Polymerase chain reaction amplifying mycobacterial DNA from aspirates obtained by endoscopic ultrasound allows accurate diagnosis of mycobacterial disease in HIV-positive patients with abdominal lymphadenopathy

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Abstract

Abdominal lymphadopathy in Human Immunodeficiency Virus (HIV) infection remains a diagnostic challenge. We performed a prospective cohort study recruiting thirty-one symptomatic HIV+ patients with abdominal lymphadenopathy assessing diagnostic yield of endoscopic ultrasound (EUS) fine needle aspiration (FNA). Mean age was 38 years, 52% were female, mean CD4 count and viral load were 124 cells/pl, and 4 log respectively. EUS confirmed additional mediastinal nodes in 26 %. Porta- hepatis was the most common abdominal site. EUS FNA was subjected to cytology, culture and polymerase chain reaction (PCR) analysis. Mycobacterial infections were confirmed in 67.7% and 31% had reactive lymphadenopathy. Cytology and culture had low sensitivity whereas PCR identified 90% of mycobacterial infections. Combining appearance of EUS FNA and cytology a diagnostic algorithm was developed to indicate when analysis with PCR would be useful. PCR performed on an EUS guided aspirate was highly accurate in confirming mycobacterial disease and determining genotypic drug resistance.

Key words: HIV, Abdominal lymphadenopathy, Endoscopic ultrasound, Tuberculosis

Background

Tuberculosis is a frequent dominant opportunistic infection in HIV-infected individuals and continues to be a leading cause of mortality in this patient population. While pulmonary tuberculosis can often be reliably diagnosed with imaging and sputum analysis, extra-pulmonary disease may often remain obscure. Tuberculosis lymphadenitis is now the most common extra-pulmonary manifestation of tuberculosis in both HIV positive and negative individuals in the USA (Fiske et al. 2010; Heller et al. 2010)

Isolated abdominal lymphadenopathy especially presents a diagnostic challenge in HIV- infected patients. The dysfunctional immune response in HIV may obscure signs of infection which may lead to atypical clinical presentations (Bos et al. 2013; Sinkala et al. 2009). In endemic areas, *Mycobacterium tuberculosis* (MTB) accounts for the majority of cases of lymphadenopathy but needs to be differentiated from infections by atypical mycobacteria, other bacterial species, fungi and parasites, conditions such as reactive lymphadenopathy and various malignancies (Giordani et al. 2013; Radin 1995; Sarma et al. 2010). Imaging studies alone fail to distinguish between different etiologies (Dos Santos et al. 2008), with the consequence of inaccurate diagnoses and empirical treatment decisions (Mendelson 2007; Navani et al. 2011). Due to the emergence of multidrug resistant tuberculosis MDR every effort should be made establishing the correct diagnosis and determine drug susceptibilities of the mycobacterium before commencing anti-tuberculosis therapy (Abbadi et al. 2009; WHO 2010).

Various methods have been described to obtain tissue samples from enlarged abdominal lymph nodes that vary in complexity and invasiveness. In general, percutaneous, endoscopic and surgical techniques are available (Gupta and Madoff 2007; Uzunkoy et al.2004; Yasurfuku et al. 2007). Although surgical lymph node biopsy has been considered the gold standard (Bhutani and Logrono 2005), its invasive nature and related morbidity preclude its general use. Percutaneous-guided

biopsies are often not feasible if lesions are in close proximity to major vessels or where there are overlying organs.

Endoscopic ultrasound (EUS)- guided fine needle aspiration (FNA) is a minimally invasive procedure that can be performed under conscious sedation, enabling the sampling of different lymph node stations in the mediastinum and abdomen in a single session. EUS-FNA has become an established procedure in obtaining tissue from suspicious gastrointestinal and mediastinal lesions and has a low complication rate (Al-Haddad et al. 2009; Bhutani and Logrono 2005; Saftoiu and Vilmann 2009).

The role of endoscopic ultrasound in the assessment of abdominal lymphnodes in HIV infected individuals has not been adequately studied. We hypothesized that in HIV, due to an attenuated dysfunctional immune response, the typical cytological findings associated with mycobacterial disease such as granuloma formation and the presence of acid fast bacilli may be absent or altered. This study examined the diagnostic efficacy of EUS-FNA in a population of advanced HIV-infected patients and the safety of the procedure in this setting. After obtaining EUS FNA samples the accuracy of various downstream diagnostic modalities were compared and the impact that the results of these investigations had on treatment decisions was examined.

Materials and methods

Study population

A multicenter prospective cohort study was performed and symptomatic HIV-1–infected individuals with abdominal lymphadenopathy were recruited between September 2009 to February 2012 from the HIV Comprehensive Care Clinic and from the Infectious Diseases Units, at Steve Biko Academic and Pretoria East Hospitals, Gauteng, South Africa. These tertiary referral centres specialize in advanced HIV care and therapeutic endoscopy. All symptomatic HIV infected patients seen in follow-

up were screened by ultrasonography or computed tomography for the presence of abdominal lymph nodes.

Patients were included in the study if abdominal lymph nodes were larger than 1 cm were present and if a microbiological diagnosis could not be established through standard examination of sputum, fluid, blood or superficial lymph node fine needle aspiration. Patients who were symptomatic despite at least three months of empiric treatment were also included. The most common symptoms observed in such patients were fever, weight loss and night sweats.

A presumptive diagnosis, based on the clinical and radiological information, was formulated by the infectious disease specialist. The endoscopist and pathologist were blinded to this. By comparing the presumptive diagnosis to the final diagnosis the impact of EUS FNA on decision making was examined. Due to the previously described absence of a diagnostic gold standard in such patients, a diagnostic composite was employed that included cytological analysis, culture and PCR. This was done as per the NICE guidelines (Nice Clinical Guidelines 2006). This protocol followed the principles of the Declaration of Helsinki and was approved by the University of Pretoria Ethics Committee. Written informed consent was obtained from each participant.

EUS-FNA technique

The procedure was performed under conscious sedation by expert endoscopists (FP, JOJ, SvdM) with more than 10 years experience in echoendoscopy performing more than 500 procedures per year. Linear array echoscopic ultrasound (Pentax Hitachi 7500) using a standard frequency setting of 7.5Mhz and standard 22-gauge needles (Cook Endoscopy, Limerick, Ireland) were used. The location of lymph nodes (celiac, porta-hepatis, retroperitoneal, para-aortic, mediastinal), number of nodes, echogenicity (hypo-echoic, hyper-echoic, isodense), form (round, matted, elongated), the absence or presence of necrosis were prospectively recorded. In addition tissue elastography was performed

and the lymph node classified as hard or soft. FNA was performed by trans-esophageal, gastric or duodenal approach depending on the location of the lymph node groups (figure 1 a-d).

The aspirate was characterized as 'bloody' or 'yellow' and smears were prepared and fixed on glass slides for cytological analysis by the gastroenterologist. No pathologist was present in endoscopy suite. Additional aspirates were placed in three sterile tubes containing 500 µL of physiological saline for culture, PCR and flow cytometry. Post-procedure, patients were observed until stable and were monitored telephonically after 24 hours and at day 7 for possible procedure-related complications. Patients were followed for 3 months following the procedure to correlate endoscopic ultrasound findings with clinical outcome before returning to scheduled clinic visits.

Cytology

Fine needle aspiration material was subjected to Papanicolaou staining (to evaluate morphology), as well as Giemsa, Ziehl-Neelsen (detection of acid fast bacilli [AFB]), periodic acid-Schiff and Warthin-Starry stains. The cytological specimens were interpreted by an expert pathologist (TS) blinded to the presumptive diagnosis and endoscopic findings.

PCR technique

DNA was extracted from the aspirate using QIAamp® DNA Mini kits (Qiagen, Hilden, Germany). Detection of Mycobacterial DNA was performed using the Roche LightCycler® *Mycobacterium* PCR kit (Roche Diagnostics, Mannheim, Germany) allowing amplification and differentiation of *Mycobacterium tuberculosis* (MTB) complex from *Mycobacterium avium* (MAC) and *Mycobacterium kansasii* by means of a post-PCR melt analysis. Genotypic rifampicin and isoniazid susceptibility testing was performed on MTB positive samples (Genotype® MTBDR*plus* assay, Hain Lifesciences, Nehren, Germany).

Culture

Mycobacteria were isolated from the aspirated samples using BacT/ALERT liquid culture media (bioMérieux, Marcy-l'Etoile, France). Identification of positive cultures and genotypic rifampicin and isoniazid susceptibility testing were then performed. Non-tuberculous mycobacteria from positive cultures were identified using the GenoType® Mycobacterium CM reverse line probe assay (Hain Lifesciences, Nehren, Germany). Fungi were isolated from the biopsy samples by inoculation onto Sabouraud dextrose agar and incubation at 25^{o}C for up to 3 weeks. Identification of isolates was performed by standard phenotypic methods (Zeng et al. 2007).

Flow cytometry

Fine-needle aspiration specimens were washed twice with RPMI 1640 tissue culture medium, centrifuged at 250 g for 10 minutes. Enumeration of the cells was performed using Flow-Count™ Fluorospheres (Beckman Coulter, Miami, FL, USA) and the cells were then resuspended in RPMI 1640 tissue culture medium. Immunophenotyping was performed on a Beckman Coulter Cytomics FC500 (Beckman Coulter, Miami, FL, USA). Monoclonal antibodies were used to identify aberrant B and T-cell populations.

Statistics

All data were entered into a Microsoft Excel 2010 spreadsheet and descriptive statistics calculated. The accuracy of the diagnostic methods was assessed using two approaches. Firstly, due to the high burden of tuberculosis (TB) observed in this study, the ability of the methods to identify TB, and secondly, their ability to identify mycobacterial infections of any kind. That is, the results of the presumptive diagnoses and the individual diagnostic methods were binary classified in terms of their identifying any mycobacteria or TB in particular. In the TB-only analysis, we controlled for those patients undergoing prior anti-tubercular treatment (ATT) or in whom a lymphoma was found to be

the final diagnosis. Using Statistix 9 (Analytical software, Tallahassee, Florida, USA) comparisons were made by means of cross tabulation, two-tailed Fischer exact tests and the calculation of positive and negative predictive values, between the results of each of the methods versus the final diagnosis. A $p \leq 0.05$ value was considered significant.

Results

EUS-FNA assessment was conducted on 31 HIV-1-infected patients with pathologically enlarged abdominal lymph nodes. All patients had advanced HIV disease. The characteristics and the findings for the patients studied are given in Table 1, with more detailed individual patient findings given in the supplementary text.

EUS detected enlarged lymph nodes in all patients. Para-aortic and porta-hepatis nodes were the most common locations found in 20 (64,5 %) and 17 (54,8 %) of patients respectively, while 8 (25,8 %) of patients had additional mediastinal nodes. The mean diameter of the nodes were 28mm (15-63mm). The endoscopic ultrasound appearance of lymph nodes was highly variable including evidence of necrosis (inhomogenous with central hypo-echogenicity) (32%), hypoechoic (48%), iso and hyper-echoic appearances (20%) (figure 1 a-d). Tissue elastography was not useful in separating reactive lymph nodes from tuberculous lymphadenitis. Extra-nodal lesions were also found in 7 (22.5 %) of patients including: an infected sub-diaphragmatic multi-loculated cyst (5,6cm x 2,3cm) that was drained, one peri-pancreatic cold abscess and pericardial effusions. Only one pericardial effusion was considered clinically important and was aspirated under EUS guidance where PCR and culture confirmed MTB. The procedure was found to be safe in this patient population and no immediate or post-procedural (48 hours) complications were reported.

Cytology yielded the following results in patients with a final diagnosis of a mycobacterial infection: AFB were detected in 38%, culture was positive in 67% and PCR in 90%. The mean time to positive culture was 25 days (13-49). Overall MTB was diagnosed in 17/31 (54.8%) patients, MAC in 5/31

(16.1%) and mixed TB/MAC in 1/31 (3%) of patients. In all but two MTB PCR positive patients, there was sufficient target DNA to perform direct drug sensitivity genotyping, leading to two patients being diagnosed with drug resistant tuberculosis. In all cases the final diagnosis was based on a combination of at least 2 of the following: cytology, culture and PCR. In one case, flow cytometry was unable to characterize a lymphoma and a surgical excision biopsy was required for confirmation of the subtype.

The accuracy of the diagnostic methods relative to identifying TB is given in Table 2.1. In the total patient group cytology, culture and PCR were all independently significantly associated with a Final_TB diagnosis. When the ATT-naïve patient group was compared, only culture and PCR remained significantly associated with the Final_TB diagnosis. The accuracy of the diagnostic methods relative to identifying *any* mycobacterial infection is given in Table 2.2. A PCR (+) for mycobacteria, cytology-with a finding of necrosis and a positive culture of mycobacteria were all independently significantly associated with a Final_diagnosis of Mycobacteria. A finding of AFB was not. When cytology, AFB and culturing were combined into a composite variable, the resulting variable's diagnostic accuracy increased to nearly that of PCRs' and was significantly associated with a Final_mycobateria diagnosis. Following EUS-FNA aspirate investigations, the final diagnosis changed from the presumptive diagnosis in a total of 13/31 (41.9%) patients and made additional diagnoses in 4/31 (12.9%) patients. The use of EUS-FNA therefore resulted in a change in the management of 17/31 (54.8%) patients. In addition 7/31 (23%) patients were diagnosed with a fungal infection. Fungal species identified included *Candida Albicans* (57% of 4/7), *Candida Glabrata* (29% of 2/7) and *Candida Krusi* (14% of 1/7). Forty three percent (3/7) were PCR and culture positive and 29% (2/7) were culture and cytology positive. Fungal infections were only considered clinically relevant if the sample that yielded the positive result was obtained from the stomach or duodenum. It however remains difficult to differentiate mucosal contaminants from invasive infections, at least to regional lymph nodes in this population.

In general, the main findings of this study were: i. Neither the size nor the EUS appearance could differentiate reactive from pathological lymph nodes ii. The cytological analysis of the aspirate alone had a low diagnostic accuracy even in patients not on anti-tuberculous therapy. iii. PCR had a high diagnostic accuracy in mycobacterial infection allowing a rapid diagnosis and early initiation of therapy. Following the various procedures all patients were placed on treatment according to the final diagnosis and followed up for 3 months by study protocol before returning to scheduled clinic visits. One patient, diagnosed with 4 opportunistic infections, including *Cytomegalovirus* (CMV), *MTB* pericarditis, *Mycobacterium avium complex* (*MAC*) and *C. Glabrata*, died during the observational period despite the initiation of appropriate therapy. All other patients stabilized or improved on specific therapy during the observational period.

Discussion

The safety and diagnostic accuracy of endoscopic ultrasound in the assessment of mass lesions and lymphadenopathy in immuno-competent individuals is well established. In HIV-infected individuals the aberrant immune response may affect EUS interpretation of lymph nodes and the diagnostic accuracy of the aspirate. Such patients are predisposed to a multitude of opportunistic infections as well as hematological and other malignancies, frequently with atypical clinical presentations (Sinkala et al. 2009). Additionally, cytological findings characteristic of bacterial infection, such as granuloma formation or the presence of acid fast bacilli, may be absent or altered (Mendelson 2007).

In general, two strategies can be followed when managing HIV/AIDS infected patients with sputum smear negative tuberculosis with abdominal lymphadenopathy. Firstly, patients may be placed on empirical therapy. In MTB endemic settings, anti-tubercular drugs may be initiated however this approach may risk adverse drug reactions and interactions, hepatotoxicity and miss other pathology (Mendelson 2007). Indeed, a study from India using EUS FNA to assess abdominal lymphadenopathy in HIV-uninfected patients, confirmed that almost half of the patients in this high endemic region

were found to have non-tubercular pathology (Dhir et al. 2011) Similarly, our study confirmed that 31% of HIV infected patients with enlarged nodes, even in a high endemic area such as South Africa, had reactive lymphadenopathy. This implies that empirical ATT, prescribed solely on the basis of the presence of enlarged lymph nodes, may be associated with considerable treatment-related risks and should consequently be avoided.

Secondly, a tissue sample may be obtained allowing treatment decisions to be based on a laboratory confirmed diagnosis. Multiple sampling modalities are available including percutaneous FNA and minimally invasive surgery; neither has been extensively investigated in an HIV-positive population. Percutaneous ultrasound-guided FNA in HIV-positive patients has been reported to have a high technical success rate and diagnostic accuracy but only allows assessment of large, superficial nodes (Veerapand et al 2004). In patients with overlying organs, where multiple lymph nodes sites are affected, or smaller and deeper nodes, percutaneous sampling becomes challenging (Gupta and Madoff 2007). In this study we report that endoscopic ultrasound is safe and effective in evaluating abdominal lymphadenopathy and obtaining tissue samples in HIV infected patients.

Classic EUS findings that have been proposed to differentiate between pathological and reactive nodes, include size greater than 1 cm, a round shape, hypo-echoic texture and sharp margins (Catalano et al. 1994). These findings were common in our study and not useful in differentiating between tuberculous lymphadenitis and reactive lymphadenopathy. Recent studies of mediastinal MTB in HIV-negative patients have found the presence of hypo-echoic nodes in the majority of patients (Song et al. 2010), and that the lymph node appearance alone could not reliably differentiate tuberculous nodes from nodes due to sarcoidosis (Fritscher-Ravens et al. 2011). In our study the EUS appearance of lymph nodes in HIV-infected patients was highly variable, including a hypo-echoic texture, particularly when nodes smaller than 2cm were observed. Nodes also demonstrated central breakdown, extensive necrosis and with a matted or hyper-echoic appearance (Figure 1).

In HIV-uninfected patients, FNA aspirates obtained by EUS are routinely analyzed by cytology. A large study from India assessing mediastinal lymph nodes by EUS FNA based on cytology only, found an overall diagnostic yield of 93% (Puri et al.2010). Navani and coworkers (2011) showed, using endobronchial ultrasound-guided transbronchial needle aspiration, cytological findings consistent with tuberculosis in 86% of cases. Yet culture was only positive in 47% of patients implying that treatment decisions were based mainly on cytological findings without the opportunity to differentiate MTB from MAC or to assess drug resistance. In our study, findings typically associated with mycobacterial disease such as granuloma formation were often absent, most likely due to HIV-associated immunosuppression. We found the presence of necrosis on cytology to be predictive of a positive mycobacterial culture and PCR. Additionally, although highly specific for mycobacterial disease, microscopy in the form of Ziehl-Nielsen staining for acid-fast bacilli (AFB), cannot reliably differentiate between MTB and MAC nor identify drug resistant strains.

Culture has historically been considered the gold standard in identifying and differentiating mycobacterium species but may take up to six weeks and is therefore of little value in early patient management, particularly in immune-compromised patients prone to rapid progression and early mortality (Mendelson 2007). In our study mycobacterial culture was positive in only 67% of patients with the mean time to a positive culture of 25 days that would considerably delay initiation of effective therapy. A recent study in patients with isolated mediastinal lymphadenopathy suggests that culture yields may be lower in HIV positive patients (Navani et al. 2011). In that study in a subgroup of 17 HIV positive patients only 36% had positive mycobacterial cultures. Collectively the published data suggest that FNA subjected to cytology and culture only, is inadequate in obtaining a final diagnosis in the majority of HIV positive patients with abdominal lymphadenopathy. In contrast PCR amplifying mycobacterial DNA had a diagnostic accuracy of 94%. Combining all modalities we could show that the final diagnosis changed from the presumptive diagnosis as formulated by the infectious disease specialist leading to a change in the management in 42% of patients.

Based on the calculated positive and negative predictive values of the various post EUS-FNA modalities we defined a diagnostic algorithm (figure 2). Our results demonstrated that the presence of a yellow aspirate in combination with caseous necrosis on cytology had a PPV of 96% whereas a bloody aspirate in the absence of caseous necrosis had a 77% NPV which can be used to determine in which patients additional PCR should be performed. PCR remains imperative to differentiate MDR-TB strains and MTB from MAC.

Our detailed prospective analysis which assessed the diagnostic accuracy of EUS guided FNA was performed on a relatively small sample size of patients with advanced HIV infection. The results of our study need to be validated in a larger cohort of patients. We also realize that endoscopic ultrasound and PCR may not be readily available in many resource limited settings. This implies that in such settings that percutaneous ultrasound-guided FNA relying on cytology may remain an important diagnostic tool. Irrespective, our study provides a clear description of the value of EUS and the utilization of PCR in the management of patients with advanced HIV with abdominal lymphadenopathy.

In summary, in addition to analyses of EUS-FNA by cytology and culture, polymerase chain reaction amplification of mycobacterial DNA was found to be invaluable in providing a final diagnosis and guiding therapy. If mycobacterial infection was confirmed by PCR, sequencing and genotypic drug susceptibility testing could also be performed, enabling early diagnosis and the prompt initiation of appropriate therapy.

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Conflict of interest

Potential competing interests: None. Financial support: South African Gastroenterological Society (SAGES)/ Astra Zeneca Fellowship in Gastroenterology awarded to Schalk van der Merwe.

Figure legends

Figure 1:

Endoscopic ultrasound (EUS) images of lymph nodes in the abdomen and mediastinum in advanced HIV patients. The appearances of lymph nodes are highly variable. (A.) EUS image of typical retroperitoneal lymph nodes (black arrow) in HIV populations contrasting the more hypo-echoic adrenal gland to the less hypo-echoic lymph nodes; (B.) Smaller lymph nodes of <2cm (white arrowhead) typically appeared round and hypo-echoic and simulated malignant nodes. Tissue elastography (left image) showing the lesion to be predominantly "soft". Tissue elastography was however not useful in distinguishing tuberculous lymphadenitis from reactive lymph nodes. (C). Mediastinal lymph node groups (black arrow head) matted together so that individual lymph nodes cannot be distinguished. In a third of patients matted nodes were seen (D.) Presence of large peri-pancreatic retroperitoneal lymph nodes (white arrow) in a patient negative by PCR and culture for mycobacterial disease. FACS analysis showed normal T- and B-cell populations. Left image shows the appearance of the node using tissue elastography.

Figure 2:

Proposed diagnostic algorithm to assess samples obtained from lymph nodes in symptomatic HIV positive patients with abdominal and or mediastinal lymphadenopathy. All patients with lymphadenopathy should be subjected to FNA irrespective of the appearance of the lymph nodes. A "yellow" aspirate in the presence of necrosis on cytology is highly predictive of mycobacterial

disease. These samples should be subjected to further analysis with culture and PCR to differentiate MTB from MAC and exclude genotypic resistance. If the aspirate is “bloody” and necrosis is not detected on cytology mycobacterial disease is highly unlikely. Such a sample should be subjected to culture and the patient carefully followed. In event of progression an excision lymph node biopsy should be performed to exclude lymphoma.

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Supplementary text

Polymerase chain reaction amplification of mycobacterial and fungal DNA

The MTBDR*plus* assay is a PCR-reverse line probe assay that detects mutations associated with rifampicin resistance within an 81-bp 'hot spot' region of the *rpoB* gene. In addition, it detects mutations in codon 315 of the *katG* gene and in the *inhA* promoter region that are associated with INH resistance. This assay has been shown to have a sensitivity of >95% and >90% for detecting rifampicin and INH resistance respectively in South African TB isolates, as compared to the gold standard of phenotypic drug susceptibility testing (Barnard et al. 2008).

A broad range fungal PCR targeting the conserved internal transcribed spacer region 2 (ITS-2) of the fungal ribosomal RNA gene was used to analyze each sample for the presence of fungal DNA. This PCR incorporated primers flanking the fungal ITS-2 region as described by Zeng et al (2007). The real-time PCR was performed with a 50µl reaction mixture containing 10µl extracted DNA, 25µl SensiMix dT (containing reaction buffer, heat-activated Taq DNA polymerase, dNTPs, 3mM $MgCl_2$) [Quantace, London, United Kingdom], 1x Sybr Green (Quantace, London, United Kingdom), 25pmol of primers its3Sb (5' GTGAATCATCGARTCTTTGAACG-3'; positions 271-279) and its4Ab (5'-GTTGGTTTCTTTTCCTCCGCTTAT TGATATGC-3'; positions 710-741). Primer positions refer to *Candida albicans* sequences corresponding to Genbank accession numbers AF455524 and L28817 respectively. Amplification was performed on a RotorGene 6000 thermal cycler (Corbett Research, Sydney, Australia), with the following conditions: 1 cycle of 94°C for 10 minutes, 30 cycles of 95 °C for 15s, 50°C for 30s, and 72°C for 30s. The presence of specific amplified product was determined by means of a post-PCR melt analysis. DNA sequences were compared with sequences from GenBank, EMBL, and DJB database using the gapped BLASTN 2.0.5 program.

Individual patient diagnostic findings

			EUS-FNA		***Biopsy examination***				
Pat.	*Presumptive diagnosis*	*Medi-cation*	*Echo-appearance*	*Sample*	*AFB*	*Cytology*	*PCR*	*Culture*	***Final Diagnosis***
1	• MTB	• Non	• Hypo-echoic nodes	• Bloody	• Negative	• atypical lymphoid proliferation	• negative	• No growth	• Non Hodgkins lymphoma[1]
2	• *MTB* • lymphoma	• ATT	• Hypo-echoic nodes	• Yellow	• Positive	• caseous necrosis	• MTB (Sensitive) • C. Albicans	• MTB • C. Albicans	• MTB • *C. albicans*
3	• MTB	• AB	• Hypo-echoic nodes	• Bloody	• Negative	• Non specific reactive	• Negative	• MTB (Sensitive)	• MTB
4	• MTB	• ART	• Hypo-echoic nodes • Pericardial effusion	• Bloody • fluid	• Positive	• candida spp	• MAC • MTB (Sensitive)[2]	• C. glabrata • MAC	• Co-infection MAC, MTB[2] • *C. glabrata*
5	• MTB	• AB	• Hypo-echoic nodes	• Bloody	• Positive	• Non specific reactive	• MAC	• MAC	• MAC
6	• MDR-MTB • lymphoma	• ART • MAC	• Central breakdown	• Yellow	• Positive	• AFB positive • Caseous necrosis	• MTB (Rifampicin resistant)	• No growth	• Resistant MTB
7	• MDR-MTB	• ART • ATT	• Central breakdown	• Yellow	• Negative	• Non specific reactive	• MTB (Sensitive)	• No growth	• MTB
8	• MDR-MTB	• ART • ATT	• Central breakdown	• Yellow	• Negative	• Caseous necrosis	• MTB (Sensitive)	• No growth	• MTB
9	• MTB	• AB	• Central breakdown	• Yellow	• Negative	• Caseous necrosis	• MTB (Sensitive)	• MTB	• MTB
10	• MDR-MTB	• ART • ATT	• Central breakdown • Multi-loculated cyst	• Bloody • Cyst fluid	• Negative	• Non specific reactive	• negative	• No growth • α-hemolytic strep + coagulase neg staph[3]	• Reactive lymph-adenopathy • Infected multi-loculated cyst
11	• MTB • Lymphoma	• Non	• Central breakdown	• Bloody	• Negative	• Non specific reactive	• negative	• no growth	• Reactive lymph-adenopathy
12	• MTB	• AB	• Central breakdown	• Yellow	• Positive	• Granulomas • Caseous necrosis	• MTB (Sensitive) • C. Albicans	• MTB	• MTB • *C. Albicans*
13	• MDR-MTB	• ATT	• Hypo-echoic nodes	• Yellow	• Positive	• Caseous necrosis	• MTB (Sensitive)	• MTB	• MTB
14	• MTB • Lymphoma	• AB	• Hypo-echoic nodes	• Bloody	• Negative	• *Candida spp*	• MTB	• MTB (Sensitive) • C. Albicans	• MTB • C. Albicans
15	• Hospital acquired infection	• ART	• Hypo-echoic • Peri-pancreatic cold abscess	• Yellow[4]	• Negative	• Caseous necrosis[4]	• MTB[4]	• No growth	• MTB[4]
16	• Resistant MAC • MTB	• ART • MAC	• Hyper-echoic	• Yellow	• Positive	• Caseous necrosis	• MAC	• ***C.*** *Albicans* • MAC	• MAC • C. Albicans
17	• MTB	• ART	• Central breakdown	• Bloody	• Negative	• Non specific reactive	• MTB (sensitive)	• MTB	• MTB
18	• MTB	• ART	• Hypo-echoic	• Bloody	• Negative	• Non specific reactive	• negative	• No growth	• Reactive lympha-denopathy
19	• Reactive	• ART	• Hypo-echoic	• Bloody	• Negative	• Non specific reactive	• negative	• No growth	• Reactive lymph-adenopathy
20	• MTB	• ART	• Hypo-echoic	• Bloody	• Negative	• Non specific reactive	• negative	• MAC	• MAC
21	• MTB	• Non	• Central breakdown	• Yellow	• Negative	• Non specific reactive	• MTB (sensitive)	• No growth	• MTB
22	• MTB	• Non	• Hypo-echoic	• Yellow	• Negative	• Caseous necrosis	• MTB (sensitive)	• No growth	• MTB

23	• Lymphoma	• Non	• Hypo-echoic	• Yellow	• Negative	• Caseous necrosis	• MTB (sensitive)	• MTB (Sensitive)	• MTB
24	• MTB	• Non	• Hypo-echoic	• Bloody	• Negative	• Non-specific reactive	• Candida glabrata	• Candida glabrata	• Candida glabrata
25.	• MAC	• ART	• Hypo-echoic	• Bloody	• Positive	• Caseous necrosis	• MTB (sensitive INH, Rif)	• MTB (sensitive INH, Rif°	• MTB
26.	• Lymphoma	• ART	• Hypo-echoic	• Yellow	• Negative	• Caseous necrosis	• MAC • Candida krusi	• MAC • Candida krusi	• MAC • Candida krusi
27.	• MTB	• ART	• Hypo-echoic	• Bloody	• Negative	• Non-specific reactive	• Negative	• No growth	• Reactive lymph-adenopathy
28.	• Lymphoma	• ART	• Iso-echoic	• Bloody	• Negative	• Non-specific reactive	• Negative	• No growth	• Reactive lymph-adenopathy
29	• MTB	• ART	• Iso-echoic	• Bloody	• Negative	• Non-specific reactive	• Negative	• No growth	• Reactive lymph-adenopathy
30	• MTB	• Non	• Hypo-echoic	• Bloody	• Negative	• Caseous necrosis	• MTB (sensitive INH, Rif)	• No growth	• MTB
31	• Lymphoma	• ART	• Hypo-echoic	• Bloody	• Negative	• Non-specific reactive	• Negative	• No growth	• Reactive lymph-adenopathy

Abbreviations: AFB = acid-fast bacilli, ATT= anti-tubercular treatment, MTB= Mycobacterium Tuberculosis, MAC = Mycobacterium Avium Complex, MDR = Multi drug resistance, ART = Antiretroviral therapy, AB = antibiotics

[1]Confirmed and typed by surgical excision biopsy, type: diffuse large B-cell lymphoma
[2]FNA from pericardial effusion was positive for MTB
[3]FNA from a multiloculated cyst cultured positive for α-hemolytic streptococcus and coagulase negative staphylococcus
[4]Sample obtained from peri-pancreatic cold abscess.

Tables

Table1. Characteristics and findings of patients studied

Parameter	N (%)
Total patients	31
Male: Female	15 (48.4%): 16 (51.6%)
Mean age (year)	38.5 (range 24-60)
Mean CRP (mg/l)	128.7 (sd 104.5)
Mean albumin (g/l)	23,8 (sd 6,7)
Mean $CD4^+$ (cells/µl)	124 (range 5-414)
Mean log viral load	4.189 (sd 1.819)
WHO stage (mode)	4 (range 3 – 4)
Chronic treatment:	
ART	10/20 (50%)
ATT	6/20 (30%)
Co-trimoxazole	15/20 (75%)
MAC	2/20 (10%)
Findings:	
Cytology	
AFB	8/30 (26.7%)
Caseous necrosis	12/31 (38.7%)
Granulomas	2/31 (6.4%)
Atypical lymphoid proliferation	1/31 (3.2%)
Candida Spp	2/31 (6.4%)
Non-specific	14/31 (45.2%)
PCR	
MTB	16/31 (51.6%)
MAC	5/31 (16.1%)
Candida spp	3/31 (9.7%)
Culture	
MTB	9/31 (29.0%)
MAC	6/31 (19.4%)
Candida spp	6/31 (19.4%)
Cyst*	1/31 (3.2%)
Final diagnosis:	
MTB	17/31 (54.8%)
MAC	6/31 (19.4%)
Candida	6/31 (19.4%)
Lymphoma	1/31 (3.2%)
Multi-loculated cyst	1/31 (3.2%)

Notes:

ATT= anti-tubercular treatment, MTB = Mycobacterium Tuberculosis, MAC = Mycobacterium Avium Complex, ART= Antiretroviral therapy , sd = standard deviation

* α-hemolytic streptococcus and coagulase negative staphylococcus.

Table2. Accuracy of the diagnostic methods with respect to identifying TB (a) or any mycobacteria (b)

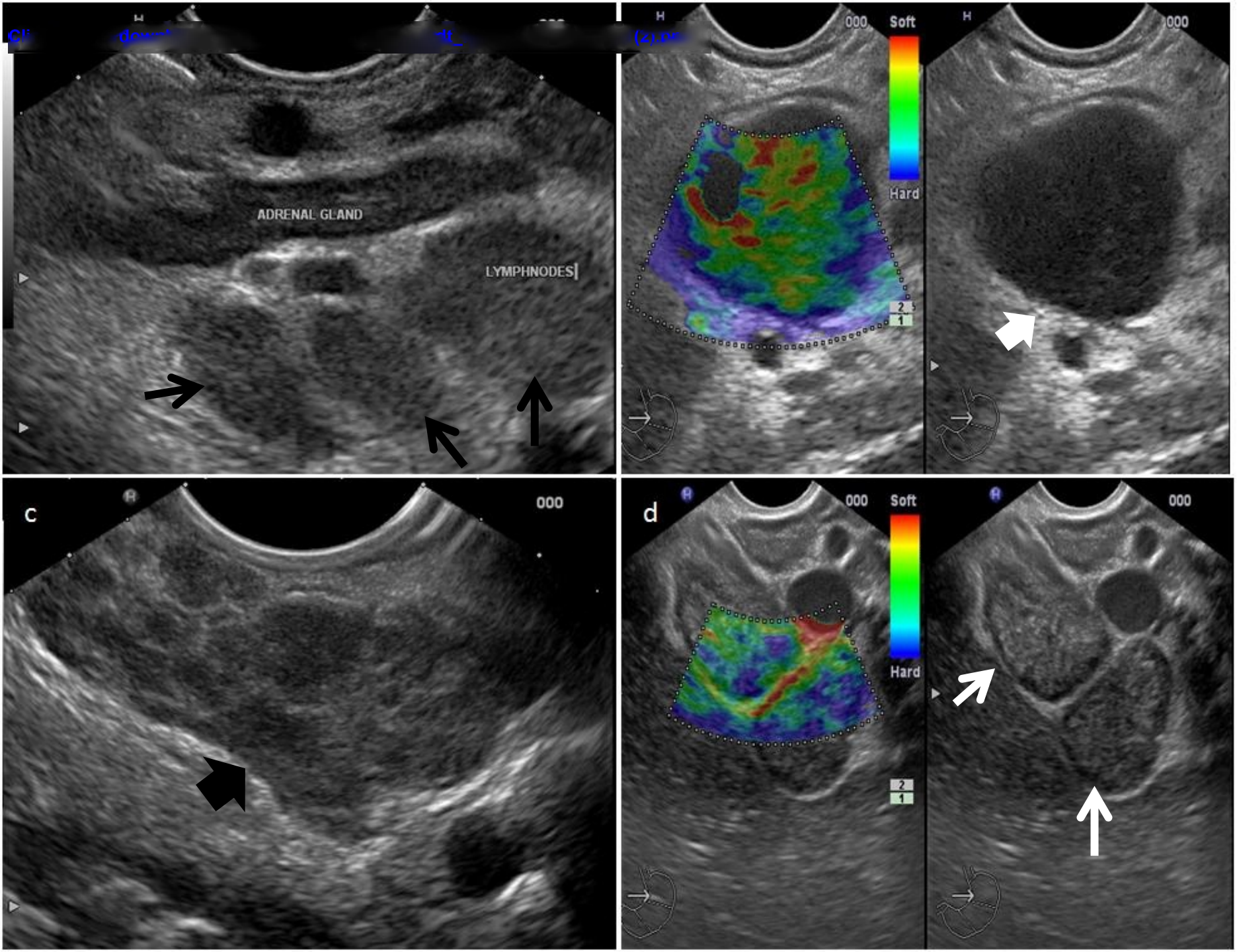
Diagnostic modality	**Patient group**					
	All patients		**association with Final_TB**	**ATT-naïve**		**association with Final_TB**
2.1. *for TB:*	identified	Predictive value	p- value	identified	Predictive value	p- value
Presumed TB - diagnosis	27/31(87.1%)	PPV = 60.7 % NPV = 87.5 %	NS	17/19(89.5%)	PPV = 57.9 % NPV = 80.0 %	NS
AFB	8/30(26.7%)	PPV = 89.5 % NPV = 54.2 %	NS	6/19(31.6%)	PPV = 84.6 % NPV = 53.3 %	NS
Cytology_necrosis	12/31(38.7%)	PPV = 89.5 % NPV = 61.9 %	0.03	8/19(42.1%)	PPV = 84.6 % NPV = 61.5 %	NS
Culturing	9/31(29.0%)	PPV = 100 % NPV = 66.7 %	<0.01	6/18(33.3%)	PPV = 100.0 % NPV = 66.7 %	0.01
PCR	16/31(51.6%)	PPV = 100 % NPV = 93.3 %	<0.01	10/19(52.6%)	PPV = 100.0 % NPV = 88.9 %	<0.01
Final_TB	17/31(54.8%)		0	11/19(57.9%)		0
2.2. for any mycobacteria:	**All patients**		**association with Final_mycobact**			
AFB	8/30(26.7%)	PPV = 100.0 % NPV = 40.9 %	NS			
Cytology_necrosis	11/30(36.7%)	PPV = 95.5 % NPV = 47.4 %	0.05			
Culture_mycobact	13/30(43.3%)	PPV = 100.0 % NPV = 58.8 %	<0.01			
Composite (of above)	18/31(58.1%)	PPV = 95.5 % NPV = 76.9 %	<0.01			
PCR_mycobact	20/31(64.5%)	PPV = 100 % NPV = 90.9 %	0			
Final_mycobact	21/31(67.7%)		0			

Notes:

Abbreviations. ATT= anti-tubercular treatment, PPV = positive predictive value, NPV = negative predictive value, AFB = acid fast bacilli staining

2.1. The Final_TB diagnosis was based on 2 or more of the modalities identifying TB. In the total patient group, PCR failed to identify one case, culturing failed in 8 cases and cytology (with a necrosis finding) failed in 5. The presence of acid-fast bacilli was less accurate than the other modalities. However, a presumed diagnosis of TB prior to any modality was the least accurate.

2.2. In the Final_mycobact diagnosis, PCR missed one mycobacteria (+) case and the composite variable missed 3. The accuracy of the composite was greater than any one of its composing variables.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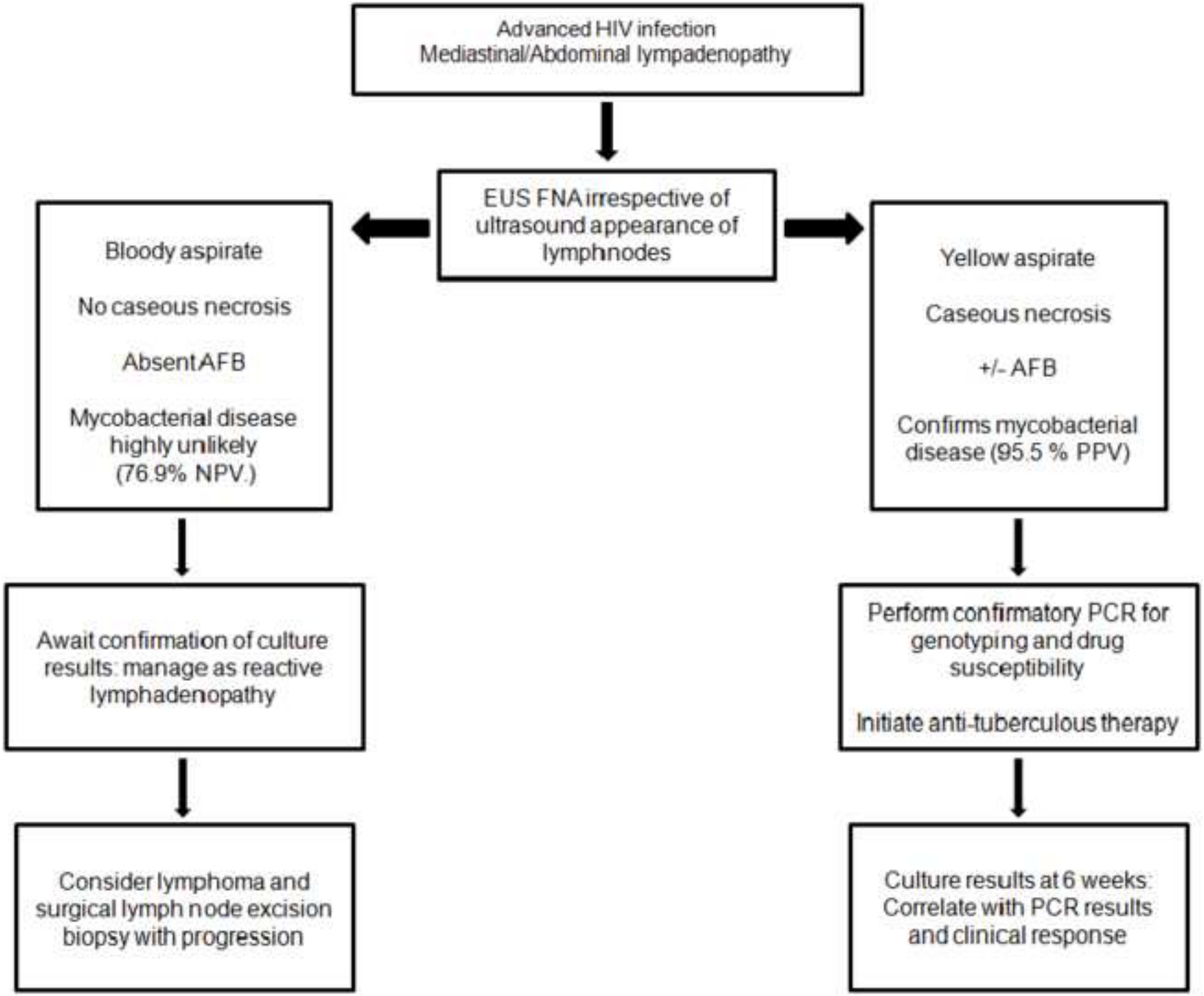
Advanced HIV infection
Mediastinal/Abdominal lympadenopathy
EUS FNA irrespective of ultrasound appearance of lymphnodes
Bloody aspirate
No caseous necrosis
Absent AFB
Mycobacterial disease highly unlikely (76.9% NPV.)
Yellow aspirate
Caseous necrosis
+/- AFB
Confirms mycobacterial disease (95.5 % PPV)
Await confirmation of culture results: manage as reactive lymphadenopathy
Perform confirmatory PCR for genotyping and drug susceptibility
Initiate anti-tuberculous therapy
Consider lymphoma and surgical lymph node excision biopsy with progression
Culture results at 6 weeks: Correlate with PCR results and clinical response