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Drug resistance in HIV-infected children living in rural South Africa: Implications of an antiretroviral therapy initiated during the first year of life

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ABSTRACT

Introduction: Management of antiretroviral-drug resistance in HIV-infected children is a global health concern. We compared the long-term virological outcomes of two cohorts of children living in a rural setting of South Africa. The first cohort initiated treatment before one year and the second after two years of age. The aim of this study was to describe the long-term consequences of early treatment initiation in terms of viral load and drug-resistance.

Methods: This retrospective study was conducted at the Edendale Hospital located in a peri-urban area of KwaZulu-Natal. Children were included during their planned appointment. Drug resistance was assessed genotypically on proviral DNA.

Results: From the 161 children included in this study, 93 samples were successfully genotyped. Both cohorts had comparable viral loads, but children treated early more often presented NRTI or NNRTI mutations, while there was no difference for PI mutations rates.

Conclusions: Treatment was highly effective when comparing virological outcomes in both early- and late-treated cohorts. The persistence of NNRTI mutations could lead to treatment failures in children older than 3 years initiating their therapy with a NNRTI, or for those switching from a PI to NNRTI based regimen. The accumulation of NRTI mutations may lead to a functional PI monotherapy and consequently to viral escape. To promote access to HIV genotyping in resource-limited settings is challenging but essential to avoid inappropriate therapy switches in case of virological failure, and to adapt national treatment guidelines in line with the epidemiology of resistance.

1. Introduction

Between 2000 and 2015, the number of children with access to combined antiretroviral therapy (cART) increased worldwide from 14.900 to 872.500 [1]. cART have transformed pediatric HIV from a rapidly fatal illness to a chronic disease but children stay at higher risk to develop treatment failure due to inappropriate drug formulations, adherence difficulties, antiretroviral toxicity or viral resistance [2–4].

Virological failure (VF) during first line cART occurs in 11 %–19 % of South African children [5–7]. Before changing to a second line cART, national guidelines recommend assessing and improve adherence but effective strategies are not always available in resource limited settings (RLS) [8–11]. Because they put current cART regimens at risk and drive up the cost of treatment, HIV drug resistances (HIVDR) testing should also be proposed. However, providing first-line HIV-1 genotypic resistance testing outside the level of provincial or national laboratories remains a challenge in most of RLS [8,10,12–14].

A few studies used genotyping to determine the level of HIVDR in South African children [6,15–22]. The present article aims to describe the long-term virological outcomes of two cohorts of children living in a rural setting of SA. The first initiated cART before one year and the second after two years of age depending on their immunological or clinical status.

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2. Methods

This retrospective study was conducted in SA between September and December 2014 at the Edendale Regional Hospital located in a peri-urban and RLS of KwaZulu-Natal.

Children were included during their planned appointment at the HIV clinic. All of them were treated with cART and followed by the local staff. They came from the same area and had the same socio-economic environment.

Children were divided in two cohorts depending on their age at treatment initiation: less than 1 year and more than 2 years of age in the Early Starters Cohort (ESC) and the Late Starters Cohort (LSC) respectively. Children in the ESC were included in a previous study about effectiveness of early cART initiation in RLS which took place between 2005 and 2008 [23]. They were younger than 1 year of age, had positive HIV-1 DNA PCR, WHO stage 2–4 and/or CD4 + % < 30 % at cART initiation. Children from the LSC initiated cART in function of their WHO clinical stage, CD4 + % or comorbidities following the successive South African guidelines between 2005 and 2012.

2.1. Study procedures

Parents or caregivers gave a written informed consent prior to any procedure. Data were collected from the medical files. The study was approved by the ethical committees «Umgungundlovu Health District Review Board (UHERB)» and «Comité d'Ethique Hospitalo-Facultaire Saint Luc – UCLouvain».

2.2. Laboratory procedures

Described in annex 1.

2.3. Statistical analysis

Mean values of patient characteristics were compared with the Student *t*-test. Proportions were compared with the exact Fisher test (R statistic software).

2.4. Definitions used in this study

Undetectable viremia: < 50 copies/mL
Low-level viremia: 50–1000 copies/mL
High-Level viremia: > 1000 copies/mL
Virological failure: > 1000 copies/mL in 2 successive blood samples taken at least 6 months apart
High-level HIVDR: as defined by the Stanford HIV drug resistance database (https://hivdb.stanford.edu/hivdb/)

3. Results

In total, 161 blood samples from HIV-infected children were analyzed in this study; 55 in the ESC and 106 in the LSC.

Children included in the ESC were younger at treatment initiation and at study implementation in 2014. They had a longer duration of follow-up. Sex ratio was comparable in both cohorts but the number of patients treated by their mothers was higher in the ESC (Table 1).

Children from the ESC presented a higher proportion of undetectable viral loads in 2014. There was no significant difference for low or high-level viremia, and both cohorts share comparable rates of virological failure (Table 2).

From these 161 blood samples, 93 proviral DNAs were successfully genotyped: 29/55 (52.7 %) in the ESC and 64/106 (47.3 %) in the LSC. When compared to the whole cohort of 161, those 93 children differ in the percentage of children not treated by their mothers and for the rate of undetectable viral loads, but without reaching statistical significance.

Both cohorts were treated with a Nucleoside Reverse Transcriptase Inhibitor (NRTI) backbone (two drugs) and one Protease Inhibitor (PI) in the ESC or one Non-Nucleoside Reverse Transcriptase Inhibitor (NNRTI) for most children in the LSC. Five children (7.8 %) aged between 2 and 3 years included in the LSC started their treatment with a PI-based regimen as recommended by the South African guidelines (Table 3).

A large proportion of children from the ESC and the LSC (100 % vs 57.8 % respectively), replaced Stavudine by another NRTI (Abacavir, Zidovudine or Tenofovir) due to revisions of the national recommendations. Switch to a second line due to VF was not significantly different between the ESC 3/29 (10.3 %) and the LSC 8/64 (12.5 %) (p = 1). In both cohorts one child changed therapy due to side effects. Treatments in 2014 are described in Table 3.

Children from the ESC had a significantly higher rate of high-level proviral HIVDR but also a lower proportion of children with no proviral mutations detected (Table 4). They more often presented any NRTI or NNRTI mutation. There was no difference for PI mutations rate between

Table 1
Cohorts' characteristics during the follow-up period.

	ESC (55)	LSC (106)	p value
Mean age at treatment initiation (months)	8.6 (2.2–11.9)	54 (24–91.2)	
Mean age in 2014 (years)	8.2 (6.9–10.2)	10.8 (6.0–14.6)	< 0.001
Mean treatment duration (years)	7.6 (6.5–9.5)	6.0 (2.0–9.5)	< 0.001
Mother is caregiver	18 (32.7 %)	53 (50.0 %)	0.0045
Females	23 (41.8 %)	48 (45.3 %)	0.86

ESC: Early starters cohort, LSC: Late starters cohort.
The values in parentheses represent the lowest and the highest range for each parameter.

Table 2
Virological outcomes in the ESC and the LSC in 2014.

	ESC (55)	LSC (106)	p-value
Undetectable VL	48 (87.0 %)	76 (71.7 %)	0.029
Low-level viremia	4 (7.5 %)	13 (12.3 %)	0.42
High-level viremia	0	4 (3.7 %)	0.30
Virological failure	3 (5.5 %)	13 (12.3 %)	0.27

ESC: Early starters cohort, LSC: Late starters cohort, VL: viral load.
All the children with virological failure had a high-level viremia which is not reported in this table.

Table 3
Treatments at cART initiation and in 2014 in the ESC and the LSC.

PI/NNRTIs	NRTIs	ESC (29)	LSC (64)
Treatments at cART initiation			
Lopinavir/ritonavir	Stavudine + Lamivudine	27	5
Lopinavir/ritonavir	Zidovudine + Lamivudine	2	/
Efavirenz	Stavudine + Lamivudine	/	44
Efavirenz	Abacavir + Lamivudine	/	15
Treatments in 2014			
Lopinavir/ritonavir	Abacavir + Lamivudine	21	6
Lopinavir/ritonavir	Zidovudine + Lamivudine	3	5
Lopinavir/ritonavir	Tenofovir + Lamivudine	1	1
Lopinavir/ritonavir	Abacavir + Zidovudine	/	2
Efavirenz	Abacavir + Zidovudine	1	/
Efavirenz	Abacavir + Lamivudine	2	42
Efavirenz	Tenofovir + Lamivudine	/	1
Efavirenz	Stavudine + Lamivudine	/	7
Efavirenz + Lopinavir/ritonavir	Abacavir + Zidovudine	1	/

NRTI: Nucleoside Reverse Transcriptase Inhibitor, NNRTI: Non-Nucleoside Reverse Transcriptase Inhibitor, PI: Protease Inhibitor, ESC: Early starters cohort, LSC: Late starters cohort.

Table 4
HIV drug resistance in the ESC and the LSC in 2014.

	ESC (29)	LSC (64)	p-value
High-level HIVDR	16 (55.2 %)	14 (21.9 %)	0.032
Low/Intermediate level HIVDR	0	5 (8.9 %)	0.32
No drug resistance mutation	13 (44.8 %)	45 (69.2 %)	0.023

ESC: Early starters cohort, LSC: Late starters cohort, HIVDR: HIV drug resistance.
Some children with high-level HIVDR had also low or intermediate level HIVDR, which is not reported in this table.

Table 5
Proviral mutations in the ESC and the LSC in 2014.

	ESC (29)	LSC (64)	p-value
Any NRTI mutation	10 (34.5 %)	9 (14.1 %)	0.030
NRTI high-level HIVDR	9 (31.0 %)	9 (14.1 %)	0.087
41 L	1	1	
67N	0	1	
70R	0	1	
74I	1	0	
115F	1	0	
184I/V	**9**	**9**	
215Y	0	1	
219E/219N	1	1	
TAMs	2	4	
Any NNRTI mutation	13 (44.8 %)	13 (20.3 %)	0.024
NNRTI high-level HIVDR	9 (31.0 %)	11 (17.2 %)	0.17
98G	0	3	
103N	**2**	**8**	
106M	2	2	
108I	0	2	
138A	2	0	
179D	2	0	
181C	**5**	**1**	
188C/H	1	1	
190A	0	2	
221Y	0	2	
225H	0	3	
227L	1	0	
230	1	0	
Any PI mutation	3 (10.3 %)	9 (14.1 %)	0.75
PI high-level HIVDR	1 (3.4 %)	2 (3.1 %)	1
15V	1	0	
30N	1	0	
33F	0	1	
36I	1	0	
46L/I	**0**	**6**	
50V	0	1	
54V	0	1	
58E	0	1	
69K	1	0	
73S	1	1	
74S	1	0	
82A/T	0	2	
83D	1	0	
Multiclass high-level HIVDR	3 (10.3 %)	7 (10.9 %)	0.75

both cohorts. Children from the ESC had higher rates of NRTI and NNRTI high-level HIVDR, but this difference was not significant. Proportion of children with multiclass high-level resistance was comparable in both cohorts (Table 5).

In the ESC and the LSC, the most frequent NRTI mutation was M184 V/I and the most frequent NNRTI mutations were Y181C and K103 N respectively. In the LSC, M46 L was the most frequent PI mutation (Table 5).

Among the ESC cohort, 13 out of 29 children who initiated a PI-based regimen had viruses with NNRTI resistance mutations, among which 9 were fully resistant to NNRTI. Interestingly, only 2/9 (22 %) were treated with NNRTI in a second line therapy. In the LSC, 9 children had a PI HIVDR and 2 a high-level HIVDR. One of these 2 children was treated with PI.

Among children that switched to a second line therapy due to VF, 1 out of 3 children in the ESC and 6 out of 8 children in the LSC switched to a second line therapy without any high-level HIVDR.

4. Discussion

Management of HIVDR in adults and children is a global health concern. Prevention, monitoring and response to HIV drug resistances are essential to achieve the 2020 UNAIDS targets that include diagnosing 90 % of people with HIV infection, providing treatment to 90 % of those diagnosed and ensuring that 90 % of people on treatment achieve virological suppression.

In 2010, the WHO recommended an immediate start of cART in all patients diagnosed with HIV before 2 years of age [24]. This recommendation was extended to children younger than 5 years of age in 2013 [25] and to all children and adults in 2015 [26]. Benefits of early treatment initiation on infant mortality [27,28], neurodevelopmental outcome [29], growth recovery [30], immunologic restoration and virological suppression [31,32], have been demonstrated in short term follow-up studies implemented in RLS.

Knowing the major benefits of an early treatment initiation in infants and the growing efforts to diagnose HIV as early as possible in life, most of the future HIV infected children should initiate treatment in infancy.

Our results monitor HIVDR in children living in a RLS of sub-saharan Africa. Children treated before one year of age with a PI-based regimen, as recommended by the international guidelines, had at least comparable virological outcomes in terms of undetectable VL, low and high-level viremia and VF than children treated after two years of age with a NNRTI based regimen. Despite this favorable outcome, children from the ESC had surprisingly higher rates of NRTI and NNRTI HIVDR.

Higher NRTI mutations rates in the ESC may be explained by different factors: early treatment initiation complications, longer duration of cART and transmitted drug resistances. First, children from the ESC were dependent on their caregiver to take their treatment during a longer period of time because they initiated cART earlier in life. The caregiver's biologic relationship to the child was associated with better adherence in some studies [33,34] and worse adherence in another [35]. Caregivers who are also on ART may draw from their own experiences to support their child's adherence [36] and non-adherent HIV infected caregivers have more non-adherent children [37]. Presence of a unique caregiver [35] and caregiver's age between 25 and 44 was associated with better adherence [38]. Treatment initiation early in life is associated to other risk factors of suboptimal cART administration. Emesis is a common cause of under-dosing for infants because re-dosing is not always done [39]. Low-level drug concentration in children's blood due to rapid growth [40], drug interaction with rifampicin [41], inadequate intake due to poor drug palatability or unadapted formulations are involved [42,43]. Finally, transport costs to the medical center or the need for a refrigerator to store some drugs may also lead to treatment failures. Second, despite them being younger in 2014, children from the ESC had a longer duration of therapy, which is associated with decreased adherence and increased risk of resistance [44]. Moreover, infants and young children tend to be maintained a longer time than adults on failing regimens because of difficulties with adherence and limited treatment options [45]. Finally, the presence of HIVDR, acquired from the mother during pregnancy, delivery or breastfeeding may explain the higher rate of NRTI mutations in the ESC.

Higher NNRTI mutation rates in the ESC could only be explained by the presence of resistant virus, acquired from their mothers or during prevention of the mother to child transmission (PMTCT) with NVP, before cART initiation. In case of failure of this prevention, infants are at high risk to develop NNRTI resistances [46–48], which could lead to treatment failure of NNRTI based treatment during the next years. Pre-

treatment resistances were certainly present in both cohorts. Among the 9 patients with high-level NNRTI HIVDR in the ESC, only 2 (22 %) were treated with a NNRTI. The 7 others acquired for sure a resistant virus from their mothers or during the PMTCT. NNRTI pre-drug resistance (PDR) is dramatically increasing in RLS. A recent meta-analysis about pretreatment HIVDR in children living in sub-saharan Africa described a rate of 42.7 % of PDR among PMTCT-exposed children and 12.7 % among PMTCT-unexposed children [49]. However, some authors stated that viral resistance selected by prior exposure to single-dose NVP might wane with time and allow switching to a NNRTI based regimen [50]. Consequently, some studies have pleaded for a switch to a NNRTI based regimen in children virologically suppressed under PI based regimen [51,52] due to reduced treatment costs, less metabolic toxicities and limited second line options. But in our study, switching from a PI to a NNRTI based regimen could have led to VF for at least 7/29 (24 %) children included in the ESC, due to presence of persistent primary NNRTI mutations. Long-lived NNRTI-resistant viruses were also described in a prospective South African Study including children who failed HIV-prophylaxis. NNRTI-resistant HIV persisted in 26/27 (96 %) of children with a median age of 21 month, which suggests that resistance will likely persist through 36 months of age, when children qualify for NNRTI-based ART [53]. Our results, added to those of other studies [52,54], encourage a close monitoring of viral load in all children, irrespective of their PMTCT exposition when switching therapy. The current first-line ART regimen with a NNRTI for children older than 3 years could also be revised due to a prevalence of 24 % of NNRTI PDR in our ESC. In fact, WHO recommends countries to consider changing their first-line regimens if levels of PDR reach 10 % [55].

The switch to a second line therapy because of VF occurred in 1/3 of children from the ESC and 6/8 of children from the LSC without any HIVDR. This highlights the difficulties to assess adherence of children despite the presence of experienced counselors. In case of recurrence of VF, most of these children will not be able to access a third line therapy due to the unavailability of the newest drugs in RLS. Genotyping allows assessing the need to switch to another treatment in case of first- or second-line failure in children with suspected suboptimal adherence. Decentralized genotypic resistance testing at least at provincial level and training for clinicians to interpret results of genotyping is an increasing need in RLS [14].

In terms of proviral mutation prevalence, 18/93 (19.4 %) children harbored the M184 V NRTI mutation, 11/93 (11.8 %) the K103 N NNRTI mutation and 3/93 (3.2 %) harbored high-level PI HIVDR. Another study published in 2014, analyzing HIVDR in children at VF in the KwazuluNatal [22] also described M184 V and K103 N as the most prevalent NRTI and NNRTI mutations. Only 1 out of 84 (1.2 %) child had one major PI resistance. This low rate of PI-resistance probably explains the comparable virological outcomes in the ESC and the LSC despite a higher rate of NRTI and NNRTI in the ESC. Previous studies demonstrated the effectiveness of LPV/r in monotherapy to treat HIV infected people even after firs-line cART failure in RLS [47,48,56,57]. However, LPV/r monotherapy demonstrates lower rates of virological suppression when compared with LPV/r based triple-ARV regimen and therefore should not be considered as a preferred treatment option for widespread use in antiretroviral-naive patients or for second line-therapies [57,58].

This retrospective study has some limitations. We excluded all rapid progressor-infants who died before treatment initiation in the LSC. This could underestimate the rate of HIVDR in the LSC. The real rate of PDR could be an underestimate, because we couldn't assess the rate of NNRTI PDR in children from the LSC who had NNRTI as part of their cART. We could not determine for sure if cells from the ESC children more often showed NRTI and NNRTI proviral mutations as a consequence of the early treatment initiation or if children were infected with a resistant virus transmitted from their mothers, because we had no pre-treatment viral genotyping available. Due to the high maternal mortality rate and the lack of maternal medical files, we couldn't assess if the mothers benefited of cART during pregnancy or breastfeeding which could explain a higher rate of HIVDR. One technical limitation of our nested-PCR protocol prior to the sequencing reaction itself is the low DNA copy number input. We could speculate that children from the ESC cohort were treated when the viral diversity was lower compared to the LSC cohort, therefore enabling an easier detection of resistant variant proviruses. Finally, we sequenced proviruses because most of the children had undetectable VL. The proviruses represent all the viral genomes included in the human cells but not all replicative viruses, which are better detected with viral RNA. Therefore proviral DNA sequencing can pick up some hypermutated defective viruses, introducing the theoretical bias of detecting mutations at codons involved in drug-resistance that were introduced due to the APOBEC activity in cells rather than selected because of incomplete VL suppression under drug pressure. As the number of substitutions compared to reference strains was low in our sample set, that effect have few consequences if any on the global patients' cohort results. Proviruses HIVDR are thus interesting from an epidemiological point of view to assess the risk of HIVDR in the general population before VF occurs.

5. Conclusions

The recommendation to treat all HIV-infected children as early as possible will increase the number of infants under lifelong treatment. The present study underscored the long-term benefits of cART as both cohorts were virologically suppressed.

However, it must be emphasized that proviruses sequenced from children included in the ESC more often harbored NRTI and NNRTI mutations due to multiple factors as their young age at treatment initiation, longer duration of therapy and pre-existing drug resistances.

The long-term persistence of NNRTI mutation is a risk factor of treatment failure in case of NNRTI initiation even after 3 years of age. Guidelines for cART initiation and switch should consider this limit in RLS and assess the possibility to introduce other classes of antiretroviral drugs like integrase inhibitors. Moreover, higher rates of NRTI mutations in the ESC could lead to a functional PI-monotherapy, which is a risk factor for viral escape.

Better access to HIV genotyping in RLS will be essential in the next years to avoid unnecessary therapy switches in case of VF or to assess susceptibility to NRTI and NNRTI as part of the cART.

Authors' contributions

JCB, PG, JR and DVDL participated to the redaction of the questionnaires, interpretation of the results and redaction of the article. JCB recruited the patients in the HIV family clinic. All authors have read and approved the final manuscript.

Declaration of Competing Interest

The authors have no conflict of interest to declare.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jcv.2020.104547.

References

[1] World Health Organization. Number of children (15 years) receiving ART globally, and by WHO region, 2000–2015. (http://www.who.int/hiv/data/pedartregions2016.png?ua=1).

[2] M. Brady, J. Oleske, P. Williams, P. Elgie, L. Mofenson, W. Dankner, et al., Declines in mortality rates and changes in causes of death in HIV-1-infected children during the HAART era, J. Acquir. Immune Defic. Syndr. 53 (1) (2010) 86–94 Jan 1.

[3] D. Wamalwa, D. Lehman, S. Benki-Nugent, M. Gasper, R. Gichohi, E. Maleche-Obimbo, et al., Long-term virologic response and genotypic resistance mutations in HIV-1 infected Kenyan children on combination antiretroviral therapy, J. Acquir. Immune Defic. Syndr. 62 (3) (2013) 267–274 Mar 1.

[4] M. Penazzato, A.J. Prendergast, L.M. Muhe, D. Tindyebwa, E. Abrams, Optimisation of antiretroviral therapy in HIV-infected children under 3 years of age, Cochrane Database Syst. Rev. 5 (2014) May 22.

[5] M.A. Davies, H. Moultrie, B. Eley, H. Rabie, G. Van Cutsem, J. Giddy, et al., Virologic failure and second-line antiretroviral therapy in children in South Africa - the IeDEA Southern Africa Collaboration, J. Acquir. Immune Defic. Syndr. 56 (3) (2011) 270–278 Mar 1.

[6] T. Meyers, S. Sawry, J. Wong, H. Moultrie, F. Pinillos, L. Fairlie, et al., Virologic failure among children taking lopinavir/ritonavir-containing first-line antiretroviral therapy in South Africa, Pediatr. Infect. Dis. J. 34 (2) (2015) 175–179 Feb.

[7] J.C. Beghin, J. Ruelle, P. Goubau, M. Krishna, L. Hall, D. Van der Linden, Virological and immunological long-term outcome of human immunodeficiency virus-1 infected children treated before one year and after two years of age in a resource-limited setting of South Africa, J. AIDS Clin. Res. 8 (2017) 690.

[8] National Consolidated Guidelines for the Prevention of Mother to Child Transmission of HIV (PMTCT) and the Management of HIV in Children, Adolescent and Adults, National Department of Health, South Africa, 2015.

[9] WHO Library Cataloguing-in-Publication Data. Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection: recommendations for a public health approach – 2nd ed. June 2016. Available from: http://www.who.int/hiv/pub/arv/arv-2016/en/.

[10] E. Mills, S. Kanters, M.E. Socias, Global Evaluation Services. Systematic Literature Review Report: Which Interventions Enhance Adherence to ART for People Living with HIV? (2015) Web Supplement B.

[11] K. Jobanputra, L. Parker, C. Azih, V. Okello, G. Maphalala, B. Kershberger, et al., Factors associated with virological failure and suppression after enhanced adherence counselling, in children, adolescents and adults on antiretroviral therapy for HIV in Swaziland, PLoS One 10 (2) (2015) e0116144.

[12] Panel on Antiretroviral Therapy and Medical Management of HIV-Infected Children. Guidelines for the Use of Antiretroviral Agents in Pediatric HIV Infection. (http://aidsinfo.nih.gov/contentfiles/lvguidelines/pediatricguidelines.pdf).

[13] Paediatric European Network for Treatment of AIDS (PENTA) guidelines for treatment of paediatric HIV-1 infection 2015: optimizing health in préparation for adult life.

[14] R.J. Lessells, A. Avalos, T. de Oliveira, Implementing HIV-1 genotypic resistance testing in antiretroviral therapy programs in Africa: needs, opportunities, and challenges, AIDS Rev. 15 (4) (2013) 221–229 Oct-Dec.

[15] V. Pillay, C. Pillay, R. Kantor, F. Venter, L. Levin, L. Morris, HIV type 1 subtype C drug resistance among pediatric and adult South African patients failing antiretroviral therapy, AIDS Res. Hum. Retroviruses 11 (11) (2008) 1449–1454.

[16] C.L. Wallis, L. Erasmus, S. Varughese, D. Ndiweni, W.S. Stevens, Emergence of drug resistance in HIV-1 subtype C infected children failing the South African National Antiretroviral roll-out program, Paeiatric I nfectious Dis J. 11 (12) (2009) 1123–1125.

[17] G.U. van Zyl, L. van der Merwe, M. Claassen, M.F. Cotton, H. Rabie, H.W. Prozesky, et al., Protease inhibitor resistance in South African children with virologic failure, Pediatr. Infect. Dis. J. 11 (12) (2009) 1125–1127.

[18] O. Latinovic, J. Kuruppu, C. Davis, N. Le, A. Heredia, Pharmacotherapy of HIV-1 infection: focus on CCR5 antagonist maraviroc, Clin. Med. Ther. 11 (2009) 1497–1510.

[19] R.E. Barth, H.A. Tempelman, E. Smelt, A.M. Wensing, A.I. Hoepelman, S.P. Geelen, Long-term outcome of children receiving antiretroviral treatment in Rural South Africa, Pediatr. Infect. Dis. J. 11 (1) (2011) 52–56.

[20] B.S. Taylor, G. Hunt, E.J. Abrams, A. Coovadia, T. Meyers, G. Sherman, et al., Rapid development of antiretroviral drug resistance mutations in HIV-infected children less than two years of Age initiating protease inhibitor-based therapy in South Africa, AIDS Res. Hum. Retroviruses 11 (9) (2011) 945–956.

[21] T.N. Green, M. Archary, M.L. Gordon, N. Padayachi, Y. Lie, E.D. Anton, et al., Drug resistance and coreceptor usage in HIV type 1 subtype C-infected children initiating or failing highly active antiretroviral therapy in South Africa, AIDS Res. Hum. Retroviruses 11 (4) (2012) 324–332.

[22] S. Pillay, R.M. Bland, R.J. Lessells, J. Manasa, T. de Oliveira, S. Danaviah, Drug resistance in children at virological failure in a rural KwaZulu-Natal, South Africa, cohort, AIDS Res. Ther. 11 (1) (2014) 3 Jan 20.

[23] S.E. Purchase, D.J. Van der Linden, N.H. McKerrow, Feasibility and effectiveness of early initiation of combination antiretroviral therapy in HIV- infected infants in a government clinic of Kwazulu-Natal, South Africa, J. Trop. Pediatr. 58 (2012) 114–119.

[24] World Health Organization, UNAIDS, AIDS Epidemic Update 2010, WHO library Cataloguing-in-Publication Data, 2010.

[25] World Health Organization, UNAIDS, Consolidated Guidelines on the Use of Antiretroviral Drugs for Treating and Preventing HIV Infection: Recommendations for a Public Health Approach, WHO Library Cataloguing-in-Publication Data, 2013.

[26] World Health Organization, Guideline on When to Start Antiretroviral Therapy and on Pre-Exposure Prophylaxis for HIV, WHO Library Cataloguing- in-Publication Data, 2015.

[27] A. Violari, M.F. Cotton, D.M. Gibb, A.G. Babiker, J. Steyn, S.A. Madhi, et al., Early antiretroviral therapy and mortality among HIV-infected infants, N. Engl. J. Med. 359 (2008) 2233–2244.

[28] M. Penazzato, A.J. Prendergast, L.M. Muhe, D. Tindyebwa, E. Abrams, Optimisation of antiretroviral therapy in HIV-infected children under 3 years of age, Cochrane Database Syst. Rev. 22 (2014) 5.

[29] T. Puthanakit, J. Ananworanich, S. Vonthanak, P. Kosalaraksa, R. Hansudewechakul, J. van der Lugt, et al., Cognitive function and neurodevelopmental outcomes in HIV- infected Children older than 1 year of age randomized to early versus deferred antiretroviral therapy: the PREDICT neurodevelopmental study, Pediatr. Infect. Dis. J. 32 (2013) 501–508.

[30] S. Shiau, S. Arpadi, R. Strehlau, L. Martens, F. Patel, A. Coovadia, et al., Initiation of antiretroviral therapy before 6 months of age is associated with faster growth recovery in South African children perinatally infected with human immunodeficiency virus, J. Pediatr. 162 (2013) 1138–1145.

[31] C.G. Sutcliffe, J.H. van Dijk, C. Bolton, D. Persaud, W.J. Moss, Effectiveness of antiretroviral therapy among HIV-infected children in sub-Saharan Africa, Lancet Infect. Dis. 8 (2008) 477–489.

[32] A. Reddi, S.C. Leeper, A.C. Grobler, R. Geddes, K.H. France, G.L. Dorse, et al., Preliminary outcomes of a paediatric highly active antiretroviral therapy cohort from KwaZulu-Natal, South Africa, BMC Pediatr. 7 (2007) 13.

[33] V. Giacomet, F. Albano, F. Starace, A. de Franciscis, C. Giaquinto, G.C. Gattinara, et al., Adherence to antiretroviral therapy and its determinants in children with human immunodeficiency virus infection: a multicentre, national study, Acta Paediatr. 92 (2003) 1398–1402.

[34] Sl Marhefka, Vj Tepper, Jl Brown, Jj Farley, Caregiver psychosocial characteristics and children's adherence to antiretroviral therapy, AIDS Patient Care STDS 20 (2006) 429–437.

[35] N. Elenga, F. Rouet, R. Laguide, K.A. Kouakoussui, M. Timite, et al., Highly active antiretroviral therapies among HIV-1-infected children in Abidjan, Côte d'Ivoire, AIDS. 18 (2004) 1905–1913.

[36] J. Haberer, C. Mellins, Pediatric adherence to HIV antiretroviral therapy, Curr. HIV/AIDS Rep. 6 (4) (2009) 194–200 Nov.

[37] G. Ricci, E.M. Netto, E. Luz, C. Rodamilans, C. Brites, Adherence to antiretroviral therapy of Brazilian HIV-infected children and their caregivers, Braz. J. Infect. Dis. 20 (5) (2016) 429–436 Sep–Oct.

[38] T. Eticha, L. Lwam Berhane, Caregiver-reported adherence to antiretroviral therapy among HIV infected children in Mekelle, Ethiopia, . BMC Pediatr. 14 (2014) 114.

[39] E.N. Menson, A.S. Walker, M. Sharland, C. Wells, G. Tudor-Williams, F.A. Riordan, et al., Collaborative HIV Paediatric Study Steering Committee Underdosing of antiretrovirals in UK and Irish children with HIV as an example of problems in prescribing medicines to children, 1997–2005: cohort study, BMJ 332 (2006) 1183–1187.

[40] A. Cupsa, C. Gheonea, D. Bulucea, S. Dinescu, Factors with a negative influence on compliance to antiretroviral therapies, Ann. N. Y. Acad. Sci. 918 (2000) 351–354.

[41] H. Sunpath, P. Winternheimer, S. Cohen, I. Tennant, N. Chelin, R.T. Gandhi, et al., Double-dose lopinavir-ritonavir in combination with rifampicin-based anti-tuberculosis treatment in South Africa, Int. J. Tuberc. Lung Dis. 18 (6) (2014) 689–693 Jun.

[42] C.M. Rademaker, L. van Dijk, A.K. Mantel-Teeuwisse, Pediatric drug formulations: a review of challenges and progress, Pediatrics 134 (2) (2014) 361–372.

[43] A. Bagenda, L. Barlow-Mosha, D. Bagenda, R. Sakwa, M.G. Fowler, P.M. Musoke, Adherence to tablet and liquid formulations of antiretroviral medication for paediatric HIV treatment at an urban clinic in Uganda, Ann. Trop. Paediatr. 31 (3) (2011) 235–245.

[44] J.A. Bartlett, Addressing the challenges of adherence, JAIDS 29 (Suppl. 1) (2002) S2–S10.

[45] F. Fitzgerald, M. Penazzato, M. Penazzato, D. Gibb, Development of antiretroviral resistance in children with HIV in low- and middle-income countries, J. Infect. Dis. 207 (Suppl. 2) (2013) S85–92 Jun 15.

[46] K.C. Sigaloff, J.C. Calis, S.P. Geelen, M. van Vugt, T.F. de Wit, HIV-1- resistance-associated mutations after failure of first-line antiretroviral treatment among children in resource-poor regions: a systematic review, Lancet Infect. Dis. 11 (2011) 769–779.

[47] N. Kumarasamy, E. Aga, H.J. Ribaudo, C.L. Wallis, D.A. Katzenstein, W.S. Stevens, Lopinavir/ritonavir monotherapy as second-line antiretroviral treatment in resource-limited settings: week 104 analysis of AIDS clinical trials group (ACTG) A5230, Clin. Infect. Dis. 60 (10) (2015) 1552–1558 May 15.

[48] J.A. Bartlett, H.J. Ribaudo, C.L. Wallis, E. Aga, D.A. Katzenstein, W.S. Stevens, et al., Lopinavir/ritonavir monotherapy after virologic failure of first-line antiretroviral therapy in resource-limited settings, AIDS 26 (11) (2012) 1345–1354 Jul 17.

[49] K.C. Sigaloff, A.S. Akanmu, S. Inzaule, M. Boele van Hensbroek, T.F. Rinke de Wit, et al., Alarming increase in pretreatment HIV drug resistance in children living in sub-Saharan Africa: a systematic review and meta-analysis, J. Antimicrob. Chemother. 72 (2) (2017) 365–371 Feb.

[50] N.A. Martinson, D.K. Ekouevi, F. Dabis, L. Morris, P. Lupodwana, B. Tonwe-Gold, et al., Transmission rates in consecutive pregnancies exposed to single-dose nevirapine in Soweto, South Africa and Abidjan, Côte d'Ivoire, J. Acquir. Immune Defic. Syndr. 45 (2007) 206–209.

[51] A. Coovadia, E. Abrams, R. Stehlau, T. Meyers, L. Martens, G. Sherman, et al., Reuse of nevirapine in exposed HIV-Infected children after viral suppression with a protease inhibitor-based treatment regimen: a randomized controlled trial, JAMA

304 (10) (2010) 1082–1090 Sep 8.
[52] L. Kuhn, A. Coovadia, R. Strehlau, L. Martens, C.C. Hu, T. Meyers, et al., Switching children previously exposed to nevirapine to nevirapine-based treatment after initial suppression with a protease-inhibitor-based regimen: long-term follow-up of a randomised, open-label trial, Lancet Infect. Dis. 12 (7) (2012) 521–530 Jul.
[53] R. Kanthula, T.M. Rossouw, U.D. Feucht, G. van Dyk, I.A. Beck, R. Silverman, et al., Persistence of HIV drug resistance among South African children given nevirapine to prevent mother-to-child-transmission, AIDS 31 (8) (2017) 1143–1148 May 15.
[54] D.L. Dahourou, M. Amorissani-Folquet, K. Malateste, C. Amani-Bosse, M. Coulibaly, C. Seguin-Devaux, et al., Efavirenz-based simplification after successful early lopinavir-boosted-ritonavir-based therapy in HIV-infected children in Burkina Faso and Côte d'Ivoire: the MONOD ANRS 12206 non-inferiority randomised trial, BMC Med. 15 (1) (2017) 85. Apr 24.
[55] HIV Drug Resistance Report 2017, World Health Organization, Geneva, 2017.
[56] L.F. Lopez-Cortes, R. Ruiz-Valderas, E. Sánchez-Rivas, A. Lluch, A. Gutierrez-Valencia, A. Torres-Cornejo, et al., Lopinavir plasma concentrations and virological outcome with lopinavir-ritonavir monotherapy in HIV-1-infected patients, Antimicrob. Agents Chemother. 57 (8) (2013) 3746–3751. Aug.
[57] J.G. Hakim, J. Thompson, C. Kityo, A. Hoppe, A. Kambugu, J.J. van Oosterhout, et al., Lopinavir plus nucleoside reverse-transcriptase inhibitors, lopinavir plus raltegravir, or lopinavir monotherapy for second-line treatment of HIV (EARNEST): 144-week follow-up results from a randomised controlled trial, Lancet Infect. Dis. 18 (1) (2018) 47–57 Jan.
[58] J.F. Delfraissy, P. Flandre, C. Delaugerre, J. Ghosn, A. Horban, P.M. Girard, et al., Lopinavir/ritonavir monotherapy or plus zidovudine and lamivudine in antiretroviral-naive HIV-infected patients, AIDS 22 (3) (2008) 385–393 Jan 30.