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ARTICLE

Incorrectly diagnosing children as HIV-infected: Experiences from a large paediatric antiretroviral therapy site in South Africa

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Objective. To assess the extent to which children may be falsely diagnosed as HIV-infected, using data from an antiretroviral therapy (ART) site in Pretoria, South Africa.

Methods. This was a retrospective patient record review of all ART-naïve children referred to Kalafong hospital's paediatric HIV clinic between April 2004 and March 2010, with detailed review of those found to be HIV-uninfected.

Results. There were 1 526 patient files analysed, with a male-to-female ratio of 1.01:1 and median age at first visit of 20 months (range 26 days - 17.5 years). Nearly half (*n*=715; 47%) of the children were aged <18 months. Fifty-one children were found to be HIV-uninfected after repeated diagnostic tests. Incorrect laboratory results for children aged <18 months included false-positive HIV DNA PCR tests (40), detectable HIV viral loads (4) and a false-positive HIV p24Ag test (1). One child above 18 months had false-positive HIV ELISA results. An additional 4 children were inappropriately referred after being incorrectly labelled as HIV-infected and 1 child aged <18 months was referred after an inappropriate diagnostic test for age was used. In summary, 1 in every 30 (3.3%) children was discharged HIV-uninfected, and below age 18 months, 1 in 16 children (6.3%) had false-positive HIV virological tests.

Conclusions. Urgency in ART initiation in HIV-infected children is life-saving, especially in infants. However, HIV tests may produce false-positive results leading to misdiagnosis of children as HIV-infected, which has serious consequences. Meticulous checking of HIV-positive status is of utmost importance before committing any child to lifelong ART.

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The HIV pandemic is an ongoing threat to the well-being of children in sub-Saharan Africa, and in high-prevalence areas such as South Africa (SA), as many as 30% of births are by HIV-infected women.[1] Interventions for prevention of mother-to-child transmission (PMTCT) reduce the risk of transmission to infants from 35% to less than 5%, even in breastfeeding populations,[2] but for those who are infected, disease progression can be rapid and it is therefore critical that these children are diagnosed early to ensure access to antiretroviral therapy (ART).[3,4]

In South Africa, the first public sector HIV treatment guidelines were released in 2004, with subsequent revision in 2010.[5] A major policy change in the updated guideline is to initiate ART in all HIV-infected infants below 1 year of age, irrespective of clinical or immunological staging, since even asymptomatic infants have a high risk of morbidity and mortality if untreated.[4,5]

According to SA guidelines, the diagnostic protocol in children above 18 months is similar to that of the adult protocol, using an initial HIV rapid or ELISA test for screening and subsequent verification with a second confirmatory rapid or ELISA test.[5] HIV diagnosis in children <18 months requires HIV DNA PCR (polymerase chain reaction) testing, which has been shown to have excellent sensitivity and specificity (98.8% and 99.4%, respectively) in a research setting in Johannesburg (SA). However, there is little information on the performance of this test when applied to the clinical setting.[6] Currently the majority of HIV DNA PCR tests on infants in South Africa are done at primary health care facilities, using the dry blood spot (DBS) technique, which greatly facilitates ease of testing, even in small babies, using the heel-prick technique.[7] The shift of care to primary health care level is done to broaden access to HIV-related care, with currently ongoing extension to also include therapeutic services with provision of ART.[5,8]

The prevalence of HIV infection in South African children was reported to be 2.8% in 2010.[9] This high burden of disease means that HIV testing of children, and subsequent access to care, has to be a national priority in order to reduce childhood morbidity and mortality. However, widespread HIV testing of children has potential pitfalls. This paper uses data from a single institution to highlight the issue of HIV-uninfected children being misdiagnosed as HIV-infected.

Patients and methods

Kalafong Hospital, an urban regional hospital in the Gauteng province of South Africa, serves as an ART treatment centre. Records of all ART-naïve children referred to the hospital's paediatric HIV clinic between April 2004 and March 2010 were included in this retrospective analysis, thus spanning the period from the start of the ART programme to the implementation of the updated 2010 HIV guidelines. The Ethics Review Committee, Faculty of Health Sciences, University of Pretoria, approved the audit study protocol.

Patient records of all children who were referred as HIV-positive but who were later found to be HIV-uninfected, were reviewed in detail. It was standard practice in the clinic to do a confirmatory HIV DNA PCR test and/or quantitative HIV RNA PCR ('viral load') on all newly referred children in whom initial testing had been done before 18 months of age. Further HIV testing was done because the second HIV DNA PCR test was negative, or the HIV viral load was undetectable, or the initial HIV test results were not available, or because the child showed no clinical and/or immunological disease progression. HIV diagnostic tests were done by the National Health Laboratory Service (NHLS).[10] Between 2004 and 2009 the HIV DNA PCR test kits used were the AMPLICOR® HIV-1 DNA Test, version 1.5 (Roche Molecular Systems, Inc.) and after 2009, the Cobas AmpliPrep/Cobas TaqMan ('CAP/CTM') HIV-1 Qual test (Roche Molecular Systems, Inc.) was used. Additional testing depended on the child's age, and entailed additional HIV DNA PCR and viral load tests on children aged <18 months, while laboratory-based HIV ELISA testing was done on children aged >18 months, using fourth-generation HIV ELISA testing since 2005. The NHLS laboratories have quality assurance protocols in place,[10] and all positive HIV ELISA tests are repeated on the same specimen using a different instrument, while additional HIV ELISA testing is done on a separate specimen if discordant results are found.

The decision to discharge a child as HIV-uninfected was made by the treating physician. Children without a non-reactive HIV ELISA result at discharge from the HIV clinic were referred to primary health care for HIV rapid or laboratory-based ELISA testing at 18 months. Efforts were also made to trace all children who were discharged as HIV-uninfected.

Results

There were 1 526 patient files included in the study. The median age at first visit of the referred children was 20 months (range 26 days - 17.5 years) with a male-to-female ratio of 1.01:1. The majority (1 063 children) started ART at a median age of 29 months (range 26 days - 17.5 years). The subgroup of children aged <18 months at first visit was 715 children (47%), of whom 393 children (55%) started ART before 18 months of age.

Fifty-one children were HIV-uninfected after diagnostic tests were repeated (Table 1). The main reason for incorrect HIV diagnosis was false-positive laboratory results found in 46 children. These included 40 children aged <18 months with false-positive HIV DNA PCR results, with another 4 children who had falsely detectable HIV viral loads (130 000 copies/ml, 50 000 copies/ml, 1 600 copies/ml and 130 copies/ml, respectively), and 1 child with a false-positive HIV p24Ag test result. Another child had false-positive HIV ELISA test results at age 20 months on two separate samples, with insufficient specimen for confirmatory testing on the first sample, while on the repeat sample the confirmatory test was non-reactive.

Table 1. Summary of cases misdiagnosed as HIV-infected

	Subtotal	Total
False-positive laboratory results		46
• False-positive HIV virological tests		
• False-positive HIV DNA PCR test results	40	
• Falsely detectable HIV viral load results	4	
• False-positive HIV p24Ag test result	1	
• False-positive HIV antibody test		
• False-positive HIV ELISA test result	1*	
Incorrect referrals		5
• Referral after incorrect labelling as HIV-infected	4	
• Age-inappropriate HIV test done†	1	
Total		51

*Insufficient specimen for confirmatory ELISA on first sample; non-reactive confirmatory ELISA on second sample.
†HIV ELISA test done as diagnostic test in age group <18 months.

Of the children with false-positive virological testing, 18 (40%) subsequently had at least one negative HIV ELISA as well as one or more negative HIV virological tests (negative HIV DNA PCR test or undetectable HIV viral load). Another 16 children (36%) were discharged after two negative virological tests and 11 children (24%) had three or more negative virological tests.

In the review of the medical files, the children considered uninfected had adequate documentation of appropriate confirmatory tests and none of the discharges was deemed to have been inappropriate. For patients discharged below 18 months of age, caregivers were advised to have a confirmatory HIV rapid or ELISA test done at a primary health care facility at 18 months. None of the discharged children was subsequently referred back as HIV-infected. Telephonic follow-up was done in August 2010 and 20 children were traceable, and reported to have remained uninfected.

Healthcare workers had inappropriately referred a further 5 patients for HIV-related care, of whom 4 children had incorrectly been labelled as HIV-infected without diagnostic proof. One child was referred as having a positive HIV DNA PCR result, although the test referred to had a negative result. Another child was referred although the HIV PCR result was equivocal, and on repeat the HIV DNA PCR was negative. One child from a children's home was referred for HIV care, implying that the child was HIV-infected, but the child was uninfected on testing. Another orphaned child with severe malnutrition was referred as HIV-infected and for ART initiation, but subsequently tested HIV-negative. In 1 child below 18 months an inappropriate diagnostic test for age was used, as he was referred as HIV-infected after a positive HIV ELISA test.

Children referred with false-positive laboratory results had a median age of 4 months (range 1 - 35 months) and a median number of 3 patient visits (range 1 - 11 visits), while the 5 children incorrectly referred for other reasons had a median age of 9 months (range 4 - 73 months), with a median of 2 visits (range 1 - 4 visits). During the 6-year period, misdiagnosis of HIV in children resulted in 193 added patient visits at the HIV clinic,

with further HIV testing done to confirm their HIV-negative status. None of the children described in this study had inadvertently been started on ART, due to the timely identification of the misdiagnosis.

Of the 46 children with false-positive HIV tests, 33 had documented PMTCT interventions, while 5 children had no PMTCT and for 8 children the information was not recorded. According to World Health Organization (WHO) HIV clinical staging done at the HIV clinic, 36 (78%) were asymptomatic or had a minor illness (stages 1 and 2), while 9 (20%) were classified as symptomatic (with stage 3 or 4 disease), and 1 child (2%) was not staged. WHO stage 3 events in the children included oral candidiasis outside the neonatal period (4), malnutrition (3), pulmonary tuberculosis (2) and history of severe infection (meningitis) (1). The child classified as WHO stage 4 had been hospitalised at the age of 5 months with severe pneumonia and a presumptive diagnosis of *Pneumocystis jirovecii* pneumonia.

In the context of all 1 526 reviewed HIV clinic patient files, the subsequent discharge of the 51 reported children means that 1 in every 30 (3.3%) referred children was later discharged as HIV-uninfected. In the age group below 18 months, 1 in every 16 children (6.3%) had been misdiagnosed as HIV-infected on a false-positive HIV virological test.

HIV disease in children has become a chronic treatable condition, and many lives can be saved by early disease detection with subsequent access to ART.

Discussion

HIV disease in children has become a chronic treatable condition, and many lives can be saved by early disease detection with subsequent access to ART. This urgency is especially important in the first year of life, with proven survival benefit with immediate ART initiation.[4] Both South African (2010)[5] and WHO guidelines[11] call for initiation of ART for all HIV-infected childen diagnosed before the age of 12 months, even if asymptomatic. For those diagnosed in the second year of life, WHO guidelines again recommend treatment of all infected children, while South African guidelines recommend treatment based on clinical disease progression or CD4 count/percentage. Widespread testing of children has its pitfalls, however, as HIV tests may produce false-positive results and misdiagnosis of children as HIV-infected has serious consequences.

A limitation to this study is that it is a clinic-based retrospective audit, and that possibly not all who were misdiagnosed as being HIV-infected were identified. On review of the medical records, none of the discharges as HIV-uninfected was deemed inappropriate. We do not have access to all follow-up test results, but all of those who were known to have had follow-up testing were confirmed uninfected and no child was subsequently referred back with HIV disease to the Kalafong ART site.

In the South African public sector, ART provision is moving from dedicated ART clinics to primary health care facilities in order to broaden access to care.[5] In the context of this study, children were referred for care at the ART site after HIV diagnosis. We found that incorrect referrals included children who had been wrongly labelled as HIV-infected or in whom age-inappropriate HIV testing had been done. It is of utmost importance that diagnostic algorithms are followed by healthcare workers and that all preceding laboratory test results which prove HIV infection are meticulously checked before initiation of ART.

In this study, false-positive test results were the most frequent reason for misdiagnosis of HIV infection; this occurred mainly in young, asymptomatic children who required HIV DNA PCR testing for PMTCT programme follow-up. The HIV DNA PCR test has been shown in South Africa to have an excellent sensitivity and specificity, achieved within a research environment with adequate staff training and sufficient staffing levels.[6] Our results show that 6.3% of children referred below age 18 months were incorrectly diagnosed as HIV-infected due to false-positive virological test results. Everyday clinical use of HIV tests in large-scale ART programmes, as is the case in South Africa, within the context of scarce human resources, increases the risk of incorrect specimen labelling and handling errors at clinics and laboratories. False-positive test results can also occur due to laboratory errors through specimen contamination and inadequate quality control.[6]

Alarmingly, one-fifth (20%) of children falsely diagnosed as HIV-infected in this study were staged as having moderate to severe (WHO stages 3 or 4) HIV disease. Incorrectly diagnosing HIV disease implies misdiagnosis of the true disease, a reminder to clinicians that other conditions, like tuberculosis and malnutrition, may mimick HIV disease in children.

The study period spanned from the start of ART availability and HIV DNA PCR testing in South Africa's public sector in 2004 to the implementation of the updated paediatric treatment guidelines in 2010. New national PMTCT guidelines were also released during 2010, intensifying interventions to reduce HIV transmission to children.[12] As the PMTCT programme improves and HIV prevalence in children subsequently decreases, the positive predictive value of the HIV DNA PCR, as with any test, declines, and even with the excellent specificity, there is an increased risk of false-positive test results.[13] The choice of test kits with different specificities can, in this context, have a profound effect on the clinical outcome achieved within programmatic testing. During our study the majority of HIV DNA PCR tests were done using the AMPLICOR HIV-1 DNA test, while during the last year of study the Cobas AmpliPrep/Cobas TaqMan HIV-1 Qual test was introduced, due to its advantages of a shorter turn-around time and being less labour intensive. The report from Maritz *et al.*[14] comparing the Amplicor and CAP/CTM tests is concerning due to reported lower specificity of the CAP/CTM test, as any decrease in test specificity would further greatly decrease the positive predictive value of any one positive HIV DNA PCR test.[13]

One major change in the SA paediatric guidelines (2010) is the immediate initiation of ART in all HIV-infected children diagnosed before age 1 year, meaning that even asymptomatic infants are eligible for ART.[5] Clinical staff can therefore no longer depend on the congruence of laboratory result and clinical picture in asymptomatic patients, with diagnosis of HIV infection thus relying fully on positive laboratory testing.[13] The guidelines recommend that HIV testing in infants is done by HIV DNA PCR testing, with an HIV RNA viral load used as confirmatory test, using a cut-off of at least 10 000 copies/ml ($\log_{10}4$) for diagnostic purposes.[5] In sub-Saharan Africa most HIV-exposed children stay far from health care centres with paediatric HIV expertise, and clinical staff may interpret low-level positive RNA viral loads as proof of HIV-positive status of an uninfected child, if training on the strengths and limitations of laboratory tests is neglected.[15]

This may become clinically more relevant within the context of ART services expanding into primary health care facilities.[5]

To minimise harm which may occur through misdiagnosis of HIV infection in HIV-exposed children who, due to PMTCT interventions, are increasingly less likely to be HIV-infected, the following recommendations are made:

- training of healthcare workers on HIV diagnostic guidelines and use of HIV diagnostic tests in children
- review of HIV testing algorithms within the context of changing circumstances, including the shifting paediatric HIV epidemic and changing ART guidelines
- adequate clinical notes and clearly written referrals between healthcare providers as standard of care
- healthcare facilities to review procedures during venepuncture to minimise risk of mislabelling of blood specimens
- continuous quality assurance in laboratories with detailed prevention of contamination
- meticulous checking of laboratory results within clinical practice
- confirming positive test results, especially in asymptomatic patients. In the South African context this would, under current guidelines, include confirming a positive HIV DNA PCR test with an HIV viral load of >$\log_{10}$4 before conclusively diagnosing a child as HIV-infected. Furthermore, if there is any doubt about the diagnosis of HIV infection, especially in an asymptomatic infant, the HIV DNA PCR test should be repeated.[5,6,13]

In conclusion, ART initiation in an HIV-infected child is life-saving, and should not be unduly postponed. Within the context of decentralisation of ART services, HIV testing algorithms need to be robust in balancing the risks of over- and under-diagnosis of the disease, especially in infants who require HIV virological testing. Staff training on the strengths and limitations of laboratory tests is vital, as clinical judgement is paramount in interpreting any positive laboratory result, even if the test is known to have an excellent sensitivity and specificity, as misdiagnosing an HIV-uninfected child as infected may mean committal to lifelong ART.

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