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Clinical predictors of HIV infection in febrile children attending health facilities in Tanzania.

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Clinical predictors of HIV infection in febrile children attending health facilities in Tanzania.

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Abstract

Introduction: In Tanzania and most Sub-Saharan countries of Africa, the HIV infection remains an important cause of child mortality. A good opportunity to test children for HIV is when they attend health facilities for a fever episode. As HIV rapid tests are not always available in resource-limited countries, the use of a clinical algorithm could provide guidance to clinicians on which febrile children should be tested in priority during these consultations. The criteria used in the actual IMCI algorithm may not necessary be the best predictors of HIV infection in children as little is known on the subject. The aim of the present study was to identify the clinical and laboratory predictors of HIV in febrile children attending outpatient clinics in Tanzania and to calculate their diagnostic performance, as well as to assess the performance of the IMCI criteria and algorithm used in Tanzania at the time of this study and determine if a combination of new predictors could have better diagnostic performance.

Methods: A nested case-control analysis in a prospective observational study on etiologies of fever in children aged less than 10 years attending health facilities in rural and urban Tanzania was performed. Detailed history taking, clinical examination, laboratory investigations and final diagnoses (based on pre-defined criteria) were obtained. The sensitivity, specificity, positive and negative likelihood ratios were calculated in bivariate analysis for all significant predictors. Stepwise backward logistic regression models were used to identify independent predictors of HIV infection and calculate their adjusted Odds Ratios. New algorithms using the best clinical predictors found in the analyses were generated and compared with the IMCI HIV branch.

Results: Of 1004 consecutive febrile children (median age 18 months), 16 were HIV positive. Regarding history taking: difficulty to breath (LR+ 6.9), chronic condition (LR+ 4.7), recent travel history (LR+ 4.1), sick contact person (LR+ 2.8) were found to be independently predictive of HIV. Regarding clinical signs: lymphadenopathy at any site (LR+ 10.8), chest indrawing (LR+ 10.7), hepatomegaly (LR+ 9.5), abdominal tenderness (LR+ 6.2), low weight for age (LR+ 4.0) and fast breathing (LR+ 3.5) were also predictive. Laboratory predictors were: hemoglobin <8 mg/dl (LR+ 2.8), naso-pharygeal pneumococcus load $\geq 10^7$ cfu/ml (LR+ 2.2) and naso-pharygeal *Staphylococcus aureus* carriage (LR+ 2.0). Children with HIV were significantly more at risk to have a severe illness of any type (LR+ 2.9), and a diagnosis of radiological end-point pneumonia (LR+ 14.8), acute otitis media (LR+ 5.6), or bronchiolitis (LR+ 3.7). On the contrary, the diagnosis of upper respiratory infection negatively predicted the HIV infection (LR+ 0.3). The HIV branch of the IMCI algorithm had a sensitivity of 93.8% and a specificity of 73.7% when using 1 criteria as cut off for testing, and a sensitivity of 56.3% and specificity of 97.2% when using 2. A new algorithm using lymphadenopathy at any site, radiological end-point pneumonia, chronic condition, abdominal tenderness, difficulty to breath, acute otitis media and oral thrush as criteria showed a sensitivity of 93.8%, and a specificity of 81.4% when using one criterion as cut-off..

Conclusion: Several clinical and laboratory predictors for HIV were found in febrile outpatient children, some of which are not included in the present IMCI criteria. These findings could serve to improve the actual IMCI algorithm and potentially increase the number of HIV positive children detected during consultations for acute care.

Table des matières

1. Introduction

As most Sub-Saharan countries of Africa, Tanzania is severely affected by the human immunodeficiency virus (HIV) infection and around 1.5 million people of all ages were living with HIV in Tanzania in 2012 (1). HIV infection and acquired immunodeficiency syndrome (AIDS) were responsible for 78'000 deaths in Tanzania in 2012 and continue to be a major cause of infant mortality in Africa (2).

If overall the commonest route of transmission remains the sexual one, a significant number of children are vertically infected around the world. Those vertical transmissions are estimated to be responsible of 18% of new infections in Tanzania, representing the second most common way of transmission with about 250'000 children aged less than 15 living with HIV in that country (3), (4). Global use of antiretroviral drug has started since 1996 and all pregnant or breastfeeding women infected with HIV should nowadays receive antiretroviral therapy (ART) in order to reduce risks of mother-to-child-transmission (MTCT). However, the estimated coverage of ART for prevention of MTCT was only 73% in 2013 (5).

Children generally acquire the virus from their mother during pregnancy, delivery or after their birth by breastfeeding. Usually they do not present any clinical features of HIV/AIDS soon after birth but develop them during infancy, except slow progressors who often only develop signs or symptoms in late childhood. Unlike in adults, the clinical presentation of HIV/AIDS can be hard to distinguish from common childhood illnesses as symptoms often overlap. Because paediatric HIV infection causes an important number of deaths and because some of them could be avoided by antiretroviral treatment, early diagnosis is important.

In an attempt to decrease the child burden of disease the World Heath Organisation (WHO) and United Nations International Children's Emergency Fund's (UNICEF) developed the Integrated Management of Childhood Illness (IMCI) that was implemented in Tanzania starting in 1996 (6). The IMCI strategy aspires to improve the management of common childhood illnesses at primary health care level by enabling early diagnosis and effective treatment. However, at first these guidelines did not specifically include HIV. In Tanzania and other high HIV prevalence countries IMCI was therefore revised to incorporate a new branch in the algorithm that would help to identify HIV exposed or infected children and provide guidelines on how to manage them. This branch, that was incorporated in the 2008 WHO generic version of IMCI, first allows to assess children potentially infected by HIV by determining if they fulfil enough criteria (at least 2 or 3 among 7, according to the version of the algorithm) of suspected HIV infection, in order to refer the child to a voluntary testing and counselling (VTC) clinic for further HIV evaluation and management. Those 7 criteria include: pneumonia, persistent diarrhoea, ear discharge, oral thrush, parotid swelling, low weight for age and enlarged lymph glands(7). In the next English WHO generic version of IMCI of 2014 the clinical criteria have been removed and the algorithm is only based on the results of the

mother and child's serological or virological tests (8). This change, which aims at increasing the number of children tested, implies however that HIV rapid tests are available at all place and time, which is often not the case in many settings in Africa. Therefore, in their locally adapted version of IMCI, many countries have decided to keep the clinical criteria to decide on HIV testing, also because of the lack of time in busy health facilities to test each child attending for acute care. For example, the Tanzanian version of IMCI algorithm of 2012 uses the clinical criteria listed above with an additional criteria: history of tuberculosis in child, siblings or parents and a cut-off of ≥3 criteria to decide on the management of the child, taking into account whether HIV testing is available or not (9).

The HIV branches of IMCI have had limited assessment since their implementation and little is known about the real performance of theirs criteria when confronted to data on documented HIV infection. Indeed, the criteria used do not necessarily correspond to the best clinical predictors of HIV infection in children, as little is know on this subject. A few studies have looked at the clinical presentation of HIV in African children and have investigated and identified clinical predictors of the infection. However, most studies were focused on children aged less than two years and only limited data regarding older children is available (10), (11), (12), (13), (14), (15), (16). Furthermore numbers of them were conducted on hospitalized children, including severely ill children with a high HIV prevalence and advanced stage of the disease (10), (17), (18), (19), (20). Two of these studies were conducted in Tanzania: one in 1995 on children aged from 18 months to 5 years, attending maternal and child health clinics in Dar es Salaam, before the beginning of IMCI implementation in 1996(21); the other one in 2000 on hospitalized children aged from 1 month to 7 years (17). It is therefore necessary to conduct new studies to assess the performance of the HIV branch of the IMCI in a larger age range and determine which are the most adequate predictors of HIV for primary health care use.

The aim of the present study is to identify the clinical predictors of the HIV infection in febrile children attending outpatient clinics in Tanzania and to calculate their diagnostic performance. For this purpose, we used data from a prospective study on etiologies of fever in children aged less than 10 years attending health facilities in rural and urban Tanzania, which was conducted in 2008. All elements of a detailed history taking, clinical examination, laboratory investigations and final diagnoses based on pre-defined criteria had indeed been collected. The other aim of this study is to assess the performance of the IMCI criteria and algorithm used in Tanzania at the time of the study and determine if a combination of new predictors have better diagnostic performance.

2. Methods

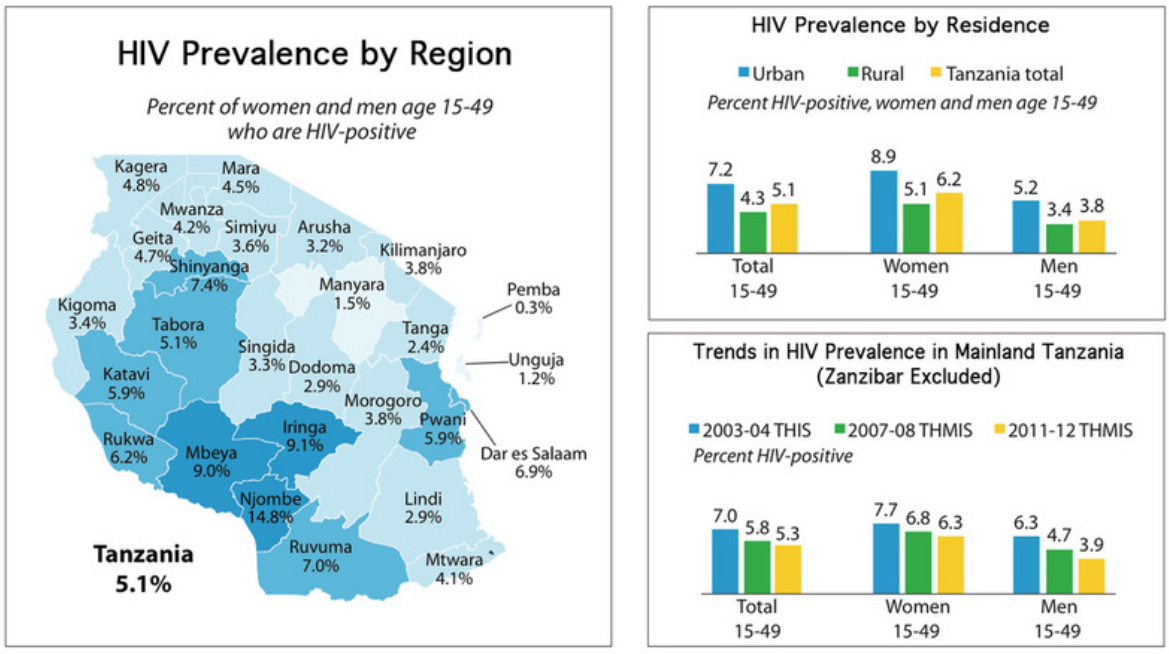
This study is a nested case-control analysis in a prospective observational study on etiologies of fever in consecutive children attending outpatient clinics. Study sites,

population, participants and procedures have already been described in detail in another article (22). Here is a summary of the methodology that is relevant for this study.

2.1 Study sites and population

The study took place in the United Republic of Tanzania, located within the great lake region in east Africa. In 2008, year of the data collection, the total population of the country was 42'354'000, 45% of which were aged under 15 years (23). The overall HIV prevalence in adults aged 15 to 49 years was 5.7% in 2009; it has then decreased to 5.1% in 2011 (Figure 1) and 5.0% in 2013(23). This decline has however been observed in men, but unfortunately not in women. As a result, in 2013 a total of 1.2 millions adults aged over 15 were living with HIV, 690'000 of which being women (4).The study on etiologies of fever included two different sites. The first site was located in urban Dar es Salaam: children were recruited from April to August 2008 at the outpatient clinic of the Amana District Hospital, one of the three district hospitals of the city. The second site was located in the rural Morogoro region: children were recruited from June to December 2008 at the outpatient clinic of the St. Francis Designated District Hospital in Ifakara, Kilombero District.

In Dar es Salaam, the Tanzania's largest and richest city which is considered the economical and administrative capital of the country, the rate of HIV infection reached 6.9% in 2012, whereas in Morogoro region, a rural region situated 500 km South-West of Dar es Salaam, the rate was only 3.8% in the same year (24).



Source: 2011-2012 Tanzania HIV/AIDS and Malaria Indicator Survey (THMIS), Key Findings, p. 7

Figure 1: HIV prevalence by region and by residence in 2011-2012, and trend in HIV prevalence in the Mainland of Tanzania (25).

2.2 Participants

Consecutive patients aged from 2 months to 10 years presenting with an axillary temperature of 38°C or higher were screened for study inclusion. Children with severe malnutrition or presenting with emergency signs requiring immediate care were excluded. Four additional criteria of inclusion were applied: children were included if it was their first visit for the present disease, if fever duration was 1 week or less, if the main reason of their visit was neither trauma nor injury and if they had not received antimalarials or antibiotics during the preceding week.

2.3 Clinical assessment

All relevant elements related to an acute fever episode, including a detailed clinical history and physical examination, were obtained using standardized case-report forms. Chief reason for the visit, 23 clinical symptoms and their duration, 49 clinical signs as well as travel history, contact with sick persons and known chronic conditions were assessed.

2.4 Sample collection and laboratory testing

For every child, pooled nasal and throat swabs as well as blood samples (5ml) were gathered for microbiological testing (rapid tests and cultures) on site and additional molecular and serologic work up in Switzerland and the United States. In all cases, a systematic set of investigations was made that included: full blood count, creatinine, alanine transaminase (ALT), polymerase chain reaction (PCR) on naso-pharyngeal swabs for 14 viruses and 4 bacteria, PCR on blood for parvovirus B19 and human herpes virus 6 (HHV6), blood slide for Malaria and Borrelia, rapid diagnostic test for Malaria and Salmonella typhi. Further investigations, including chest x-ray and additional microbiological testing, were done according to several predefined non-mutually exclusive decision charts elaborated for the chief symptoms of the child. Final diagnoses were established using sets of predefined criteria (clinical and microbiological) based on systematics reviews and on WHO and Infectious Diseases Society of America guidelines.

Regarding HIV infection, during the study VCT was recommended according to the IMCI guidelines available at the time of the study, i.e. every time that at least 2 of the 7 IMCI clinical criteria for suspected HIV infection were met. Those 7 criteria were: history of persistent diarrhoea, ear discharge, very low weight for age, oral thrush, parotid swelling, pneumonia and enlarged lymph nodes in two or more of the following sites: neck, armpit or groin. With the approval of their guardians, those children were referred to the VCT clinic of the hospital in order to be tested for HIV.

HIV testing was systematically conducted later in Switzerland on the blood of all children, independently of the IMCI criteria, using SD BIOLINE HIV-1/2 3.0 Rapid Test (Standard Diagnostics Inc, Korea). Children under 18 months whose results came back positive were then tested by PCR to differentiate those HIV exposed from those actually infected. INSTI HIV-1/HIV-2 Rapid Antibody Test (bioLytical Laboratories Inc, Canada)

was used to confirm all positive results in children aged over 18 months. Discordant results were submitted to immunoblots, INNO-LIA HIV I/II, Innogenetics, to determine the final status of the child. Positive HIV cases were defined as a child with a positive rapid test result and a PCR positive result in children aged less than 18 months and as either a positive rapid test result and an immunoblots positive result or two positive rapid tests results for children over 18 months. HIV exposed children were defined as those aged less than 18 months with a positive rapid test result and a negative PCR result.

2.5 Analysis

Data were analysed using Stata/MP version 13.0 (college station, Texas USA). All available variables were included in the analysis except those that were present or abnormal in less than 10 children to avoid results with very large confidence intervals.

Variables were tested in bivariate analysis using chi-squared test to measure differences between HIV-positive and HIV-negative children. Simple proportions were used to calculate sensitivity, specificity, positive and negative likelihood ratios. Predictors were considered as significant if the p-value was lower than 0.1. Continuous parameters were recoded in dichotomous ones using acceptable threshold for normality. Significant variables were then divided in four different groups, the first including context factors and symptoms that could be gathered during history taking, the second clinical signs obtained by the physical examination, the third the laboratory findings and the fourth final diagnoses based on predefined criteria. When two parameters had similar clinical meaning, only the most relevant one was included in the model. Each variables group was analysed separately in multivariate analyses using stepwise backward logistic regression models to calculate its variables adjusted Odds Ratios (aOR) and 95% confidence intervals (95%CI).

Then, the diagnostic performance of the Tanzanian IMCI HIV branch and criteria used at the time of the study was calculated. New algorithms using the best clinical predictors found in the analysis were generated to compare their performance with the actual IMCI HIV branch.

3. Results

3.1 Description of the study sample

A total of 1005 children were included in the study on etiologies of fever, 507 in Dar es Salaam and 498 in Ifakara. A child enrolled in Ifakara was then excluded of the analyses because its HIV status could not be determined due to the lack of blood. The overall median age was 18.2 months and 48.5% of the children were female.

Among the 1004 febrile children, 16 were found to be HIV positive and 8 HIV exposed. The overall HIV infection prevalence was 1.6% and respective sites prevalence were 1.2% in Dar es Salaam and 2.0% in Ifakara (p-value=0.3). Demographic and clinical characteristics of the febrile children of both sites are listed in Table 1.

Table 1: *Demographic and clinical characteristics of the patients according to study site*

Characteristic	**Dar es Salaam (N=507) No. (%)**	**Ifakara (N=497) No. (%)**
Female sex	237 (46.7)	250 (50.3)
Age groupe		
2 to <12 months	150 (29.6)	176 (35.4)
12 to <36 months	228 (45.0)	226 (45.5)
36 to <60 months	75 (14.8)	85 (17.1)
5 to <8 years	36 (7.1)	8 (1.6)
8 to <11 years	18 (3.6)	2 (0.4)
Weight for age		
Normal	277/498 (55.6)	291/496 (58.7)
1 SD below mean	150/498 (30.1)	147/496 (29.6)
2 SD below mean	52/498 (10.4)	48/496 (9.7)
3 SD below mean	16/498 (3.2)	10/496 (2.0)
4 SD below mean	3/498 (0.6)	0/496 (0.0)
HIV-positive	6 (1.2)	10 (2.0)
HIV-exposed	4 (0.8)	4 (0.8)
Chief symptom reported by the guardian		
Fever	313/497 (63.0)	353/494 (71.5)
Cough	56/497 (11.3)	47/494 (9.5)
Vomiting	47/497 (9.5)	28/494 (5.7)
Diarrhea	33/497 (6.6)	22/494 (4.5)
Abdominal pain	15/497 (3.0)	19/494 (3.8)
Other	33/497 (6.6)	25/494 (5.1)
Diagnoses		
Acute respiratory infection	248 (48.9)	376 (75.7)
Malaria	60 (11.8)	44 (8.9)
Gastroenteritis	45 (8.9)	58 (11.7)
Urinary tract infection	39 (7.7)	20 (4.0)
Viral disease	307 (60.6)	401 (80.7)
Bacterial disease	128 (25.2)	93 (18.7)
Parasitic disease	61 (12.0)	47 (9.5)
Severe	90 (17.8)	43 (8.7)

3.2 Characteristics of HIV positives cases

For the 16 children diagnosed with HIV infection, 44% (7/16) were girls and 38% (6/16) were attending the outpatient clinic of Dar es Salaam. The age of the subjects ranged from 4 to 59 months and the median age was 18 months. The chief reason for the visit mentioned by the guardian was fever in 6 cases, cough in 4 cases, diarrhea in 2 cases, vomiting in 2 cases and running nose in 1 case (unknown for one child). 5 (31%) of the 16 HIV infected children were already known to suffer from a chronic condition: 2 had presented recurrent fever, 1 recurrent chest infection, 1 scalp fungus and 1 was already known to be HIV infected. Clinical pneumonia was the most frequent final diagnosis for the febrile episode and was present in 9 of the children, 4 of which were classified as severe clinical pneumonia. Of those 9 clinical pneumonias, 6 were radiological end-point pneumonias (as defined by WHO) (26), while the chest x-ray was normal for the other 3. Other diagnoses (7/16 children had multiple diagnoses) were 3 gastroenteritis, 3 typhoid, 2 jaundice, 2 exanthemas of unknown origin, 2 bronchiolitis, 2 acute otitis media, 1 HHV6 acute infection, 1 malaria and 1 elevated liver enzymes of unknown origin.

3.3 Clinical predictors of HIV infection

A total of 6 symptoms, 18 clinical signs, 5 laboratory findings and 7 final diagnoses were found to be significantly associated with HIV infection in the bivariate analysis (see Table 2). Overall, the strongest predictors of HIV infection were: radiological end-point pneumonia (OR 23.1, 95% CI 7.5-71.1), lymphadenopathy at any site (OR 18.4, 6.3-53.6) - armpit being the localisation with the strongest association (OR 32.6, 8.6-124.1), followed by ≥ 2 sites (OR 25.1, 5.9-106.8), groin (OR 25.0, 6.9-91.0) and neck (OR 7.6, 2.0-28.5) -, hepatomegaly (OR 14.6, 4.9-43.2), fast breathing (respiratory rate ≥50/min for children <12 months or ≥40/min for children ≥12 months) (OR 14.3, 4.0-51.5), chest indrawing (OR 14.0, 4.1-47.6), nose flapping (OR 11.8, 3.1-45.4) and splenomegaly (OR 11.1, 3.6-33.9).

Table 2: *Demographics and symptoms, clinical signs, laboratory findings and final diagnoses predictive of HIV infection in 1004 febrile children (bivariate analysis).*
SD=Standard Deviation; NP=Naso-Pharyngeal

Demographics and symptoms	Overall (N=1004) N (%)	HIV+ (N=16) N (%)	HIV- (N=988) N (%)	OR	(95% CI)	p-value
Sick contact person	91/1003 (9.1)	4 (25.0)	87/987 (8.8)	3.4	1.1-11.0	0.03
Chronic condition	70/1003 (7.0)	5 (31.3)	65/987 (6.6)	6.4	2.2-19.3	<0.001
Recent travel history	48/1001 (4.8)	3 (18.8)	45/985 (4.6)	4.8	1.3-17.6	0.008
Difficulty to drink/breastfeed	12 (1.2)	1 (6.3)	11 (1.1)	5.9	0.7-49.1	0.06
Breahting difficulty	20 (2.0)	2 (12.5)	18 (1.8)	7.7	1.6-36.7	0.002
Cough	458 (45.6)	11 (68.8)	447 (45.2)	2.7	0.9-7.7	0.06

Clinical signs	Overall N (%)	HIV+ N (%)	HIV- N (%)	OR	(95% CI)	p-value
Low weight for age (≤ 2SD)	129/994 (13.0)	8 (50.0)	121/978 (12.4)	7.1	2.6-19.4	<0.001
High Temperature (≥ 40°C)	37 (3.7)	2 (12.5)	35 (3.5)	3.9	0.8-17.8	0.06
Tachychardia[1]	193/991 (19.5)	9 (56.3)	184/975 (18.9)	5.5	2.0-15.2	<0.001
Fast breathing[2]	243 (24.2)	13 (81.3)	230 (23.3)	14.3	4.0-51.5	<0.001
Abnormal chest auscultation	108 (10.8)	5 (31.3)	103 (10.4)	3.9	1.3-11.5	0.008
Chest indrawing	27 (2.7)	4 (25.0)	23 (2.3)	14.0	4.1-47.6	<0.001
Nose flapping	22 (2.2)	3 (18.8)	19 (1.9)	11.8	3.1-45.4	<0.001
Abdominal tenderness	33 (3.3)	3 (18.8)	30 (3.0)	7.4	2.0-27.5	<0.001
Hepatomegaly	45 (4.5)	6 (37.5)	39 (3.9)	14.6	4.9-43.2	<0.001
Splenomegaly	44 (4.4)	5 (31.3)	39 (3.9)	11.1	3.6-33.9	<0.001
Jaundice	42 (4.2)	2 (12.5)	40 (4.0)	3.4	0.7-15.4	0.09
Oral thrush	13 (1.3)	1 (6.3)	12 (1.2)	5.4	0.7-44.6	0.08
Oral ulcers	39 (3.9)	2 (12.5)	37 (3.7)	3.7	0.8-16.8	0.07
Lymphadenopathy						
- any site	47 (4.7)	7 (43.8)	40 (4.0)	18.4	6.3-53.6	<0.001
- ≥ 2 sites	12 (1.2)	3 (18.8)	9 (0.9)	25.1	5.9-106.8	<0.001
- neck	32 (3.2)	3 (18.8)	29 (2.9)	7.6	2.0-28.5	<0.001
- armpit	14 (1.4)	4 (25.0)	10 (1.0)	32.6	8.6-124.1	<0.001
- groin	17 (1.7)	4 (25.0)	13 (1.3)	25.0	6.9-91.0	<0.001

Laboratory findings	Overall N (%)	HIV+ N (%)	HIV- N (%)	OR	(95% CI)	p-value
Anemia (Hb<8g/dl)	138 (13.7)	6 (37.5)	132 (13.4)	3.9	1.4-10.9	0.005
Presence of NP Enterovirus	40 (4.0)	1 (6.3)	39 (3.9)	1.6	0.2-12.6	0.6
Presence of NP Influenza virus	190 (18.9)	0 (0.0)	190 (19.2)	0.0	.-.	0.05
Presence of NP *Staphylococcus aureus*	212/908 (23.3)	7/15 (46.7)	205/893 (23.0)	2.9	1.0-8.2	0.03
High NP *Streptococcus pneumoniae* load[3]	144/1000 (14.4)	5 (31.3)	139/984 (14.1)	2.8	0.9-8.1	0.05

[1] Heart rate >140/min for children <12 months, >130/min for children 12-≤24 months, >120 for
[2] Respiratory rate ≥50/min for children <12 months or ≥40/min for children ≥12 months.
[3] $\geq 10^7$ cfu/ml

Final diagnoses	Overall N (%)	HIV+ N (%)	HIV- N (%)	OR	(95% CI)	p-value
Severe illness	133 (13.2)	6 (37.5)	127 (12.9)	4.1	1.4-11.4	0.004
Bacterial disease	221 (22.0)	9 (56.3)	212 (21.5)	4.7	1.7-12.9	0.001
Clinical pneumonia[4]	151 (15.0)	9 (56.3)	142 (14.4)	7.7	2.8-21.1	<0.001
Radiological end-point pneumonia	31 (3.1)	6 (37.5)	25 (2.5)	23.1	7.5-71.1	<0.001
Bronchiolitis	35 (3.5)	2 (12.5)	33 (3.3)	4.1	0.9-19.0	0.05
Upper respiratory tract infection	438 (43.6)	2 (12.5)	436 (44.1)	0.2	0.0-0.8	0.01
Acute otitis media	24 (2.4)	2 (12.5)	22 (2.2)	6.3	1.3-29.5	0.008

3.3.1 Demographics and symptoms

In the bivariate analysis, a total of 6 variables were found to be significantly associated with the HIV infection: difficulty to breath (OR 7.7, 1.6-36.7), suffering from a chronic condition (OR 6.4, 2.2-19.3), difficulty to drink or breastfeed (OR 5.9, 0.7-49.1), having travelled out of the district in the last month (OR 4.8, 1.3-17.6), having a contact person presently sick (OR 3.4, 1.1-11.0) and cough (OR 2.7, 0.9-7.7) (see Table 2). The most sensitive predictor of HIV infection was cough [sensitivity (se) 68.8%] and the most specific was difficulty to drink or breastfeed [specificity (sp) 98.9%].

Four of these predictors were still significantly associated with HIV in the multivariate analysis: chronic condition (aOR 6.7, 2.2-20.4), difficulty to breath (aOR 6.4, 1.2-32.9), recent travel history (aOR 4.9, 1.3-18.8) and sick contact person (aOR 3.1, 0.9-10.2). Their respective sensitivity, specificity, positive likelihood ratio (LR+), negative likelihood ratio (LR-), aOR and 95% CI are presented in Table 3.

Table 3: *Context factors and symptoms predictive of HIV infection (multivariate analysis).*

Demographics and symptoms	Sensitivity (%)	Specificity (%)	LR+	LR-	aOR	95% CI
Chronic condition	31.3	93.4	4.7	0.7	6.7	2.2-20.4
Difficulty to breath	12.5	98.2	6.9	0.9	6.4	1.2-32.9
Recent travel history	18.8	95.4	4.1	0.9	4.9	1.3-18.8
Sick contact person	25.0	91.2	2.8	0.8	3.1	0.9-10.2

3.3.2 Clinical signs

Analysis of the clinical signs in the bivariate analysis showed that lymphadenopathy at any site (OR 18.4, 6.3-53.6) - armpit being the localisation with the strongest association (OR 32.6, 8.6-124.1), followed by ≥ 2 sites (OR 25.1, 5.9-106.8), groin (OR 25.0, 6.9-91.0) and neck (OR 7.6, 2.0-28.5) -, hepatomegaly (OR 14.6, 4.9-43.2), fast

[4] according to WHO criteria

breathing[5] (OR 14.3, 4.0-51.5), chest indrawing (OR 14.0, 4.1-47.6), splenomegaly (OR 11.1, 3.6-33.9), abdominal tenderness (OR 7.4, 2.0-27.5), weight for age ≤2 standard deviation (OR 7.1, 2.6-19.4), tachycardia[6] (OR 5.5, 2.0-15.2), oral thrush (OR 5.4, 0.7-44.6), abnormal chest auscultation (OR 3.9, 1.3-11.5), temperature ≥40°C (OR 3.9, 0.8-17.8), oral ulcers (OR 3.7, 0.8-16.8) and jaundice (OR 3.4, 0.7-15.4) were significantly more common in HIV infected children compared to the negative children (see Table 2). The predictor with highest sensitivity was fast breathing[5] (se 81.3%), and the one with highest specificity was lymphadenopathy ≥ 2 sites (sp 98.9%).

In the multivariate analysis, clinical signs predictive of HIV infection were: lymphadenopathy at any site (aOR 21.0, 5.3-84.2), abdominal tenderness (aOR 16.6, 3.0-91.3), fast breathing[5] (aOR 10.7, 2.5-45.5), weight for age ≤2SD (aOR 7.1, 2.1-24.6), chest indrawing (aOR 6.9, 1.3-38.4) and, hepatomegalia (aOR 4.5, 1.1-19.0). Their respective sensitivity, specificity, positive likelihood ratio (LR+), negative likelihood ratio (LR-), aOR and 95% CI are detailed in Table 4.

Table 4: *Clinical signs predictive of HIV infection (multivariate analysis).*

Clinical signs	Sensitivity (%)	Specificity (%)	LR+	LR-	aOR	95% CI
Lymphadenopathy at any site	43.8	96.0	10.8	0.6	21.0	5.3-84.2
Abdominal tenderness	18.8	97.0	6.2	0.8	16.6	3.0-91.3
Fast breathing[5]	81.3	76.7	3.5	0.2	10.7	2.5-45.5
Low weight for age (≤2SD)	50.0	87.6	4.0	0.6	7.1	2.1-24.6
Chest indrawing	25.0	97.7	10.7	0.8	6.9	1.3-38.4
Hepatomegaly	37.5	96.1	9.5	0.7	4.5	1.1-19.0

3.3.3 Laboratory findings

Regarding laboratory findings, anemia (Hb<8g/dl) (OR 3.9, 1.4-10.9) was found to be significantly more present in HIV infected children. The comparison of the analyses of various respiratory virus and bacteria PCR on nasopharyngeal (NP) swabs showed some significant differences: the presence of *Staphylococcus aureus* (OR 2.9, 1.0-8.2) was predictive of HIV infection, the prevalence of *Streptococcus pneumoniae* was the same in both HIV positive and negative children (94% and 84% respectively); however the bacteria load was significantly higher in HIV positive children (OR 2.8, 0.9-8.1) with a threshold of 10^7 cfu/ml). The presence of enterovirus (OR 1.6, 0.2-12.6) in NP swabs was as well found to be associated with HIV infection. On the contrary, the presence of Influenza virus (OR 0.0) was found to be more frequent in HIV negative than in HIV positive children (see Table 2). The most sensitive predictor of HIV infection was found

[5] Respiratory rate ≥50/min for children <12 months or ≥40/min for children ≥12 months.
[6] heart rate >140/min for children <12 months, >130/min for children 12-≤24 months, >120 for children 24-≤58 months, >110 for children ≥58 months

to be the presence of NP *Staphylococcus aureus* (se 46.7%) and the most specific the presence of nasopharyngeal enterovirus (sp 96.1%).

In the multivariate analysis, anemia (hb<8g/dl) (aOR 4.1, 1.4-12.0), high NP *Streptococcus pneumoniae* load (aOR 3.6, 1.2-11.0) and presence of NP *Staphylococcus aureus* (aOR 3.5, 1.2-10.3) were found to be independent predictors of HIV infection. Their respective sensitivity, specificity, positive likelihood ratio (LR+), negative likelihood ratio (LR-), aOR and 95% CI are listed in Table 5.

Table 5: *Laboratory findings predictive of HIV infection (multivariate analysis).*

Laboratory findings	Sensitivity (%)	Specificity (%)	LR+	LR-	aOR	95% CI
Anemia (Hb<8g/dl)	37.5	86.6	2.8	0.7	4.1	1.4-12.0
Presence of NP *Staphylococcus aureus*	46.7	77.0	2.0	0.7	3.5	1.2-10.3
High NP *Streptococcus pneumoniae* load[7]	31.3	85.9	2.2	0.8	3.6	1.2-11.0

3.3.4 Final diagnoses

Comparison of final diagnoses by bivariate analysis showed that radiological end-point pneumonia (OR 23.1, 7.5-71-1), acute otitis media (OR 6.3, 1.3-29.5), suffering from any bacterial diseases (OR 4.7, 1.7-12.9), bronchiolitis (OR 4.1, 0.9-19.0) and presenting a severe illness (OR 4.1, 1.4-11.4) were statistically associated with HIV infection. On the contrary, suffering from an upper respiratory tract infection (OR 0.2, 0.0-0.8) was significantly more frequent in HIV negative children (see Table 2). Suffering from a bacterial infection and being diagnosed with a clinical pneumonia were the most sensitive predictors of HIV infection both with a sensitivity of 56%. The most specific predictor was acute otitis media (97.8%).

Table 6: *Final diagnoses predictive of HIV infection (multivariate analysis).*

Final diagnoses	Sensitivity (%)	Specificity (%)	LR+	LR-	aOR	95% CI
Acute otitis media	12.5	97.8	5.6	0.9	82.2	5.8-1159.6
Radiological end-point pneumonia	37.5	97.5	14.8	0.6	19.5	5.7-66.5
Bronchiolitis	12.5	96.7	3.7	0.9	4.9	0.9-25.9
Severe condition	37.5	87.1	2.9	0.7	2.8	0.9-8.8
Upper respiratory tract infection	12.5	55.9	0.3	1.6	0.1	0.0-1.4

[7] $\geq 10^7$ cfu/ml

Independent diagnoses predictive of HIV infection were acute otitis media (aOR 82.2, 5.8-1159.6), radiological end-point pneumonia (aOR 19.5, 5.7-66.5), bronchiolitis (aOR 4.9, 0.9-25.9) and severe illness (aOR 2.8, 0.9-8.8). Having an upper respiratory tract infection (aOR 0.1, 0.0-1.4) was independently associated with the fact of being HIV negative. Their respective sensitivity, specificity, positive likelihood ratio (LR+), negative likelihood ratio (LR-), aOR and 95% CI are detailed in Table 6.

3.3.5 HIV testing according to IMCI criteria

The performance of the IMCI criteria used in Tanzania at the time of the study to decide on HIV testing are the following (in descending order of sensitivity): clinical pneumonia (se 56.3%, sp 85.6%), weight for age ≤2SD (se 50.0%, sp 87.6%), lymphadenopathy at ≥ 2 sites including neck, armpit and groin (se 18.8%, sp 99.1%), ear discharge (se 12.5%, sp 99.5%), oral thrush (se 6.3%, sp 98.8%), persistent diarrhea (se 6.3%, sp 100%) and parotid swelling (found in only one child) (se 0%, sp 99.9%). Their respective sensitivity, specificity, positive likelihood ratio (LR+) and negative likelihood ratio (LR-) are listed in Table 7.

Table 7: *Performance of the criteria used in the HIV branch of the IMCI algorithm*

Criteria	**HIV + N=16 N (%)**	**HIV- N=988 N (%)**	**Sensitivity (%)**	**Specificity (%)**	**LR+**	**LR-**
Clinical pneumonia[8]	9 (56.3)	142 (14.4)	56.3	85.6	3.9	0.5
Weight for age ≤2SD	8 (50.0)	121/978 (12.4)	50.0	87.6	4.0	0.6
Lymphadenopathy ≥2 sites[9]	3 (18.8)	9 (0.9)	18.8	99.1	20.6	0.8
Ear discharge	2 (12.5)	5 (0.5)	12.5	99.5	24.7	0.9
Oral thrush	1 (6.3)	12 (1.2)	6.3	98.8	5.1	0.9
Persistant diarrhea ≥14 days	1 (6.3)	0 (0)	6.3	100	-	0.9
Parotid swelling	0 (O)	1 (0.1)	0	99.9	0.0	1.0
Algorithm	**HIV + N=16 N (%)**	**HIV- N=988 N (%)**	**Sensitivity (%)**	**Specificity (%)**	**LR+**	**LR-**
1 or more criteria for HIV	15 (93.8)	260 (26.3)	93.8	73.7	3.6	0.1
2 or more criteria for HIV	9 (56.3)	28 (2.8)	56.3	97.2	19.8	0.5
3 criteria for HIV	0 (0)	2 (0.2)	0	99.8	0	1.0

[8] according to WHO criteria
[9] Including neck, armpit and groin

Performance of the whole HIV branch was also assessed. When using only one criterion to classify the child for suspected HIV-infection, 15 HIV positive children were detected by the algorithm (se 93.8%, sp 73.7%). When using 2 or more criteria, the algorithm detected 9 out of the 16 HIV-positive children (se 56.3%, sp 97.2%) and when using 3 criteria none of the HIV-positive children were detected (se 0%, sp 99.8%)(see Table 7).

3.3.6 New HIV algorithm

New clinical HIV algorithms were generated using the best predictors found in the above analyses. The algorithm found to have the best global performance is presented here. Its diagnostic performance is the following: when using only one criterion to classify the child for suspected HIV-infection, 15 out of 16 HIV-positive children were detected by the algorithm (se 93.8%, sp 81.4%). When using 2 or more criteria, the algorithm detected 10 HIV-positive children (se 62.5%, sp 97.4%) and when using 3 criteria only 1 HIV-positive children was detected (se 6.3%, sp 99.8%) (see table 8). The respective sensitivity, specificity, positive and negative likelihood ratios of each criteria used in the new algorithm are presented in table 8.

Table 8: *Performance the improved HIV algorithm and of each of the criteria used*

Criteria	**HIV + N=16 N (%)**	**HIV- N=988 N (%)**	**Sensitivity (%)**	**Specificity (%)**	**LR+**	**LR-**
Lymphadenopathy at any site	7 (43.8)	40 (4.0)	43.8	96.0	10.8	0.6
Radiological end-point pneumonia	6 (37.5)	25 (2.5)	37.5	97.5	14.8	0.6
Chronic condition	5 (31.3)	65/987 (6.6)	31.3	93.4	4.7	0.7
Abdominal tenderness	3 (18.8)	30 (3.0)	18.8	97.0	6.2	0.8
Difficulty to breath	2 (12.5)	18 (1.8)	12.5	98.2	6.9	0.9
Acute otitis media	2 (12.5)	22 (2.2)	12.5	97.8	5.6	0.9
Oral thrush	1 (6.3)	12 (1.2)	6.3	98.8	5.1	0.9
Algorithm	**HIV + N=16 N (%)**	**HIV- N=988 N (%)**	**Sensitivity (%)**	**Specificity (%)**	**LR+**	**LR-**
1 or more criteria for HIV	15 (93.8)	184 (18.6)	93.8	81.4	5.0	0.1
2 or more criteria for HIV	10 (62.5)	26 (2.6)	62.5	97.4	23.8	0.4
3 criteria for HIV	1 (6.3)	2 (0.2)	6.3	99.8	30.9	0.9

4. Discussion

The HIV infection prevalence of 1.6%, found in febrile children in the present study, is very close to the 1.7% reported in a study realized in 1995 in Tanzania on children aged from 18 months to 5 years, attending maternal and child health clinics in Dar es Salaam (21). However, it is lower than previous studies conducted in Tanzania in 2000 in Nigeria, or Zimbabwe which included hospitalized children with more severe conditions, probably leading to a higher HIV prevalence and advanced AIDS stage (17), (27), (10). Nevertheless, it remains twice higher than the national estimated 2015 burden of HIV infection in children less than 15 years (whether sick or not) (28).

Some of the clinical symptoms, signs and diagnoses found in the present study to be predictive of HIV infection correlate well with the known presentation of paediatric HIV infection. Several predictors were similar to those already published in other studies such as: low weight for age (10) (12) (17) (21) (27), lymphadenopathy (10) (12) (15) (17) (21) (27) (29), hepatomegaly (10), (12) (18), (27), splenomegaly (10), (12), (18), (27), jaundice (10), (27), pneumonia (13) (29) acute otitis media (13) (27), oral thrush (10), (12), (17), (18), (27), (29), and oral ulcers (10), (17), (29).

Others predictors that we found were new and had to our knowledge, never been reported in previous studies. Sick contact person for example, was found to be an independent predictor of HIV infection. As HIV infection in children is most of the time due to mother-to-child-transmission, this predictor can be explained by the consequence of the HIV infection on the health of other family members. Indeed, mother, father and/or other siblings may as well be HIV-positive and consequently more often sick, or might be simply exposed to more infectious diseases brought in by the HIV-positive member (as known for tuberculosis). The fact that travel history out of the district was a predictor may be explained by the possible suspicion by the guardians about their or the child's positive HIV status that motivated them to travel to a district hospital having an HIV specialized clinic with the aim to get appropriate care.

Anemia is a known feature of HIV infection but had to our knowledge not been formally reported as predictive of the infection. Difficulty to breath, cough, abnormal chest auscultation, fast breathing, chest indrawing and nose flapping are explained by the increased risk of HIV infected children to suffer from severe respiratory infections. The presence of *Staphylococcus aureus* and a high load of *Streptococcus pneumoniae* in the nasopharynx are very interesting predictors that can be explained by the tendency of HIV infected children to be colonised by bacteria at a higher rate, even before developing a symptomatic infection. Upper respiratory tract infection was found to be less prevalent in HIV positive children, whereas bronchiolitis and radiological end-point pneumonia were predictive of the HIV infection, highlighting the tendency of HIV infected children to present more severe respiratory infections, possibly also more often due to bacteria than viruses. Indeed, the diagnosis of bacterial disease was found to be associated with the HIV infection.

Other predictors such as difficulty to drink or breastfeed, temperature ≥40°C and severe illness were found to be associated with the HIV infection and could reflect the tendency of HIV infected children to have a worse presentation or more complications of a common illness compared to HIV negative children.

Abdominal tenderness was also found to be significantly associated with the HIV infection and could be explained for example by painful sensation caused by an expansion and inflammation of the liver or the spleen frequent in HIV-infected children. It could be as well due to an underlying abdominal infectious process, or simply to a more severe presentation of the disease. The interesting point of this predictor is that it is an easier sign to understand and assess than hepato-splenomegaly for health workers who do not have much experience. Hepato-splenomegaly had not been included in the HIV branch of the IMCI algorithm because of its difficulty to assess, abdominal tenderness could therefore be a good alternative and contribute to improve its performance.

Regarding the criteria included in the HIV branch of IMCI, pneumonia, oral thrush, very low weight for age and enlarged lymph gland were also found to be significantly predictive of HIV infection in the present study. Ear discharge was found to be significant but was not included in the analyses as it was present in less than 10 children. Persistent diarrhoea, which generally does not present with acute fever and parotid swelling, were both found in only 1 child and could therefore not be included in the analysis. If the decision of HIV testing had been made based on the IMCI criteria's used in Tanzania at that time, only 9 out of 16 children would have been referred to a VCT clinic. This represents only 56% of the HIV positive children, which is largely insufficient taking into account the dramatic consequences of not being diagnosed for this disease. If only one criterion would be used to refer the child, 15 (94%) of the HIV positive children would have been detected, but with a quite high number needed to test (27% of all children should be tested). In a country with high HIV prevalence and high child mortality rate, a highly sensitive algorithm is desirable for early identification and referral in order to limit disease progression and mortality. Nevertheless, a highly specific algorithm is wished as well to save tests in settings suffering of constant shortage of tests and medicines.

When using one criterion as cut-off, the new HIV clinical algorithm generated in this study improves the specificity of the algorithm while keeping the same sensitivity when compared to the IMCI algorithm (using also 1 criteria as cut-off). When using 2 or more criteria, the sensitivity improves while the specificity remains the same when compared to the IMCI algorithm (using also 2 criteria as cut-off) . WThe new algorithm thus enables to reduce the number of children needed to test from 275 (27%) with IMCI to 199 (20%). This would enable to save some tests in periods of shortage by providing better guidance to clinicians on which febrile children should be tested in priority without decreasing the sensitivity of the algorithm. This new algorithm is not aimed to replace the IMCI HIV branch, but more to show that it is still possible to improve by

introducing new predictors such as abdominal tenderness, difficulty to breath or chronic condition for example, or by changing some of the actual criteria.

Furthermore, in order to diagnose the highest number of positive children it would be better to only use, for the new algorithm, one criterion as cutoff instead of 2. It would increase the number of children to test from 36 (3.6%) to 199 (19.8%) but this number remains reasonable.

The clinical presentation of paediatric HIV infection is very variable and may often overlap with common childhood illnesses symptoms. Some of the infected children will develop severe symptoms during their first year of life while others will live years with an asymptomatic infection. Due to those facts, the use of a clinical algorithm to classify weather a child is potentially infected or not, remains risky and complicated. It might be able to detect children with significant immunosuppression but not those with normal or close to normal CD4 counts. This is why in theory, as recommended in the Tanzanian fourth edition (April 2012) of the national guidelines for the management of HIV: *"Any sick child, whether qualifying by the IMCI algorithm or not, should be offered HIV testing by provider-initiated testing and counselling (PITC) to establish the infection status as early as possible*" (30). However, in Tanzania and other resource-limited countries where diagnostic tools may not always be widely available, a clinical identification using combinations of specific and sensitive signs and symptoms is crucial to provide guidance for focused HIV testing and further referral. This study has identified several predictors, some already described in other studies and other rather new. Those predictors could serve to adjust actual algorithm to assess HIV infection.

5. Limitations

The main limitation of this study is the low number of children that were HIV infected. Indeed, the presentation of those 16 cases may not be representative of the entire HIV paediatric population, which might have introduced a bias, and some interesting predictors may have been absent in the children studied because too rare.

Another limitation comes from the fact that only febrile children presenting with an axillary temperature ≥ 38°C were included in the present study, and not all children presenting with an acute medical problem to health facilities. However, in Tanzania 80% of the children consulting in medical centre have a history of fever (but not necessarily a temperature above 38°C) (31).

6. Conclusion

The clinical presentation of paediatric HIV infection is wide and various. If in theory all febrile children consulting health facilities should be offered HIV testing, in Tanzania and other resource-limited settings where testing might not be always available, the use of a clinical algorithm remains important to enable an early detection and treatment of infected children. This study has identified several interesting predictors of the HIV infection in febrile children attending heath facilities in Tanzania. The predictors found in the present study and by other studies could therefore assist in predicting HIV infection among children attending heath facilities and improve the performance of the actual algorithm. Considering that IMCI has started to be used in an electronic format running on mobile phones and tablets in Tanzania and Burkina Faso, a more complex score with higher accuracy, which would be calculated automatically by the machine, could be afforded. This would be a simple and thus very attractive tool to develop in the near future.

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