

Tuberculosis Lymphadenitis, A Diagnostic Problem in Areas of High Prevalence of HIV and Tuberculosis

M J Boeree, A. Kamenya, G Liomba, B Ngwira, V R Subramanyam, A D Harries

- 1 College of Medicine, Blantyre
- 2 National Tuberculosis Program, Lilongwe

AUTHOR FOR CORRESPONDENCE:

M J Boeree
Private Bag 360
Chichiri
Blantyre 3

Acknowledgement

This article has previously been published in Transactions of the Royal Society of tropical Medicine and Hygiene by Bekedam et al 1997, 91, 294 - 297. The editorial board of the Malawi Medical Journal are grateful to Royal Society of Tropical Medicine and Hygiene for permission to republish in a modified form.

Introduction

Malawi has high human immunodeficiency virus (HIV) infection rates. In 1993, 32% of mothers visiting the antenatal clinic in an urban area, and 12% in rural areas, were sero-positive for HIV (1). The HIV epidemic is associated with a marked increase in the number of cases of tuberculosis in general (2) and extra-pulmonary cases in particular (3, 4, 5). In Malawi the number of tuberculosis cases has increased threefold from 6301 to 19195 over the last 10 years (1986 - 1995). In the same period, the Queen Elizabeth Central Hospital in Blantyre registered 4 times as many tuberculosis cases in total, and extra-pulmonary tuberculosis increased twelvefold to 893 in 1995 (6). The greater increase at the hospital reflects the higher HIV infection rates in urban Malawi (7).

The influence of HIV infection on diagnostic methods for tuberculous lymphadenitis is uncertain, despite clear descriptions of the non-reactive histopathology related to HIV infection (8). To develop recommendations for a protocol that takes into account limited laboratory facilities and availability of personnel at the district level., a prospective study was conducted to compare basic diagnostic procedures for tuberculous lymphadenitis with the results of histology and/or culture. The influence of HIV infection on these findings was assessed. The study took place in a routine outpatient department setting. Except for HIV testing, no additional resource was made available or used to carry out the research.

Methods

Selection of patients

One hundred outpatients aged 15 - 55 years attending the Queen Elizabeth Central Hospital in Blantyre were selected during the period April 1994 to April 1995. Patients who presented with lymphadenopathy in one or more extra-inguinal sites were issued with general antibiotics for 7-10 days and reviewed after a week. Patients who did not improve on treatment were included in the study. After pre-counselling and consent, blood for HIV testing was taken, and an appointment was made for needle aspiration and lymph node excision.

Procedures

Under local anaesthesia, wide needle aspiration of an enlarged lymph node was performed with a 19-gauge needle (GEM et al., 1993). The aspirate was spread on slides and dried in air. The same lymph node was then removed, cut in half, and macroscopically examined for caseation. Fluid from the cut surface of the lymph node was spread on slides and air-dried. Slides were stained by the Ziehl-Neelsen (ZN) method and examined for acid-fast bacilli. Half of the lymph node was fixed in formalin and sent for histological examination. The other half was used to inoculate tuberculosis cultures. Blood sera were tested for HIV antibodies.

Definition of tuberculous lymphadenitis

The diagnosis of tuberculous lymphadenitis was established when the culture yielded mycobacteria and/or a histology result showed granulomatous and caseating lymphadenitis consistent with tuberculosis (this was the definitive standard technique). Non-tuberculous lymphadenitis was diagnosed when both histology and culture results were inconsistent with tuberculosis.

Results

From 100 patients enrolled in 52 cases all procedures were carried out in accordance with the protocol. Incomplete results were related to unrecorded observations, lost specimens, sending incorrect specimens, and the fact that 10 patients failed, for unknown reasons, to report for their surgical procedures. Thirty-eight of these 52 patients (73%) were diagnosed as tuberculous lymphadenitis, and 32 of the 38 (84%) were HIV seropositive. Non-tuberculous lymphadenitis patients had slightly lower HIV seropositivity (11/14 or 79%). The HIV status of the 48 patients excluded from further analysis was similar, 85% (40/47) were HIV seropositive.

Acid-fast bacilli were detected in 3 wide needle aspirates and 4 biopsy smears, and histology or culture, giving a positive predictive value of 100% confirmed all. In all these cases, macroscopic caseation of the excised lymph node was observed (Table 1). Macroscopic caseation was observed in 35 cases and tuberculosis was confirmed in 31 of them, giving a positive predictive value of 89%. All the isolates grown in the culture were identified as *Mycobacterium tuberculosis*.

Table 1. Sensitivity, specificity and predictive values of diagnostic procedures for tuberculous lymphadenitis compared to histology and/or culture.

	Sensitivity	Specificity	Predictive value	
			Positive	Negative
Wide needle aspiration				
Direct smear	8% (3/38)	100% (14/14)	100% (3/3)	29% (14/49)
Biopsy				
Caseation	82% (31/38)	71% (10/40)	89% (31/35)	59% (10/17)
Direct smear	11% (4/38)	100% (14/14)	100% (4/4)	29% (14/48)
Histology	82% (31/38)	100% (14/14)	100% (31/31)	67% (14/21)
Culture	61% (23/38)	100% (14/14)	100% (23/23)	48% (14/29)
Combination*	82% (31/38)	71% (10/14)	89% (31/35)	59% (10/17)

*wide needle aspiration direct smear+ biopsy caseation + biopsy direct smear.

Yield of diagnostic procedures

Histology, caseation and culture detected a high proportion of cases (Table 2). The contribution of wide needle aspiration and biopsy smears was low.

Table 2 Yield of diagnosis procedures for Tuberculous lymphadenitis

	Tuberculosis patients		
	All	HIV ^a positive	HIV ^a negative
No. of patients	38	32	6
Wide needle aspiration			
Direct smear	3 (8%)	3 (9%)	0
Biopsy			
Caseation	31 (82%)	26 (81%)	5 (83%)
Direct smear	4 (11%)	4 (13%)	0
Histology	31 (82%)	25 (78%)	6 (100%)
Culture	23 (61%)	18 (56%)	5 (83%)
Combination ^b	31 (82%)	26 (81%)	5 (83%)

^aHuman immunodeficiency virus

^bWide needle aspiration direct smear + biopsy caseation + biopsy direct smear.

Table 2. Yield of diagnostic procedures for tuberculous lymphadenitis

Diagnostic yield related to HIV status

All confirmed tuberculosis patients with acid-fast bacilli found in aspirates and biopsy smears were sero-positive for HIV. All 6 HIV seronegative tuberculosis patients were histologically diagnosed as tuberculous lymphadenitis. Only 25 of the 32 HIV seropositive patients were diagnosed by histology (Table 2). No statistically significant association was found between the results of aspiration, biopsy smears or histology and HIV status.

Results of histology and culture were both indicative of tuberculous lymphadenitis in 16 of 38 cases, 5 of the 16 were HIV seronegative and 11 were seropositive. With the remaining 22 cases; only one of the 2 procedures gave a positive result for tuberculosis, 7 by culture and 15 by histology. One patient with a histology result consistent with tuberculous lymphadenitis (but a negative culture) was seronegative for HIV; all the other 21 cases were HIV positive. The HIV status of the tuberculous lymphadenitis patients suggest a negative influence of HIV infection on the possibility of both histology and culture being indicative of tuberculosis (odds ratio [OR]=0.10)

Histology and culture: sensitivity and negative predictive values

The results of histology and culture on their own were compared with the combined outcome of both tests to estimate sensitivity and negative predictive values (Table 1)

Histology was indicative of tuberculosis in 31 of 38 diagnosed cases of tuberculous lymphadenitis, giving a sensitivity of 82%. Growth of *M. tuberculosis* occurred with 7 of 21 histologically non-tuberculosis cases. Histological examination therefore had a negative predictive value of 67% (14/21).

Cultures gave positive results for tuberculosis in 23 of the cases, a sensitivity of 61%. Histology was positive for tuberculosis in 15 of the 29 patients with negative cultures, a negative pre-

dictive value of 48% (14/29) for cultures.

Discussion

Wide needle aspirations and biopsy smears made only small contributions to the diagnosis of tuberculous lymphadenitis (8% and 11%, respectively). Others have reported higher sensitivities, varying from 28% (9) to 35% for aspiration and 53% for biopsy smears (10). There are three possible reasons for the higher yield in other reports. Firstly, other studies include in-patients (9, 10), who present in a later stage of HIV infection and tuberculosis (11) with a more highly compromised immune system. More acid-fast bacilli can be detected in their lymph nodes. Secondly, other studies included patients for whom only smears were examined to confirm tuberculous lymphadenitis (10), thus increasing the number of positive results. Thirdly, our study excluded sputum positive patients since further investigations would not have resulted in different treatment of the patient (10). A study of out-patients in rural Zambia (12) also reported relatively low yields from smear results, 12% for aspirates and 15% for biopsies.

In contrast to the low yield of aspirate and biopsy smears, macroscopic caseation of excised lymph nodes gave a high yield in our out-patient department setting, similar to that of histology (82%) and higher than culture (61%). The overall positive predictive value of diagnosing tuberculosis by macroscopic caseation was 89%, but it improved during the second part of the study (patients no. 51 - 100) to give a positive predictive value of 100% (15/15). The improvement could have been due to experience gained, and to the fact that only one surgeon carried out the procedures, instead of 4. This is an encouraging result, but it reminds us of the need for proper supervision and guidance when new protocols are introduced.

If the "stepwise" addition of the 3 simple diagnostic methods was followed (10) a cumulative total of 31 of the 38 proven tuberculous lymphadenitis patients was detected retrospectively: firstly, (i) ZN staining of wide needle aspirates diagnosed 3 patients., (ii) macroscopic caseation found 28 more, (iii) biopsy smears did not detect any case of tuberculosis that had not been found by the previous 2 methods.

The contribution of aspiration might be improved by adding an observation described (9): in 41% of the cases with macroscopic caseation, caseation was also seen in the wide needle aspirate, with a positive predictive value of 100% for tuberculous lymphadenitis. This aspect was not covered in our study although the surgeons sometimes spontaneously reported (but did not record) macroscopic caseation of the aspirate.

Negative predictive value of histology

In many sub-saharan African countries, results of histological examination are often used as the standard. Our study revealed a negative predictive value of 67%, implying that tuberculosis was not identified in 33% of patients with negative histology results. If histology had been the only available diagnostic procedure, 18% of the tuberculosis cases would not have been detected and treated. This raises concerns about the effectiveness of histology as a single tool with which to diagnose tuberculous lymphadenitis.

Does HIV infection influence results of diagnostic procedures?

There is no dispute that the HIV epidemic has contributed to changing patterns of tuberculosis infection. More contentious is the effect of HIV infection on diagnostic methods (13, 14, 15, 16, 17). Although hyporeactive or anergic responses, with numer-

ous bacilli in the lymph nodes of tuberculous lymphadenitis patients infected with HIV, have been described clearly (8), the influence of HIV on the yield of diagnostic methods for tuberculous lymphadenitis is less certain (9, 10).

Our study suggested that HIV seropositivity of tuberculous lymphadenitis patients decreased the possibility of histology and culture both being indicative of tuberculosis (OR=0. 10). In addition, the results tentatively suggested that histology is a more sensitive tool to diagnose tuberculosis in HIV seronegative patients, all of whom were diagnosed by histology, compared with only 78% of those who were HIV seropositive. However, due to the low numbers in general, and especially of HIV seronegative patients, no significant association was found. Our finding that HIV infection might decrease the sensitivity of diagnostic procedures for tuberculous lymphadenitis needs further investigation.

Recommendations

The low return rates in our setting reflect the difficulties of research in routine settings outside a research environment. A protocol for diagnosing tuberculous lymphadenitis should therefore be simple and pro-active, reducing the chance of a missed diagnosis due to difficulties in protocol procedures. Therefore, we suggest primarily the use of 4 simple methods to diagnose tuberculous lymphadenitis as shown in the Figure.

Lymphadenopathy patients not responding to general antibiotics

Wide Needle Aspiration

1. Macroscopic caseation -> positive->Tuberculous lymphadenitis
negative
2. Smear for AFB ->positive ->Tuberculous lymphadenitis
negative

Lymph Node Excision

3. Macroscopic caseation -> positive ->Tuberculous lymphadenitis
negative
4. Smear for AFB -> positive -> Tuberculous lymphadenitis
negative

Histology and Culture

Figure. Protocol for diagnosis of tuberculous lymphadenitis using 4 simple techniques

These 4 methods have achieved 100% positive predictive values for diagnosing tuberculous lymphadenitis in earlier studies (9, 10), and can easily and safely be carried out at district level. If any of these tests is positive, the diagnosis of tuberculous lymphadenitis can be made and there is no need to make further investigations, thus saving both cost and time. The recording of caseation of the wide needle aspirate should increase the usefulness of wide needle aspiration in an out-patient department setting. We expect that 80 - 95% of tuberculous lymphadenitis cases can be diagnosed by these methods in a timely and cost-effective manner in areas with high prevalences of tuberculosis and HIV infection like Malawi.

Conclusions

The contribution of aspirate and biopsy smears in diagnosing tuberculous lymphadenitis in out-patients was low. In contrast, examination for macroscopic caseation of the cut surface of lymph nodes had high sensitivity. Our study raised concern that sophisticated procedures such as histology and culture, which are

developed to diagnose tuberculosis, fail to do so, especially with HIV-associated tuberculous lymphadenitis. Histology missed 18% of the tuberculosis cases, all of whom were seropositive for HIV.

There is a need to develop simple, pro-active methods to diagnose tuberculous lymphadenitis in out-patient departments. Four simple methods, which can easily and safely be carried out at district level, should diagnose 80-95% of tuberculous lymphadenitis cases in a timely and cost effective manner.

Acknowledgements

We are grateful to the Ministry of Health and Population of Malawi who, through the Health Science Research Committee, gave approval to the research. The World Health organisation personnel generously made HIV serological reagents available and facilitated the testing. Special thanks to the pathology laboratory staff of Queen Elizabeth Central Hospital, the staff of the Department of Microbiology, College of Medicine, and Mr Gondwe, who helped to set up the research.

References

1. Chilongozi DA, Costello Daly C, Franco L, Liomba, NG and Dallabeta G (1996). Sexually transmitted diseases: a survey of case management in Malawi. *International Journal of STD and AIDS*, 7, 269-275.
2. Harries AD, Maher, D, Mvula B and Nyangulu DZ (1995). An audit of HIV testing and HIV serostatus in tuberculosis patients, Blantyre, Malawi. *Tubercle and Lung Diseases*, 76, 413-417.
3. Ravignone MC, Narain JP and Kochi A (1992). HIV-associated tuberculosis in developing countries. clinical features, diagnosis and treatment. *Bulletin of the World Health Organisation*, 70, 515-526.
4. Ravignone MC, Snider DE and Kochi A (1995). Global epidemiology of tuberculosis, morbidity and mortality of a worldwide epidemic. *Journal of the American Medical Association*, 273, 220-226.
5. Drobniewski AF, Pozniak AL and Uttley AHC (1995). Tuberculosis and AIDS. *Journal of Medical Microbiology*, 43, 85-91.
6. Anonymous (1996). National Tuberculosis Programme. Lilongwe, Malawi: Community Health Science Unit, Ministry of Health.
7. Harries AD (1990). Tuberculosis and human immunodeficiency virus infection in developing countries. *Lancet*, 335, 387-390.
8. Nambuya A, Sewankambo N, Mugerwa J, Goodgame R and Lucas S (1988). Tuberculous lymphadenitis associated with human immunodeficiency virus (HIV) in Uganda. *Journal of Clinical Pathology*, 44, 93-96.
9. GEM C, Patil PS, Elliot AM, Namaambo KM, Bharucha H and Porter JDH (1993). The value of wide needle aspiration in the diagnosis of tuberculosis lymphadenitis in Africa. *AIDS*, 7,1221-1225.
10. Perenboom RM, Richter C, Swai ABM, Kitinya J, Mtoni I, Chande H, Kazema RR, Mwakyusa DH and Maselle SY (1994). Diagnosis of tuberculosis lymphadenitis in an area of HIV infection and limited diagnostic facilities. *Tropical and Geographical Medicine*, 46, 288-292.
11. Richter C, Koelemay MJW, Swai ABM, Perenboom R, Mwakyusa DH and Oosting J (1995). Predictive markers of survival in HIV-seropositive and HIVseronegative Tanzanian

- patients with extrapulmonary tuberculosis. *Tubercle and Lung Disease*, 76, 510-517.
12. Voetberg A and Lucas SB (1991). Tuberculosis or persistent generalised lymphadenopathy in HIV disease? *Lancet*, 337, 56-57.
 13. Long R, Scalcini M, Manfreda J, Jean-Baptiste M and Hershfield E (1991). The impact of HIV on the usefulness of sputum smears for the diagnosis of tuberculosis. *American Journal of Public Health*, 81, 1326-1328.
 14. Nunn P, Gicheha C, Hayes R, Gathua S, Brindle R, Kibuga D, Mutie T, Kamunyi R, Omwega M, Were J and McAdam (K1992). Cross sectional survey of HIV infection among patients with tuberculosis in Nairobi, Kenya. *Tubercle and Lung Disease*, 73, 45-51.
 15. Elliot AM, Halwiindi B, Hayes RJ, Luo N, Tembo G, Machiels L, Bem C, Steenbergen G, Pobe JOM, Nunn PP and McAdam KP (1993). The impact of human immunodeficiency virus on presentation and diagnosis of tuberculosis in a cohort study in Zambia. *Journal of Tropical Medicine and Hygiene*, 96, 1-11.
 16. De Cock KM (1994). Impact of interaction with HIV. In: *Tuberculosis: Back to the Future*, Porter JDH and McAdam KPWJ (editors). Chichester: John Wiley and Sons, pp. 35-52.
 17. Smith RL, Yew K, Berkowitz KA and Aranda CP (1994). Factors affecting the yield of acid-fast sputum smears with HIV and tuberculosis. *Chest*, 106, 684-686.