Prevention, suppression, and resistance

Antiretroviral treatment for children with HIV in sub-Saharan Africa

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CHAPTER 5:

Pretreatment HIV drug resistance results in virological failure and accumulation of additional resistance mutations in Ugandan children

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[submitted]
ABSTRACT

Background
Pretreatment HIV drug resistance can impair virological response to antiretroviral therapy (ART), jeopardizing effective treatment for children.

Methods
Children aged ≤12 years initiated first-line ART in Uganda in 2010-2011. Baseline and 6-monthly viral load (VL) and genotypic resistance testing if VL>1,000 copies/ml was done. The 2015 IAS-USA mutation list and Stanford algorithm were used to score drug resistance mutations (DRMs) and susceptibility. Virological failure was defined as two consecutive VLs >1,000 copies/ml or death after at least six months of ART. Factors associated with failure and acquired drug resistance were assessed in a multivariable logistic regression analysis.

Results
Among 317 children enrolled, median age was 4.9 years and most (91.5%) received NNRTI-based regimens. Pretreatment drug resistance was detected in 47/278 (16.9%) children of whom 22 (7.9%) had resistance against their first-line regimen and were therefore on a partially active regimen. After 24 months of follow-up, 92/287 (32.1%) had experienced virological failure. Children with pretreatment drug resistance had a higher risk of virological failure (odds ratio: 15.25, p<0.001) and acquired drug resistance (odds ratio: 3.58, p=0.01).

Conclusions
Almost one third of children experienced virological failure within 24 months of NNRTI-based first-line treatment. Pretreatment drug resistance was the strongest predictor of virological failure and acquired drug resistance. Our results stress the importance of fully-active first-line regimens. Routine VL testing is needed to detect children with virological failure and switch to second-line in time, in order to prevent clinical deterioration and accumulation of additional drug resistance.
INTRODUCTION

Sub-Saharan Africa has the highest burden of HIV infected children in the world. There has been unprecedented acceleration of access to antiretroviral therapy (ART) in the last 10 years in low- and middle-income countries (LMIC). By the end of 2015, an estimated 823,000 HIV-infected children were receiving ART in LMIC, the large majority on first-line regimens. In Uganda, of the estimated 95,649 children living with HIV, 60,029 children were accessing ART at the end of 2015.

Although access to ART has conferred substantial benefits on survival and quality of life, it has also caused the emergence of both acquired and transmitted drug resistance, especially in east and southern Africa. As most infected children have acquired HIV from their mother, they are particularly at risk for HIV drug resistance in the context of prevention of mother-to-child transmission (PMTCT) or via transmission of resistant strains from their mothers. Our group has previously shown high rates of HIV drug resistance among children initiating ART in Uganda.

Pretreatment drug resistance has the potential to contribute to increasing rates of virological failure at a population level, thus compromising long-term effectiveness of recommended first-line regimens. Treatment failure is more frequent among children and adolescents compared to adults and this translates into higher risk of acquired drug resistance development. Studies in sub-Saharan Africa have shown that virological failure rates on NNRTI-based first-line ART were significantly higher in children compared with adults. In another study from Côte d’Ivoire, only 66% of children achieved virological success after a median of 10.2 months of ART. Amongst those children experiencing virological failure, 71% of viruses displayed resistance to at least one antiretroviral drug.

In view of the fact that HIV drug resistance is a growing problem, and given the fact that children are especially vulnerable, this study seeks to describe virological outcomes of children on first-line treatment, evaluate the effect of pretreatment drug resistance on treatment failure and on accumulation of DRMs. The results will provide important information to develop appropriate interventions that enhance the effectiveness of currently available first-line regimens.
METHOIDS

Study Design and Setting
The Monitoring Antiretroviral Resistance in Children (MARCH) study is a multicenter prospective observational cohort study of HIV-1 infected children who received HIV treatment and care at three Joint Clinical Research Center (JCRC) Regional Centers of Excellence (RCEs) based in Kampala, Mbale and Fort Portal, Uganda. The ethical committees of JCRC and the Uganda National Council of Science and Technology, and the Academic Medical Center of the University of Amsterdam in The Netherlands approved the study protocol before commencement of the study.

Study participants
Study methods of this cohort have previously been described in detail. We enrolled HIV infected children aged ≤12 years from January 2010 to August 2011. For this analysis, participants starting on first-line ART were included and followed up for 24 months. Children were started on ART based on 2006 World Health Organization (WHO) treatment guidelines and by August 2010, the clinics adopted the revised treatment guidelines for 2010. Following the revision, all children <24 months of age were eligible for ART irrespective of their clinical stage or CD4 cell counts. A combination of two nucleoside reverse transcriptase inhibitor (NRTI) drugs and one non-NRTI (either efavirenz or nevirapine), were the preferred combinations of choice and recommended by the Uganda guidelines. Protease inhibitor (PI)-based regimens were prescribed for young children exposed to PMTCT. Efavirenz was only given to children above 3 years.

Follow-up procedures and variables measured
Socio-demographic and clinical data were collected at enrollment and at subsequent 3-monthly follow-up visits and aggregated in a web-based database. At baseline and 6-monthly thereafter, viral load (VL) testing was done as well as genotypic resistance testing on specimens with VL>1,000 copies/ml. Susceptibility to the prescribed regimen was determined by calculating the genotypic sensitivity score (GSS) using the Stanford algorithm (Version 7.0). Reduced susceptibility to the prescribed regimen was defined as GSS<3; i.e. <3 fully susceptible drugs. Thirty-day adherence was based on caregiver’s report; adherence over time was calculated as the mean of these adherence reports, and was categorized as being suboptimal (≤95% adherence) or optimal (>95% adherence). Virological failure was defined according to the WHO as two consecutive detectable VL...
>1,000 copies/ml taken at least 6 months after treatment initiation. A VL >1,000 copies/ml at the last available measurement, or death after at least 6 months of treatment was also considered as failure. Children were excluded from the analysis if they had less than two VL measurements during follow-up. Acquired drug resistance was defined as a new DRM following initiation of ART for both children with or without pretreatment drug resistance.

**Statistical analysis**

Baseline characteristics are presented as proportions or medians with inter-quartile ranges (IQR). Group comparisons for categorical data were performed using the chi-square or Fisher’s exact tests, and for continuous data using the t-test or the Wilcoxon rank-sum test. Logistic regression was performed to model the association of presence of pretreatment drug resistance with the outcomes virological failure and acquired drug resistance. Explanatory variables considered in the analysis were age, sex, WHO clinical stage at study entry, pretreatment drug resistance, viral load at study initiation, adherence, NNRTI used, exposure of child to drugs for PMTCT and immunodeficiency for age (defined as diminished if age <5 years and CD4% <25% or age ≥5 years and CD4 count<500, and as immunodeficient if age<5 years and CD4%<10% or age ≥5 years and CD4 count<100). Explanatory variables associated with the outcome variables (p<0.10) in the univariable analysis were included in the multivariable model in a step-wise approach. Biologically plausible interactions were examined. Results were expressed as odds ratios (ORs) with 95% confidence intervals and p-values, with p<0.05 regarded statistically significant. Analyses were performed using the statistical software package STATA version 12 (STATA Corp LP, TX, USA).

**RESULTS**

Between January 2010 and August 2011, 317 children aged ≤12 years initiating first-line ART were enrolled into the MARCH study in Kampala (n=90), Mbale (n=108) and Fort Portal (n=119) and followed up for 24 months. Baseline characteristics of the children are summarized in Table 1. Of all participants, 290 (91.5%) initiated a NNRTI-based regimen, 12 (3.8%) a PI-based regimen, and 15 (4.7%) started a triple-NRTI-regimen, because of PMTCT exposure and tuberculosis treatment, respectively. Genotype results before treatment initiation were available for 278 (87.7%) children. At least one DRM was detected in
47 (16.9%) participants; of these children, 22 (7.9%) had predicted reduced susceptibility to at least one drug of their first-line regimen (Table 1). Among participants with drug resistance who initiated a fully active regimen, the E138A mutation was detected in 18/25 (72%) children, which confers resistance to the second generation NNRTI rilpivirine.

At 12 and 24 months of follow-up respectively, a total of 287 (90.5%) and 261 (82.3%) participants were still on first-line ART and in care. An additional 7 participants were still in care but had switched to second-line therapy by 24 months. The participants who did not complete follow-up included 13 (4.1%) who died (8 in the first 6 months), 12 (3.8%) who were lost to follow-up and 24 (7.6%) who transferred out of the recruiting ART centers (Figure 1). VL at the 6, 12, 18 and 24 month visits was assessed for 81.3% (239/294), 90.2% (259/287), 89.1% (246/276) and 96.2% (251/261), respectively, of the participants who were still receiving first-line therapy. Virological suppression (VL<1,000 copies/ml) was achieved by 80.3% (191/239), 77.0% (197/256), 76.8% (189/246), and 71.3% (179/251) at months 6, 12, 18 and 24, respectively (Figure 1).

Overall, 92/287 (32.1%) children experienced virological failure during 24 months of follow-up, of which 1 (1.1%) while on PI-based treatment. Thirty children (10.5%) had an unknown status of virological failure because they had less than two VL results, due to transfer out, loss to follow-up or death before 6 months of treatment. Compared to those with known status of virological failure, these participants were younger (2.1 vs 4.9 years; p=0.012) and initiated triple nucleoside therapy more frequently (16.7% vs 3.5%; p=0.008). The proportion of children with pretreatment drug resistance was not significantly different in both groups. In a multivariable analysis, children with pretreatment drug resistance were more likely to experience virological failure (adjusted odds ratio [aOR] 15.25, 95% CI: 3.77-61.67; p<0.001; Table 2). Other predictors associated with virological failure included higher baseline VL (aOR 2.28 for every log VL increase, 95% CI:1.57–3.31; p<0.001) and WHO clinical stage 2 compared to stage 1 (aOR 10.3 95% CI:1.41–75.56, p=0.022, Table 2). None of the other baseline characteristics including age, sex, CD4%/CD4 count and type of NNRTI were predictive of failure.

Sequence results were available for 39/48 (81.3%), 51/62 (82.3%), 47/57 (82.5%) and 61/72 (84.7%) children with a VL>1000 cps/ml at months 6, 12, 18 and 24 respectively (Figure 1). Of 278 children with GRT results during follow-up, 72 children (25.9%) acquired additional DRMs; 68 (24.5%) acquired additional NNRTI mutations and 67 (24.1%) acquired additional NRTI mutations. In children who met the study definition of virological failure and had GRT results available, 67/84 (79.8%) acquired additional DRMs; 64 (76.2%) acquired NNRTI mutations and 63 (75.0%) NRTI mutations. No PI
Table 1. Baseline Characteristics of a cohort of 317 HIV infected children initiating first-line ART at 3 clinics in Uganda (January 2010 – July 2011)

| Variable | Summary Statistics |
|----------|--------------------|
|          | n or median (IQR) | N | % |
| age      |                     |   |   |
| <5 years | 168                 | 317 | 53.0% |
| <3 years | 113                 | 317 | 35.6% |
| sex      |                     |   |   |
| male     | 159                 | 317 | 50.2% |
| study site |                 |   |   |
| Kampala  | 90                  | 317 | 28.4% |
| Fort Portal |           | 119 | 37.5% |
| Mbale    | 108                 | 317 | 34.1% |
| WHO stage|                     |   |   |
| 1        | 25                  | 317 | 7.9% |
| 2        | 62                  | 317 | 19.6% |
| 3        | 162                 | 317 | 51.1% |
| 4        | 68                  | 317 | 21.5% |
| Height for age z score |           |   |   |
| < -2     | 155                 | 302 | 51.3% |
| Weight for age z score |           |   |   |
| < -2     | 97                  | 266 | 36.5% |
| CD4 % (in children < 5 yr) |   |   |
| 19 (12-29) | 159             |   |   |
| CD4 count (in children ≥ 5 yr) |   |   |
| 350 (223-689) | 146     |   |   |
| Pre-treatment drug resistance¹ |   |   |
| No       | 231                 | 278 | 83.1% |
| Yes, but on fully active regimen | 25 | 278 | 9.0% |
| Yes, on partially active regimen | 22 | 278 | 7.9% |
| Log viral load at baseline | 5.1 (4.5-5.6) | 309 |   |
| Subtype |                     |   |   |
| A        | 153                 | 286 | 53.5% |
| D        | 84                  | 286 | 29.4% |
| C/G      | 10                  | 286 | 3.5% |
| Circulating recombinant form | 23 | 286 | 8.0% |
| Unique recombinant form | 16 | 286 | 5.6% |
| PMTCT exposed |   |   |
| yes      | 16                  | 317 | 5.0% |
| no       | 266                 | 317 | 83.9% |
| unknown  | 35                  | 317 | 11.0% |
| Initial ART regimen |                 |   |   |
| NNRTI-based | 290             | 317 | 91.5% |
| triple NRTI | 15              | 317 | 4.7% |
| PI-based  | 12                  | 317 | 3.8% |
| Primary caregiver |   |   |
| mother   | 178                 | 317 | 56.2% |
| father   | 18                  | 317 | 5.7% |
| other    | 121                 | 317 | 38.2% |
| primary school or higher | 247 | 317 | 77.9% |
| Parental status |     |   |   |
| both alive | 152            | 255 | 59.6% |
| both deceased | 39      | 255 | 15.3% |
| Immunological status |   |   |
| Normal² | 109                 | 305 | 35.7% |
| Diminished³ | 154       | 305 | 50.5% |
| Immunodeficient⁴ | 42    | 305 | 13.8% |

1. Based on genotypic sensitivity score; 2. age < 5 and CD4% ≥ 25% or age ≥ 5 and CD4 count > 500; 3. age < 5 years and CD4% < 25% or age ≥ 5 years and CD4 count < 500; 4. age < 5 years and CD45 < 10% or age ≥ 5 years and CD4 count < 100
317 children started first-line treatment
309 VL results
292 VL>1000 cps/ml
278 sequence results

294 children on first-line treatment after 6 months
239 VL results
48 VL>1000 cps/ml
39 sequence results

287 children on first-line treatment after 12 months
259 VL results
62 VL>1000 cps/ml
51 sequence results

276 children on first-line treatment after 18 months
246 VL results
57 VL>1000 cps/ml
47 sequence results

261 children on first-line treatment after 24 months
251 VL results
72 VL>1000 cps/ml
61 sequence results

Deceased: 8
Lost to follow-up: 3
Transfer out: 12
Switch to second-line: 0

Deceased: 3
Lost to follow-up: 2
Transfer out: 1
Switch to second-line: 1

Deceased: 2
Lost to follow-up: 3
Transfer out: 2
Switch to second-line: 4

Deceased: 0
Lost to follow-up: 4
Transfer out: 9
Switch to second-line: 2

Figure 1. Follow-up of children in this cohort
VL: viral load

mutations were detected (Figure 2). Table 3 shows predictors of acquired drug resistance in the multivariable model. Children with pretreatment drug resistance had significantly higher odds to develop acquired drug resistance (aOR: 3.58, 95% CI 1.35-9.51; p=0.010). The other predictors for acquired drugs resistance included higher VL before treatment initiation (aOR 2.16, 95% CI:1.46-3.22; p<0.001) and WHO stage 2 compared to stage 1 (aOR 10.14, 95% CI: 1.12-91.71; p=0.039).
Table 2. Factors associated with virological failure among children in this cohort

|                  | Univariable | Multivariable |
|------------------|-------------|---------------|
|                  | VF OR 95%CI | p             | OR 95%CI | p |
| age              |             |               |
| ≥3 years         | 59/192      | 1             |           |   |
| <3 years         | 33/95       | 1.2 0.71-2.02 | 0.494     |   |
| sex              |             |               |
| Male             | 53/145      | 1             |           |   |
| Female           | 39/142      | 0.66 0.40-1.08 | 0.100     |   |
| WHO stage at treatment initiation |           |               |           |   |
| 1                | 2/24        | 1             |           |   |
| 2                | 22/57       | 6.91 1.48-32.34 | 0.014 | 10.3 1.41-75.56 | 0.022 |
| 3                | 43/148      | 4.50 1.01-20.0 | 0.048 | 3.5 0.49-24.92 | 0.212 |
| 4                | 25/56       | 8.33 1.79-38.79 | 0.007 | 3.5 0.51-28.82 | 0.193 |
| Activity of first-line regimen¹ |           |               |           |   |
| Fully active regimen | 69/240 | 1             |           |   |
| Partially active regimen | 15/20 | 8.9 2.9-27.8 | <0.001 | 15.25 3.77-61.67 | <0.001 |
| Baseline VL (log) |           |               |           |   |
| >95%             | 65/215      | 1             |           |   |
| ≤95%             | 27/72       | 1.38 0.79-2.42 | 0.254 | 1.97 0.95-4.12 | 0.070 |
| NNRTI used       |             |               |           |   |
| Nevirapine       | 61/164      | 1             |           |   |
| Efavirenz        | 25/104      | 0.53 0.31-0.92 | 0.023 |   |
| PMTCT exposed    |             |               |           |   |
| Yes              | 4/15        | 1             |           |   |
| No               | 80/241      | 1.37 0.42-4.43 | 0.603 |   |
| Immunological status |         |               |           |   |
| Normal²          | 29/106      | 1             |           |   |
| Diminished³      | 44/137      | 1.26 0.72-2.19 | 0.423 |   |
| Immunodeficient⁴ | 16/34       | 2.36 1.06-5.24 | 0.035 |   |

Analysis is corrected for study site (Kampala, Fort Portal or Mbale). 1. based on calculation of genotypic sensitivity score (GSS); 2. age<5 and CD4%≥25% or age≥5 and CD4count>500; 3. age <5 years and CD4% <25% or age ≥5 years and CD4 count<500; 4. age<5 years and CD4count<10% or age ≥5 years and CD4 count<100; NNRTI: non-nucleoside reverse transcriptase; PMTCT: prevention of mother-to-child transmission; OR: odds ratio; VF: virological failure; VL: viral load; 95% CI: 95% confidence interval

**DISCUSSION**

This study of 317 ART-naïve Ugandan HIV-infected children evaluated the effect of pretreatment drug resistance on virological outcomes and on developing acquired drug resistance. Pretreatment drug resistance was common as it was observed in one out of...
every six ART-naïve children starting ART. This has important consequences, as a third of all children failed on first-line ART within two years and pretreatment drug resistance was the most important predictor for this failure. Children with pretreatment drug resistance were more than 15 times as likely to experience virological failure compared to children who received a fully active regimen. In addition, children with pretreatment drug resistance were 3 times more likely to acquire additional drug resistance mutations.

The effects of pretreatment drug resistance on treatment outcomes we found, confirm results mainly from adult cohorts on treatment for 6-18 months in developed countries and developing countries. The results also indicate that the effect of pretreatment drug resistance on treatment outcomes might be even stronger in children than in adults as we found the odds ratio of failure was 15.3 in children with pretreatment drug resistance, while a large cohort study in adults previously conducted by our research group showed an odds ratio of 2.1. The odds ratio for acquired drug resistance was 3.6 in our study, compared to 2.3 in adults.

Selecting an initial regimen has longstanding consequences for future therapy. Hence, in order to provide optimal treatment and enhance effectiveness and benefits of ART, international ART guidelines recommend individualization of initial regimens according to resistance testing results. In resource-limited settings, however, individual resistance testing before treatment initiation is not (yet) feasible in routine care due to costs.
and complexity. In the context of a public health approach in these settings, the WHO ART guidelines recommend ritonavir-boosted lopinavir (LPV/r)-based regimens for all children ≤3 years of age irrespective of PMTCT exposure\textsuperscript{29}. This recommendation is based on evidence of the superiority of a LPV/r-based regimen for infants and young children\textsuperscript{26,49} and studies that have demonstrated compromised response to nevirapine-containing first-line ART in children exposed to NNRTI used for PMTCT\textsuperscript{103,104}. Current Ugandan guidelines\textsuperscript{105}, however, still recommend NNRTI-based regimens as the preferred first-line treatment for PMTCT-unexposed children. In Uganda and other developing countries where genotypic testing is not routinely available or feasible, first-line regimens containing boosted-PI should be implemented for all children ≤3 years as recommended by WHO\textsuperscript{29}. In our study,

|            | Univariable | Multivariable |
|------------|-------------|---------------|
|            | ADR (n/N)   | OR      | 95% CI | p      | OR      | 95% CI | p      |
| age        |             |         |        |        |         |        |        |
| ≥3 years   | 48/183      | 1       |        |        |         |        |        |
| <3 years   | 24/95       | 0.95    | 0.54-1.68 | 0.862 |         |        |        |
| sex        |             |         |        |        |         |        |        |
| Male       | 43/140      | 1       |        |        |         |        |        |
| Female     | 29/138      | 0.60    | 0.35-1.03 | 0.066 |         |        |        |
| WHO stage  |             |         |        |        |         |        |        |
| at baseline|             |         |        |        |         |        |        |
| 1          | 1/20        | 1       |        |        |         |        |        |
| 2          | 17/59       | 7.69    | 0.95-62.07 | 0.056 | 10.14   | 1.12-91.71 | 0.039 |
| 3          | 35/145      | 6.05    | 0.78-46.80 | 0.085 | 6.37    | 0.73-55.90 | 0.095 |
| 4          | 19/54       | 10.31   | 1.28-83.14 | 0.028 | 6.19    | 0.68-56.68 | 0.107 |
| Activity of first-line regimen\textsuperscript{1} |             |         |        |        |         |        |        |
| Fully active regimen | 61/256 | 1       |        |        |         |        |        |
| Partially active regimen | 11/22 | 3.20 | 1.32-7.74 | 0.010 | 3.58 | 1.35-9.51 | 0.010 |
| Baseline VL (log) |             | 1.77 | 1.25-2.52 | 0.001 | 2.16 | 1.46-3.22 | <0.001 |
| Adherence  |             |         |        |        |         |        |        |
| >95%       | 54/208      | 1       |        |        |         |        |        |
| <=95%      | 18/70       | 0.99    | 0.53-1.83 | 0.967 | 1.46    | 0.69-3.07 | 0.325 |
| NNRTI used |             |         |        |        |         |        |        |
| Nevirapine | 47/148      | 1       |        |        |         |        |        |
| Efavirenz  | 19/105      | 0.47    | 0.26-0.87 | 0.016 |         |        |        |
| PMTCT exposed |             |         |        |        |         |        |        |
| yes        | 2/14        | 1       |        |        |         |        |        |
| no         | 64/232      | 2.29    | 0.50-10.50 | 0.288 |         |        |        |

Analysis is corrected for study site (Kampala, Fort Portal or Mbale). 1. based on calculation of genotypic sensitivity score (GSS); ADR: acquired drug resistance; NNRTI: non-nucleoside reverse transcriptase; PMTCT: prevention of mother-to-child transmission; OR: odds ratio; VL: viral load; 95% CI: 95% confidence interval.
only 12 children started a PI-based first-line regimen, of which 1 experienced treatment failure. It was not possible to assess the association of drug class with virological failure or acquired drug resistance, because the number of children that started PI-based ART was too small. However, given that the presence of NNRTI-associated mutations, resulting in a partially active first-line regimen, was associated with first-line failure, it is expected that a fully-active regimen, such as PI-based ART, might have prevented treatment failure. Current WHO guidelines only recommend PI-based first-line ART for children <3 years of age. However, the high rate of pretreatment drug resistance and its association with treatment failure we found in our cohort of older children (median age 4.9 years) suggest that PI-based first-line ART for children >3 years might need to be considered as well. Another potential alternative to NNRTI-based first-line treatment could be the use of integrase inhibitors. The most recent WHO guidelines recommend the use of the integrase inhibitors raltegravir and dolutegravir as part of an alternative first- or second-line treatment for children and adolescents. Potential advantages of integrase inhibitors include a high genetic barrier (dolutegravir), few side effects and few drug-drug interactions. Dolutegravir is currently only approved for use in adolescents aged ≥12 years, but studies in younger children are ongoing. Although availability of integrase inhibitors in low- and middle-income countries is still very limited, this drug class might represent a promising pediatric treatment option in the future.

Long-term treatment success requires maximizing effectiveness of first-line therapy and minimizing the emergence of resistant virus. The findings from this study further support the need for children to be initiated on at least three fully active antiretroviral drugs to ensure long-term effectiveness of first-line ART. Despite expanded access to ART in Uganda and other developing countries, pre-therapy genotypic resistance testing is generally not available for clinical use. We observed that children with pretreatment drug resistance were often prescribed first-line regimens to which the virus was not fully susceptible. Especially in the absence of access to tests to determine pretreatment drug resistance and without the widespread use of alternatives to NNRTI-based first-line ART, children need to be monitored closely using VL to determine treatment failure, prompting timely switch to a second-line regimen.

The major strength of our study is that this is the largest pediatric prospective cohort study in Africa that has evaluated the effect of pretreatment drug resistance on treatment response and acquired drug resistance. The study participants are representative of children seeking HIV care in treatment programs from three regions of the country. Our study also has potential limitations. First, there was no testing for minority mutations. Most
children in this cohort were perinatally infected with HIV, but the median age at study entry was 4.9 years. Therefore, it is possible that we have underestimated the prevalence of pretreatment drug resistance and its effect on treatment outcomes as mutations might be archived in older children in the absence of selective drug pressure. Furthermore, data were missing on outcome of virological response and acquired drug resistance for 30 children (9.5%) because of death in the first 6 months of treatment, transfer out and loss to follow-up. However, this rate is lower than observed in other observational treatment cohorts in sub-Saharan Africa\textsuperscript{107,108}. The rate of pretreatment drug resistance in the group with missing data was similar to the children with VL data so it is expected that the relationship with failure would not change. Finally, we could not analyze the effect of drug class (NNRTI, PI, or triple NRTI) on virological failure or acquired drug resistance, because the numbers of children who were not on a NNRTI-based regimen were too small.

In conclusion, in the largest study evaluating the effect of pretreatment drug resistance in children in Africa, we found that pretreatment drug resistance is common and strongly associated with virological failure and acquired drug resistance. This emphasizes the need for at least three fully active antiretroviral drugs and underlines that, in the absence of testing for pretreatment drug resistance, children ≤3 years should be initiated on a PI-based regimen as per WHO guidelines.

**Acknowledgements**

We would like to thank all study participants and their caregivers, doctors and nurses, and support staff at JCRC and AIGHD: Annet Nandudu, James Nkalubo, Isaac Egau, and Lincoln Mugarura (JCRC Kampala); Michael Owor, Christine Matama, and Florence Nambaziira (JCRC Fort Portal); Mary Abwola, Fred Senono, Ronald Namisi, and Sylvia Nakusi (JCRC Mbale); Cees Hesp, John Dekker (PharmAccess); Corry Manting, Desiree Lathouwers, Nadine Pakker, Bram Prins, Elske van Schijndel, Marloes Nijboer (AIGHD-Amsterdam); Cathy Nalubwama, Martin Omello (AIGHD-Kampala).

**Funding**

This work was supported by the European & Developing Countries Clinical Trials Partnership and the NACCAP program of NWO WOTRO.

**Disclosure Statement**

We have no competing interests to declare.