Optimising Specimen Collection in Children

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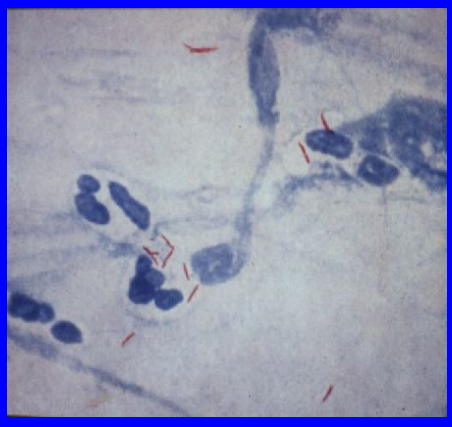
What is burden of childhood TB?

- Paediatric burden under recognised
  - 20% in high incidence
- WHO, national notifications - smear positive cases
  - 0-4 yr and 5-14 yr
- TB diagnosis in children ???



Principles for TB diagnosis in children - challenges

- Do no harm
  - don't compromise existing care or access
- Ensure access and equity
  - for all children with suspected TB
- Promote quality and efficiency
  - deliver best care within public health approach
- Ensure sustainability

Guidelines for treatment of TB in children WHO 2010

Diagnosis of TB

Detection of organism

- Smear
- Culture
- Antigen detection
- Molecular methods

Detection of immune response

- Tuberculin skin test, interferon, serology

Existing methods in children

- Clinical
- Radiological
- Immune diagnosis
  - Skin testing
- Culture confirmation
  - Smear and culture

New possibilities

Organism detection

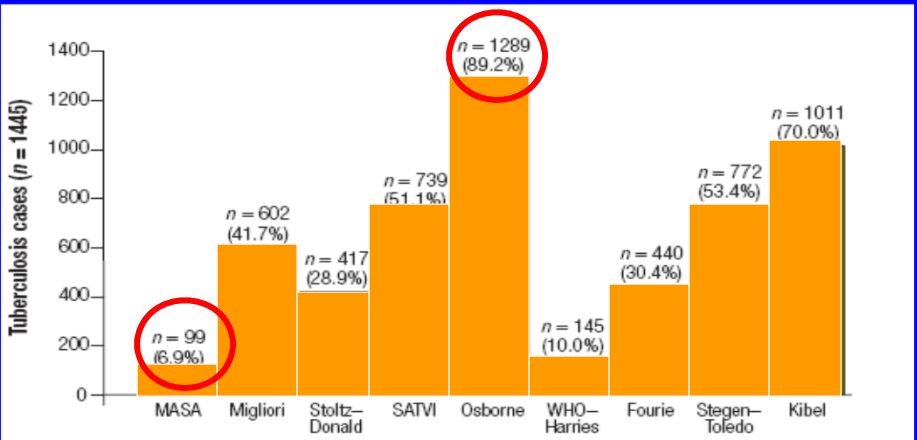
- New culture & drug susceptibility tests
- Rapid molecular methods
- Antigen detection

Immune detection

- Interferon assays
- Serology

Clinical diagnosis

Proportion of children diagnosed with TB using 9 different scoring systems



MASA, Medical Association of South Africa; SATVI, South African Tuberculosis Vaccine Initiative; WHO, World Health Organization.

Hatherhill et al Bull WHO 2010

Tuberculin skin testing

- Technique dependent
- Variability in measurement
- Standardised application, interpretation
- What constitutes a positive result?
  - Epidemiology MTB, NTM
  - HIV – 5mm
  - BCG
- Poor sensitivity in HIV-infected, malnourished

Radiological diagnosis

- Non-specific
- Hilar adenopathy – wide inter and intra-observer variation – *Swingler Arch Dis Child 2005*
  - inter-observer agreement 30%
  - accuracy not improved with lateral CXR
- High prevalence of HIV associated chronic lung disease – 1/3 by age 3-4yrs, *Norton 2001*

Microbiological diagnosis

Microbiological diagnosis

- NOT standard for diagnosis in children
- Prevailing perceptions:
  - not feasible / possible / practical
  - not useful - paucibacillary disease
  - not possible in infants or young children
  - no place in primary care settings
- Different diagnostic standard for adults

Microbiological confirmation children

- BUT micro diagnosis is
  - possible
  - feasible in very young
  - confirms diagnosis
  - especially useful for drug resistant TB
- specimens -gastric lavage, induced sputum, NPAs, ear swabs, blood, stool, nasal secretions, fine needle aspiration

Why make a microbiological diagnosis in children?

- Confirmed diagnosis
- Drug resistance
- Better, rapid methods for diagnosis – ensures timely treatment
  - > 40% children with culture confirmed TB, discharged without treatment *Moore PIDJ 2011*
- Potential for rapid deterioration and spread
- HIV and TB – pill burden, adherence, adverse reactions

But the quality and type of specimen are crucial.....

- Performance of diagnostic test dependent on quality and quantity of specimens
- Children unable to spontaneously produce produce sputum
- Requires health care worker training
- Small volume specimens obtained
- Children – smear negative
- Extra pulmonary disease

What specimen?

- Gastric lavage were standard for diagnosis
  - 3 sequential GLs
- Overnight starve
- Unpleasant for child and person doing GL
- Hospitalisation
- Yield low - positive in 10-20% children with PTB

Other (? improved) specimens?

- Induced sputum
- Nasopharyngeal specimen
- Ear secretions
- String test
- Fine needle aspiration of node
- Bronchoalveolar lavage (BAL)

Induced sputum versus gastric lavage for microbiological confirmation of pulmonary tuberculosis in infants and young children: a prospective study

Lancet 2005; 365: 130–34

See Comment page 97

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Summary

Background For microbiological confirmation of diagnosis of pulmonary tuberculosis in young children, sequential gastric lavages are recommended; sputum induction has not been regarded as feasible or useful. We aimed to compare the yield of *Mycobacterium tuberculosis* from repeated induced sputum with that from gastric lavage in young children from an area with a high rate of HIV and tuberculosis.

Methods We studied 250 children aged 1 month to 5 years who were admitted for suspected pulmonary tuberculosis in Cape Town, South Africa. Sputum induction and gastric lavage were done on three consecutive days according to a standard procedure. Specimens were stained for acid-fast bacilli; each sample was cultured singly for *M tuberculosis*.

Findings Median age of children was 13 months (IQR 6–24). A positive smear or culture for *M tuberculosis* was obtained from 62 (25%) children; of these, 58 (94%) were positive by culture, whereas almost half (29 [47%]) were smear positive. Samples from induced sputum and gastric lavage were positive in 54 (87%) and 40 (65%) children, respectively (difference in yield 5·6% [1·4–9·8%], p=0·018). The yield from one sample from induced sputum was similar to that from three gastric lavages (p=1·0). Microbiological yield did not differ between HIV-infected and HIV-uninfected children (p=0·17, odds ratio 0·7 [95% CI 0·3–1·3]). All sputum induction procedures were well tolerated; minor side-effects were increased coughing, epistaxis, vomiting, or wheezing.

Interpretation Sputum induction is safe and useful for microbiological confirmation of tuberculosis in young children. This technique is preferable to gastric lavage for diagnosis of pulmonary tuberculosis in both HIV-infected and HIV-uninfected infants and children.

Induced sputum vs gastric lavage

- 250 children with suspected PTB
  - median age 13 (6-24) months
- 3 IS vs 3 GLs
- pos smear or culture in 62 (25%)
  - **yield IS (54, 87%) better than GL (40, 65%) – OR 2.8** (1.2 - 7.2), p=0.018
  - **single IS (41, 66%) equivalent to 3 GLs** (40, 65%), OR 1.1, p=1.0

Zar et al, Lancet 2005

IS vs GL

- youngest child undergoing IS - 1 month
- youngest positive IS culture - 3 months
  - **48% younger than 1 year**
- no difference in culture rates by HIV status
- 2nd IS increased yield by 15%
- 3rd IS increased yield by 7%

Zar et al, Lancet 2005

Sputum collection is routine in adult TB programs in primary care

IS at primary care facility

- 270 children with suspected PTB at Kuyasa clinic in Khayelitsha, South Africa
  - median age 38 months
- 2 IS specimens on sequential days / same day – smear, liquid culture
- IS in 269 (99%)
- 29 (10.7%) positive for *M tuberculosis*
  - 7 (24%) HIV-infected

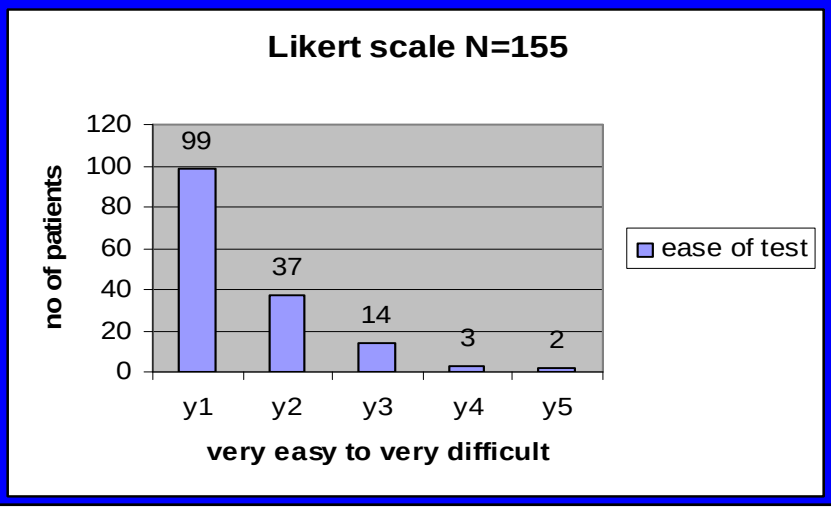
Moore et al, Int J Tub Lung Dis, 2011

IS at primary care facility

- 18 children not clinically diagnosed had positive microbiology and treated thereafter
- IS improved diagnostic yield from 65 (clinically Dx) to 83 cases
  - 22% increase
- IS well tolerated, no severe adverse events

Moore et al Int J Tuber Lung Dis 2011

How easy is it to do sputum induction?



Moore et al Int J Tuber Lung Dis in press

Nasopharyngeal aspirate (NPA)

- 218 children with suspected PTB, age 4 years
- 2 GL vs 2 NPA
  - culture (MODS, L-Jensen)
  - in-house PCR
- 22 (10%) had 1 positive culture TB
- GL detected all cases, NPA only 12 cases (54%)

Oberhelman et al, Lancet ID 2010

NPA vs GL – PCR

- PCR only 62% sensitivity, 90% specificity for culture positive cases
  - 43% sensitivity, 90% specificity on NPA
  - 62% sensitivity, 90% specificity on GL
- positive PCR on healthy controls (6%)
  - 1 PPD positive; 2% false positive

Oberhelman et al Lancet ID 2010

Molecular methods for detection of *M tuberculosis* & resistance

Genotype MTBDR or MTBDR*plus* Haine test

- use PCR reverse hybridisation to detect MTB complex & drug resistant mutations
  - *rpoB and inhA / katG* mutations
- only on smear or culture positive specimen
  - sensitivity smear negative – 33%
- good detection Rif resist; variable, lower INH
- GenoType MTBDR*sl* - resistance to fluoroquinolone, aminoglycosides, ethambutol

www.finddiagnostics.org

GeneXpert

- Adult data (1 test) – 92% sensitivity PTB
  - 72% for smear negative (85% with 2 tests)
  - specificity 99%, *Boehme NEJM 2010*
- WHO recommended 2011 *www.who.int/tb/laboratory/*
  - Xpert replace smear for PTB as initial diagnostic test in areas of high HIV or drug resistant TB
  - consider as a follow-on test to smear in other areas
- No published data in children

Accuracy of the Xpert MTB/RIF test for the diagnosis of pulmonary tuberculosis in children admitted to hospital in Cape Town, South Africa: a descriptive study

Mark P Nicol, Lesley Workman, Washiefa Isaacs, Jacinta Munro, Faye Black, Brian Eley, Catharina C Boehme, Widaad Zemanay, Heather J Zar

Summary

Background WHO recommends that Xpert MTB/RIF replaces smear microscopy for initial diagnosis of suspected HIV-associated tuberculosis or multidrug-resistant pulmonary tuberculosis, but no data exist for its use in children. We aimed to assess the accuracy of the test for the diagnosis of pulmonary tuberculosis in children in an area with high tuberculosis and HIV prevalences.

Methods In this prospective, descriptive study, we enrolled children aged 15 years or younger who had been admitted to one of two hospitals in Cape Town, South Africa, with suspected pulmonary tuberculosis between Feb 19, 2009, and Nov 30, 2010. We compared the diagnostic accuracy of MTB/RIF and concentrated, fluorescent acid-fast smear with a reference standard of liquid culture from two sequential induced sputum specimens (primary analysis).

Results 452 children (median age 19·4 months, IQR 11·1–46·2) had at least one induced sputum specimen; 108 children (24%) had HIV infection. 27 children (6%) had a positive smear result, 70 (16%) had a positive culture result, and 58 (13%) had a positive MTB/RIF test result. With mycobacterial culture as the reference standard, MTB/RIF tests when done on two induced sputum samples detected twice as many cases (75·9%, 95% CI 64·5–87·2) as did smear microscopy (37·9%, 25·1–50·8), detecting all of 22 smear-positive cases and 22 of 36 (61·1%, 44·4–77·8) smear-negative cases. For smear-negative cases, the incremental increase in sensitivity from testing a second specimen was 27·8% for MTB/RIF, compared with 13·8% for culture. The specificity of MTB/RIF was 98·8% (97·6–99·9). MTB/RIF results were available in median 1 day (IQR 0–4) compared with median 12 days (9–17) for culture (p<0·0001).

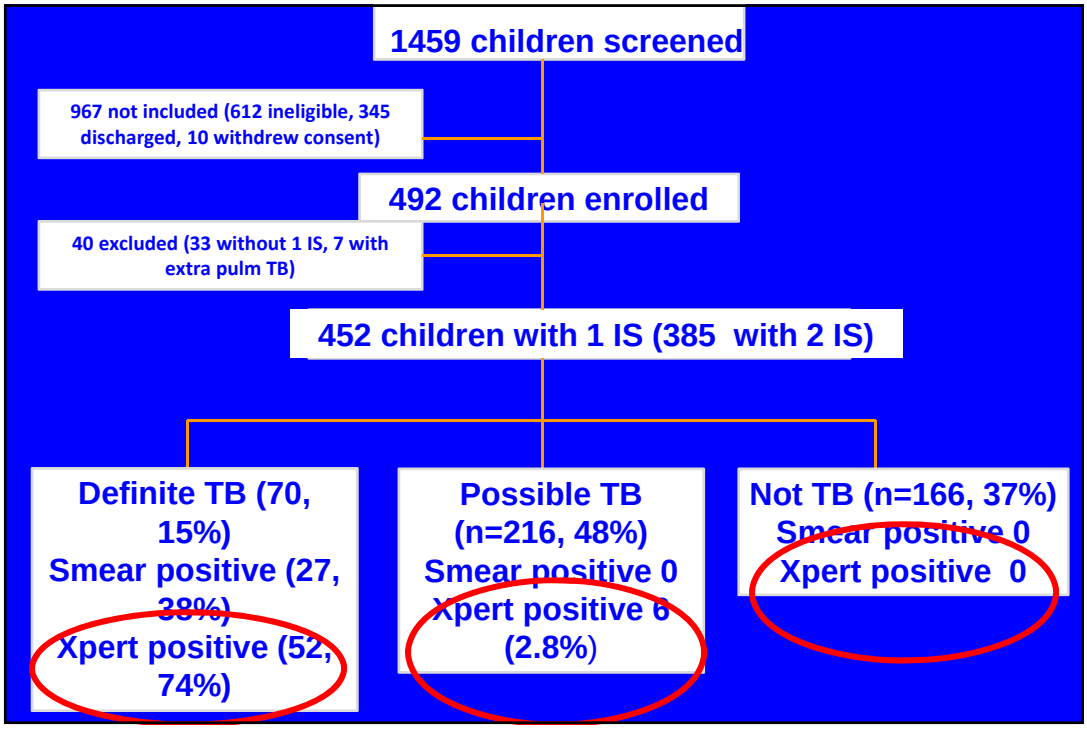
Interpretation MTB/RIF testing of two induced sputum specimens is warranted as the first-line diagnostic test for children with suspected pulmonary tuberculosis.

Lancet ID July 2011

Xpert in children

- children hospitalised with suspected PTB
- 2 IS for smear, liquid culture, Xpert
- 492 children enrolled
  - 452 (92%) had 1 IS, 385 (78%) had 2 IS
- median age – 19.4 months
- 70 (16%) culture confirmed TB
- Xpert detected 52/70 (74%) overall

Nicol et al Lancet ID 2011



Xpert results

- Xpert positive in 52/70 (74%) definite TB
- Xpert positive in 6/216 (2.8%) possible TB
- Xpert positive in 0/135 (0%) NOT TB cases

Nicol et al Lancet ID 2011

Results – single IS

- A single IS detected 62 / 70 (88%) of definite TB by culture
- Xpert positive in 41/62 (66.1%)
  - all smear positive cases (22/22, 100%)
  - 9/40 (47.5%) smear negative cases

Nicol et al Lancet ID 2011

Results – two induced sputa

- 385 (78%) children had 2 IS
- 58/385 (15.1%) culture positive cases (definite TB)
- 22/58 cases (37.9%) smear positive
- Xpert positive in 44/58 (75.9%)
  - all smear positives (22/22, 100%)
  - 22/36 (61.1%) smear negative cases

Nicol et al Lancet ID 2011

Xpert results – two sputa

- Incremental yield from 2nd specimen in smear neg
  - by culture was 8 cases **(13.8% increment)**
  - by smear was 0 cases (all pos on first IS)
  - by Xpert - 12/36 (27.8%) to 22/36 (61.1%)
    - **27.8% increment**

Nicol et al Lancet ID 2011

Xpert by HIV status

- sensitivity of Xpert higher in HIV-infected children
  - 14/14 (100%) definite HIV-TB cases detected vs 30/44 (68.2%) in HIV-uninfected, p=0·042
  - *but* more smear-positive in HIV infected [9/14 (64.3%) vs. 13/44 HIV-uninfected (29.5%), OR 2.25, 95% CI 0.93 – 5.44], p=0.072

Nicol et al Lancet ID 2011

Specificity Xpert – two sputa

- 323/327 (98.8%)
- 6 children in possible TB group has positive Xpert with negative culture - ? true positives
  - 4 treated for *M tuberculosis* with clinical improvement
- none of the children in NOT TB group had positive Xpert
  - specificity using this group – 100%

Nicol et al Lancet ID 2011

Rifampicin resistance

- Xpert correctly identified all 70 cases of Rif sensitive *M tuberculosis* compared to line probe assay on a per sample analyis
- 2 Rif resistant isolate identified by both Xpert and line probe assay
- 1 case of Rif resistance on line probe that were inconclusive on Xpert

Nicol et al Lancet ID 2011

Time to result and invalid results

- Xpert significantly faster than culture
  - median time to result 1 (0-1) day Xpert vs 12 (9-17) days culture, p<0.001
- fewer Xpert tests were recorded as failures or invalid (1/867, 0.1%) compared to cultures which were contaminated (11/867, 1.3%)

Nicol et al Lancet ID 2011

Conclusion

- 2 Xpert tests on 2 induced sputum specimens detected most cases (76%) PTB in young children
- 100% sensitive smear pos; 61% smear neg
- Xpert detected twice as many cases as smear
- 28% incremental yield with a 2nd Xpert test
- specificity 99%
- results faster than culture

Conclusion

- Xpert is an important advance in the diagnosis of PTB in HIV-infected and uninfected children
- Need for rapid up-scaling of IS in children in health facilities and for use of Xpert
- Need for improved POC test for paediatric TB

Challenges

- Upscale induced sputum in children in health facilities
  - Training
  - Operational issues
  - Laboratory issues
  - Cost
- Shift in thinking of health care professionals, policy makers etc – MICROBIOLOGICAL diagnosis possible and important in children

SA roll out of Xpert….

- Little attention to childhood cases or to algorithm for testing
- MUST include children in future diagnostic development and testing and operational research