CASE REPORT

False-negative HIV-1 polymerase chain reaction in a 15-month-old boy with HIV-1 subtype C infection

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Polymerase chain reaction (PCR) testing is the gold standard for determining the HIV status in children <18 months of age. However, when clinical manifestations are not consistent with laboratory results, additional investigation is required. We report a 15-month-old HIV-exposed boy referred to our hospital after he had been admitted several times for infectious diseases. A rapid antibody test on the child was positive, while routine diagnostic HIV PCRs using the Roche COBAS Ampliprep/COBAS TaqMan HIV Qual Test were negative at 6 weeks, 6 months, 7 months and 15 months. In addition, the same PCR test performed on the HIV-infected mother was also negative. Alternative PCR and viral load assays using different primer sets detected HIV RNA or proviral DNA in both child and mother. *Gag* sequences from the child and his mother classified both infections as HIV-1 subtype C, with very rare mutations that may have resulted in PCR assay primer/probe mismatch. Consequently, the child was commenced on antiretroviral therapy and made a remarkable recovery. These findings indicate that more reliable PCR assays capable of detecting a wide range of HIV subtypes are desirable to circumvent the clinical problems created by false-negative PCR results.

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Even at a very early stage in paediatric HIV infection, polymerase chain reaction (PCR) has been shown to be highly sensitive and specific.[1] However, when the clinical picture is inconsistent with laboratory results, additional investigation is warranted. Several published studies have reported concerns about the ability of PCR tests to detect all current subtypes as a result of HIV-1 genetic diversity.[2-6] These concerns include the COBAS AmpliPrep/COBAS Taqman (CAP/CTM) HIV-1 Qual Test (Roche Molecular Systems, Branchburg, NJ, USA) which, until recently, was widely used in South Africa (SA) and is reported to have 99.7% sensitivity and 100% specificity.[7] We describe a case of a false-negative HIV PCR using this assay to detect vertically transmitted HIV infection in an SA child. Failure to recognise HIV infection at an early stage severely compromised the health of this child. *Gag*-specific sequencing of the virus in both mother and child was carried out to explore the reason for lack of detection by the qualitative PCR assay.

Case description

In January 2014 a critically ill 15-month-old boy was transferred to Red Cross War Memorial Children's Hospital, Cape Town, SA from a secondary hospital. He had acute gastroenteritis with hypovolaemic shock, a lower respiratory tract infection, septicaemia, pyogenic myositis of his left thigh, and subungual abscesses of both thumbs caused by methicillin-sensitive *Staphylococcus aureus*. He responded to cloxacillin, fluid and electrolyte replacement and nutritional rehabilitation. Of note in his background medical history was that his mother was diagnosed serologically with HIV infection 5 months into her pregnancy. Dual zidovudine and nevirapine prophylaxis was administered antenatally and during labour, and postnatally the child received 4 weeks of zidovudine prophylaxis. He was breastfed until 4 months of age. Between the ages of 6 and 9 months he was hospitalised on three occasions for gastroenteritis and respiratory tract infection (two admissions) and tuberculosis (one admission).

Table 1 shows the HIV-related test history of the child and his mother – alternative PCR assays were initiated at the time of the child's correct diagnosis and performed retrospectively on previous archived samples. The earliest qualitative HIV PCR test using the routinely available method (COBAS AmpliPrep/COBAS Taqman (CAP/CTM) HIV-1 Qual Test version 1) at 6 weeks was negative. Repeat PCR tests during subsequent admissions were also negative. A rapid antibody test at 15 months during admission to our hospital was positive. Because of the clinical suspicion of HIV infection, an HIV viral load test (Abbott RealTime HIV-1, Abbott Molecular Inc., Des Plaines, IL, USA) was performed, which documented 1 562 169 HIV copies/mL ($\log_{10}$ 6.19). Alternative PCR assays[8,9] using different primer sets also detected HIV proviral DNA. The child's CD4 count was markedly reduced at 64 cells/μL (3.86%). He was commenced on abacavir, lamivudine, lopinavir/ritonavir and co-trimoxazole, made remarkable progress and was discharged after 14 days.

HIV *gag*-specific PCR was performed for diagnostic purposes and to investigate viral genotypic characteristics, which could inform reasons for lack of detection by the CAP/CTM assay (methodology available on request). Samples taken from both mother and child around the time of diagnosis, as well as archived samples from the child taken at 6 and 7 months of age, were all *gag* PCR-positive (Table 1). Amplification of partial *gag* (160 base pairs)[8] confirmed the presence of HIV in the child. Sequence analyses of full-length *gag*[9] amplified from infant and maternal samples confirmed that both were infected with HIV-1 subtype C, the predominant subtype in SA, with no evidence of intersubtype recombination (Fig. 1A).

We analysed the most conserved region of *gag*, the p24 capsid region, which is more likely to contain primer/probe target regions. The sequence of an HIV subtype C infectious molecular clone (IMC)

Table 1. HIV-related test results

Sample	Age	Test method	Result
Child			
C1	6 weeks	CAP/CTM Qual PCR*	Negative
C2	6 months	CAP/CTM Qual PCR*	Negative
		Full-length *gag* PCR[9]	Positive
C3	7 months	CAP/CTM Qual PCR*	Negative
		Full-length *gag* PCR[9]	Positive
C4	15 months	Determine HIV-1/2 antibody†	Reactive
C5	15 months	Enzygnost Anti-HIV 1/2 plus ELISA‡	Reactive
C6	15 months	PanLeucogating CD4 count (Beckman Coulter)[10]	64 cells/μL (3.86%)
		CAP/CTM Qual PCR*	Negative
		Partial *gag* PCR[8]	Positive
C7	15 months	Abbott RealTime HIV-1 viral load§	1 562 169 copies/mL ($\log_{10}$ 6.19)
C8, C9, C10	15 months	CAP/CTM Qual PCR*	Negative
C10	15 months	CAP/CTM Qual PCR version 2¶	Positive
		Full-length *gag* PCR[9]	Positive
Mother			
M1	19 years	Determine HIV-1/2 antibody†	Reactive
M2	19 years	ARCHITECT HIV Ag/Ab Combo ELISA‖	Reactive
		Enzygnost Anti-HIV 1/2 Plus ELISA‡	Reactive
		CAP/CTM Qual PCR*	Negative
		Full-length *gag* PCR[9]	Positive
		Abbott RealTime HIV-1 viral load§	51 977 copies /mL ($\log_{10}$ 4.72)

*COBAS AmpliPrep/COBAS Taqman HIV-1 Qual Test (Roche Molecular Systems, Branchburg, NJ, USA).
†Determine HIV-1/2 (Alere Medical, Chiba, Japan).
‡Enzygnost Anti-HIV 1/2 Plus (Siemens, Marburg, Germany).
§Abbott RealTime HIV-1 (Abbott Molecular Inc, Des Plaines, Ill., USA).
¶COBAS Amplipre/ COBAS Taqman HIV-1 Qualitative test, V2.0 (Roche Molecular Systems, Branchburg, NJ, USA).
‖ARCHITECT HIV Ag/Ab Combo (Abbott Laboratories, Wiesbaden, Germany).

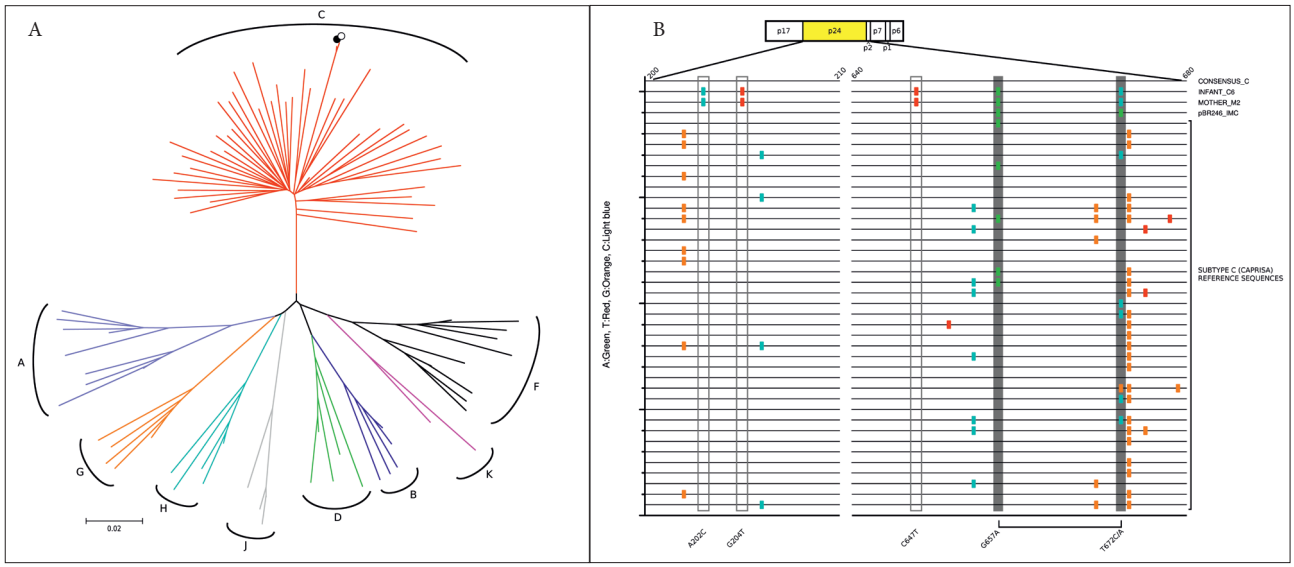

Fig. 1. Subtyping and identification of rare mutations in the mother and infant samples. (A) A neighbour-joining phylogenetic tree of global subtype reference and SA subtype C gag *sequences (www.hiv.lanl.gov). Both the infant's sample C6 (indicated by a closed circle) and the mother's sample M2 (indicated by an open circle) cluster with subtype C sequences. Scale bar = 0.02. (B) Rare mutations and a rare pairing of mutations in* gag *p24 sequences from samples C6, M2 and the subtype C IMC plasmid pBR246-F10, when compared with a consensus subtype C sequence and database subtype C sequences from the CAPRISA 002 cohort (previously detected by the COBAS Amplicor 1.5/Taqman HIV-1 test),[11,12] are illustrated in a highlighter nucleotide mismatch plot (www.hiv.lanl.gov). Rare mutations shared by the infant and mother are indicated by clear grey bars and the rare mutation pair shared by the infant, mother and IMC are indicated by shaded grey bars. Nucleotide mismatches relative to the consensus are shown as coloured blocks. Nucleic acid numbering relative to consensus C* gag *p24 is indicated.*

(pBR246F-10, provided by B Hahn), previously identified in our lab as failing to amplify on the CAP/CTM assay, was included in the analysis. Three individual nucleotide mutations (A202C, G204T and C647T, shared by the infant and mother sequences) and one pair of mutations (G657A together with T672C/A, shared by the mother, infant and IMC sequences) were identified as rare, based on their frequency within 530 database SA subtype C sequences (www.hiv.lanl.gov) (present in <1% of subtype C sequences) (Fig. 1B).

Because of the proprietary nature of the primers and probes of the Roche CAP/CTM HIV Qual assay, we were unable to obtain their sequences to compare with the primer/probe binding region sequences of the mother and infant viruses. However, Roche Diagnostics was able to confirm that the sequence of both the IMC and the mother/infant virus contained critical primer/probe mismatches that may prevent detection using the first version of the CAP/CTM HIV-1 Qual assay. They confirmed that the newer version of the CAP/CTM assay, released late in 2013, would detect the virus, which was confirmed by another laboratory evaluating the performance of version 2 of the assay.

Discussion

Bøgh *et al.*[2] reported that the Amplicor HIV-1 DNA PCR test (Roche Molecular Systems, Branchburg, NJ, USA) did not detect all subtypes with equivalent sensitivity and that 10% of the non-subtype B samples tested negative. Kline *et al.*[3] reported a false-negative Amplicor HIV-1 DNA PCR in an infant with subtype C HIV infection. Other studies have similarly shown that version 1.0 of the Roche COBAS Ampliprep/COBAS Taqman (CAP/CTM, Roche Molecular Systems, Branchburg, NJ, USA) HIV-1 viral load assay may rarely under-quantify or fail to detect non-B subtypes.[4-6] In a report[13] in which the majority of women were African and likely to have non-B subtypes of HIV-1, nearly a quarter of the 108 samples collected from pregnant women gave rise to false-negative results in the routine assay (Roche Amplicor version 1.0). In this setting, if additional tests had not been conducted, misdiagnosis in infants may have occurred.

This report describes a case of a false-negative qualitative PCR in a young child and his mother, both of whom have HIV-1 subtype C infection, confirmed by positive antibody and alternative DNA and RNA PCR assays.

It is not possible to determine the exact timing of the child's infection; however, failure of the routine PCR to detect HIV despite repeated attempts represents a missed opportunity for early diagnosis and resulted in multiple admissions and severe illness, requiring intensive care. This emphasises the need for a high index of suspicion in an HIV-exposed infant with clinical evidence of recurrent infections and failure to thrive.

To reduce the risk of false-negative PCR tests, some guidelines have suggested molecular testing of a maternal sample as part of routine paediatric diagnosis.[11] In cases where maternal HIV DNA cannot be detected on the routine assay, an alternative assay able to detect the maternal virus can be used to test the infant.[11] Testing samples with a qualitative PCR with a parallel quantitative assay has also been suggested.[11] However, in endemic settings with a high prevalence of HIV, implementation of these policies may pose logistic challenges in terms of cost and feasibility, negating their use in routine practice.

In this case report, the false-negative CAP/CTM result could likely be explained by primer or probe mismatches. We identified three rare mutations and a rare pairing of mutations in both the mother and infant sequences, which could have accounted for lack of detection. However, this could not be confirmed in the absence of primer/probe sequence information. Qualitative assays that are more reliable in detecting HIV would be the long-term solution to the problem of false-negative PCR tests.[2,3]

Additional HIV diagnostic testing is required when there are doubts about the HIV status of a symptomatic exposed infant. Continued surveillance and reporting of cases of HIV non-detection are therefore necessary. Upgrading of the amplification tests for better detection of all HIV subtypes is also essential. The new version of the qualitative CAP/CTM assay (version 2), which targets both the *gag* and LTR regions of the virus, should have an improved sensitivity and inclusivity profile.

Conclusion

Opportunities were missed for early diagnosis and treatment of HIV in this child. PCR assays that are more reliable in detecting a wide range of HIV subtypes and variants are desirable to prevent the problem of false-negative PCR tests. Clinicians should consider a negative PCR result to possibly be false in cases where the clinical picture does not match the laboratory report.

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