. Some are linked to specific ARV resistance; which makes it possible to deduce the sensitivity or otherwise of an HIV strain.

The results of the sequencing of the regions of the genome responsible for almost all the mutations leading to ARV resistance, are shown in Fig. 4. The RT and Protease regions of the genes of 80% (52 of the 65 strains) were sequenced.

In practice, no disturbance was observed during the biochemical work-up: all the children had normal renal function; only one of them showed a disturbance in liver markers.

4. DISCUSSION

Difficulties were encountered in carrying out this study, including the distance between the 2 university hospitals (Fann and Dantec) where the 3 study sites are located:

At the level of the children's care unit at the HEAR's "Pavillon des Mères": while access to the children's files posed no problem, the data that had to be collected was a different matter. Some information was missing, such as the results of various laboratory tests; we had to look for it in different places, and this search was not always conclusive.

At the CRCF of the CHUF: in the laboratory responsible for determining VL, we did not have access to all the results, especially those relating to six-monthly VL monitoring. The reason given was the confidentiality of data concerning PLHIV. We were therefore unable to fully assess changes in the VL of children on 2nd-line ART.

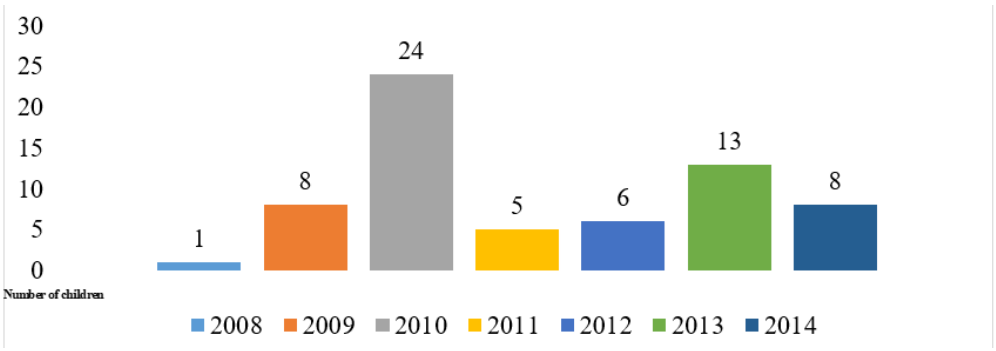

Fig. 1. Breakdown of our sample by year of initiation of 2nd-line ART

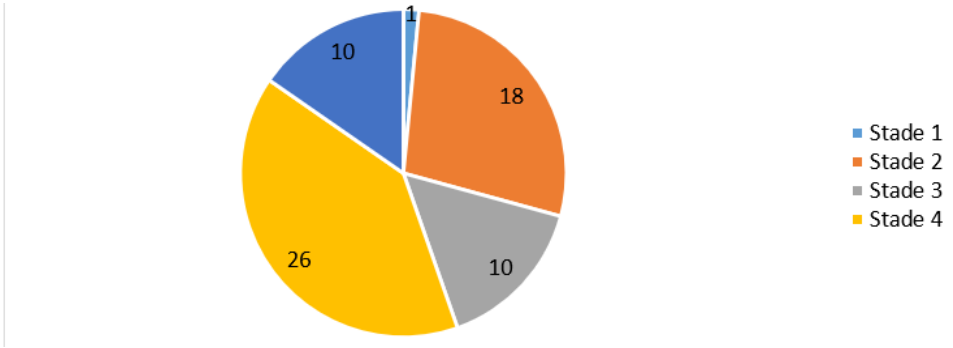

Fig. 2. Distribution of our sample according to WHO classification at the start of treatment

Table 3. Breakdown of our sample by therapeutic regimen

Therapeutic regimes	Frequency	Percentage (%)
TDF-3TC-LPV/r	16	24,61
ABC-3TC-LPV/r	20	30,76
AZT-3TC-LPV/r	17	26,15
TDF-3TC-ATZ/r	5	07,69
Combivir-LPV/r	6	09,23
ABC-3TC-ATZ/r	1	01,53
Total	65	100,00

Table 4. Clinical course of children on 2nd-line ART

Evolution	Number of children	Percentage (%)
Favourable	56	86,15
Unfavorable	2	03,07
Not specified	7	10,76
Total	65	100,00

Table 5. Results of the different therapeutic regimens obtained

Therapeutic regimes	Number of children treated	Number of successes	Percentage (%)
ABC-3TC-LPV/r	20	13	65
AZT-3TC-LPV/r	17	12	70,58
TDF-3TC-LPV/r	16	7	43,75
Combivir-LPV/r	6	4	66,66
TDF-3TC-ATZ/r	5	2	40
ABC-3TC-ATZ/r	1	1	100

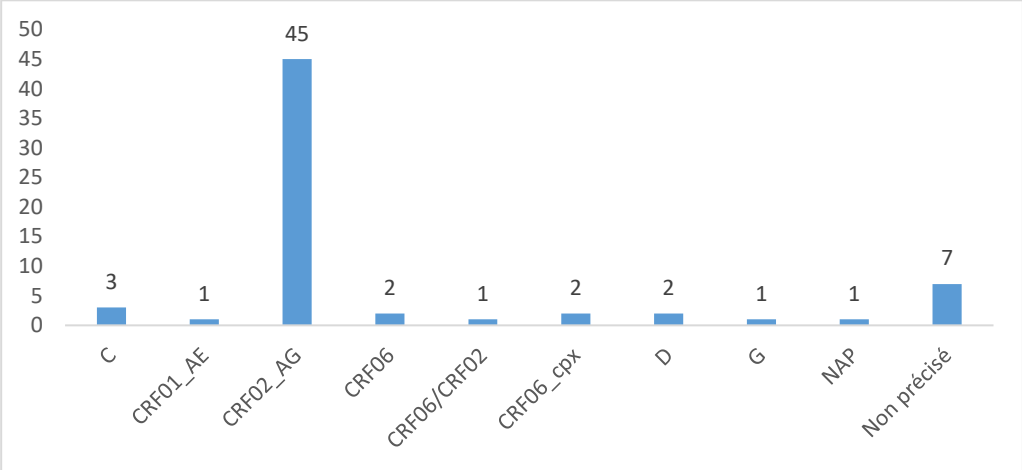

Fig. 3. Genotypes and recombinant forms observed

Fig. 4. Sequenced genome regions

At the HALD Molecular Biology Unit: access to the database was possible. However, consultation of the registers was strictly limited, and therefore not authorised to foreigners.

In terms of results, all 65 children were vertically infected, that is to say by mother-to-child transmission (MTCT). Only one child was co-infected with HIV2.

The sample comprised patients aged between 5 and 15 years, with an average age of 11.43 years. This mean age was higher than that observed (6.51 years) in a study of children who had failed first-line ARV therapy at HEAR in 2013; they were almost 15 years old at inclusion in this cross-sectional study [6, 7]. As their age increased (over 15 years), the children were transferred to the CHUF Outpatient Treatment Centre (CTA). The predominance of male children (66%) has been found elsewhere in West Africa [8].

The general objective of ART in children is the same as in adults: to achieve a sustained reduction in VL below detection thresholds, which is the only guarantee of the absence of selection of resistance mutations and of long-term virological, immunological and clinical efficacy [9]. This justifies adapting ART by prescribing a 2nd-line treatment. This fund was only introduced at HEAR in 2008, when the average inclusion rate was 9.28 children per year until 2014.

The majority of our children were in stage 4 (40%) or stage 2 (27.69%) at the start of 2nd-line ART; these 2 stages are part of the WHO criteria for mandatory initiation of ART [2]. Clinical stages were not specified in 15.38% of children according to the WHO classification and in 90% according to the CDC classification; this is a recurrent finding in Senegal's University Hospitals. A study carried out over the period 2000-2003 at HEAR showed that 39% of infected

children were at CDC stage C at the time of their inclusion [10].

For 2nd-line ART in Senegal, 2 NRTIs are combined with 1 NNRTI. Elsewhere in the world, this treatment sometimes combines 2 NRTIs and 1 protease inhibitor (PI) [2]. This resulted in IR in 56 children (86%), but was unable to prevent virological failure in 14 cases (21.53%); in all, 38 children (58.46%) had an excellent immunovirological response: VL undetectable and TCD4 >500/ml. The therapeutic regimens with a good immunovirological response were, in descending order: ABC-3TC-ATZ/r (100%), AZT-3TC-LPV/r (70.58%), Combivir-LPV/r (66.66%), ABC-3TC-LPV/r (65%); in Mali, good response was obtained with ABC-TDF-IDV, ABC-TDF-LPV/r, AZT-DDI or boosted TDF-IP regimens [11].

No deaths were recorded, although we did find 1 case of opportunistic infection such as tuberculosis; the current mortality rate linked to HIV in children worldwide is around 14% [12]. We deduce that a good IR does not mean necessarily a good immunovirological evolution.

Starting ARV must be guided if possible by the determination of the genotype of the HIV undermined [2, 6, 7]. The genotype was identified in 58 children (89.23%). The main genotypes found (CRF02-AG and C) are the same as those detected during an earlier study made to HEAR. There was a virtual absence of genotypes associated with resistance to NRTIs (M184V), NNRTIs (K103N/R/S/) and PIs (L90M and M46I) [7]. Genotyping data were not interpretable for 7 children.

The monitoring of liver and kidney parameters not shown major disruptions. They must be appreciated before and during the under ARV, some drugs used are particularly toxic.

5. CONCLUSION AND RECOMMENDATIONS

ART has considerably reduced morbidity and mortality in children, hence the WHO recommendation to treat all children under 24 months of age, regardless of their clinical or immunological stage. This poses a problem in DCs because of the delay in diagnosis and the lack of pediatric formulations of ARVs.

At HEAR, pediatricians mainly use VL, which is the earliest and most reliable way of detecting possible therapeutic failure. In order to determine the frequency of immunovirological failure during 2nd-line ART in children treated at this hospital, we conducted this retrospective, longitudinal and analytical study over a period of 6 months, in a cohort of 65 children aged 0 to 15 years.

The main findings were as follows:

- The sex ratio was 1.95, with a predominance of males (66%).
- Clinical outcome was favourable in 86.15% of cases, unfavourable in 3.07% and unspecified in 10.76%; no deaths were reported.
- The best 2nd-line treatment regimens were, in descending order ABC-3TC-ATZ/r, AZT-3TC-LPV/r, Combivir-LPV/r, ABC-3TC-LPV/r, TDF-3TC-LPV/r and TDF-3TC-ATZ/r.

This study showed that under 2nd-line ART, the incidence of virological failure was 21.53%, and that of good IR was 86.15%.

This work has enabled us to formulate the following recommendations:

For health establishments

Comply with the national guidelines issued by the Ministry of Health and Social Action on neonatal diagnosis of HIV infection

Carry out immuno-virological evaluation studies on PLHIV in order to determine the level of resistance to 2nd-line ARVs

Work towards making available a 3rd-line of ART in pediatrics

For clinicians treating patients

- Fill in patient records correctly, classifying them according to WHO and/or CDC criteria
- Take into account the therapeutic regimens that give the best results

For Biologists

- Carry out genotyping and sequencing whenever possible
- Update registers and databases

For PLHIV, parents and/or carers

- Seek advice as soon as possible from specialist services
- Enrol in ART

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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