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Research article

Utility of anti-*Mycobacterium tuberculosis* antibody (ab905) for detection of mycobacterial antigens in formalin-fixed paraffin-embedded tissues from clinically and histologically suggestive extrapulmonary tuberculosis cases

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HIGHLIGHTS

- Diagnosing EPTB is difficult due to its paucibacillary nature and indistinct symptoms.
- IHC with anti-PPD antibody is more sensitive than ZN in detecting TB in fixed tissues.
- ZN staining has low specificity as it cannot differentiate between Mtb and NTM.

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ABSTRACT

Background: The detection of acid-fast bacilli in extrapulmonary tissue samples is challenging due to its paucibacillary nature. The present study assessed the utility of immunohistochemistry (IHC) using anti-*Mycobacterium tuberculosis* antibody (ab905) for detecting the presence of mycobacterial antigens in archived formalin-fixed paraffin-embedded (FFPE) tissues.

Methods: FFPE tissues [surgical biopsies (n = 32) and post-mortem tissues (n = 8)] from clinically and histologically suggestive extrapulmonary tuberculosis (EPTB) cases at the Korle Bu Teaching Hospital, Accra, Ghana from 2015 to 2020 were stained with IHC (anti-*Mycobacterium tuberculosis* antibody) and Ziehl-Neelsen (ZN) stain. The staining outcomes of IHC and ZN were compared, and their sensitivity and specificity determined against histopathology as reference standard.

Results: Lymph nodes were about 40% (16/40) of the samples analyzed. IHC stained positive in 43.8% (7/16) biopsies and 87.5% (4/5) post-mortem samples ranging from 43.8% (7/16) in lymph nodes to 80% (4/5) in gastrointestinal organs. The overall sensitivity for IHC was 52.50% (95% CI: 36.13%–68.49%) and 0% (95% CI: 0.00%–8.81%) for ZN. Specificity was 72.50% (95% CI: 56.11%–85.40%) and 75% (95% CI: 58.80–87.31%) for IHC and ZN respectively.

Conclusions: IHC using anti-*Mycobacterium tuberculosis* antibody (ab905) can detect mycobacterial antigens in diverse range of paucibacillary extrapulmonary tissue sections. It is potentially a useful tool for the diagnosis of EPTB in FFPE tissues in a routine pathology laboratory.

1. Introduction

Globally, approximately 18% of the 5.8 million tuberculosis (TB) cases recorded in 2020 were extrapulmonary TB (EPTB) – affecting sites other than the lungs (WHO, 2021). Diagnosing EPTB is challenging due to its extremely wide clinical presentations, paucibacillary nature and the use of invasive methods to obtain specimens from often inaccessible sites (Purohit and Mustafa, 2015). The gold standard for EPTB diagnosis

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includes histopathology, composite reference standard and polymerase chain reaction (PCR). Ziehl–Neelsen (ZN) staining is relatively cheap and rapid at detecting the presence of acid-fast mycobacteria but is not specific for *Mycobacterium tuberculosis* complex (MTBC) and has very low sensitivity (0%–40%) (Njau et al., 2019). Culture may be regarded as the gold standard but requires resource intensive infrastructure such as a Biosafety Level 3 (BSL-3) laboratory and only fresh unfixed tissues can be used (Polepole et al., 2017). Moreover, culture has a wide range of variable sensitivity (0%–80%) and long turnaround time of usually 4–8 weeks or up to 12 weeks on Lowenstein-Jensen (LJ) solid media (Orvankundil et al., 2019; Vongthilath-Moeung et al., 2021). Alternatively, liquid culture – mycobacterial growth indicator tube (MGIT) takes about 18–21 days for identification but relatively expensive to use in resource limited areas. The World Health Organization (WHO) has endorsed the use of the Xpert MTB/RIF Ultra (Xpert Ultra), a semi-automated, integrated, cartridge-based molecular assay with improved sensitivity but decreased specificity over earlier approved Xpert MTB/RIF (Xpert) for diagnosing EPTB and other paucibacillary forms of TB (Dorman et al., 2018; WHO, 2014). However, reported sensitivities of Xpert Ultra for EPTB samples are highly heterogeneous and vary widely across different sample types (Aurilio et al., 2022; Dorman et al., 2018; Hoel et al., 2020). Other molecular assays including in-house and commercially available PCR-based assays have been used to improve detection rates of TB in formalin-fixed paraffin-embedded (FFPE) tissues (Johansen et al., 2004; Kim et al., 2015). These tests are rapid with improved sensitivity and specificity but are costly requiring enhanced laboratory infrastructure and technical expertise. Loop-mediated isothermal amplification (LAMP) assay is a temperature-independent DNA amplification test, recommended by WHO for diagnosing TB. However, very few studies available regarding its use in the diagnosis of EPTB show variable sensitivities and specificities (Kamra et al., 2022; Nagdev et al., 2011; Sharma et al., 2017). Immunological tests such as interferon gamma release assays (IGRAs) may be used as adjunct methods for diagnosing EPTB, but cannot distinguish between latent infection and active EPTB, and negative results cannot entirely exclude the disease (Rumende et al., 2018; Shin et al., 2015). Conventional histopathological diagnosis of EPTB by haematoxylin and eosin (H&E) staining of tissue sections usually depends on the presence of classical granulomas with caseous necrosis and Langhans-type giant cells. However, some non-TB pathologic conditions could mimic TB with similar classical TB inflammation patterns (Njau et al., 2019). Immunohistochemistry (IHC), using monoclonal and polyclonal antibodies against mycobacterial whole organisms, certain antigens or purified components in their cell wall or cytoplasm can be used easily on both fresh and FFPE paucibacillary tissues without the need for prior culture (Ihama et al., 2012; Ince et al., 2011). A positive IHC result rules out the differential diagnosis of sarcoidosis and other nonspecific granulomas when H&E-stained tissue sections show non-classical tuberculous granulomatous inflammation (Kohli et al., 2014). The polyclonal rabbit anti-*Mycobacterium tuberculosis* antibody (ab905; Abcam, Cambridge, UK) is a commercially available antibody raised against mycobacteria purified protein derivative (PPD)- a heat-inactivated culture filtrates of *Mycobacterium tuberculosis* for detecting mycobacterial antigens in human tissue sections. Unlike MTBC-specific antigens, PPD contains a number of shared antigens with other mycobacterial species which is useful in establishing mycobacterial aetiology of extrapulmonary diseases with moderately high sensitivity and specificity. Also, it is relatively inexpensive and easily performed, which is suitable to laboratories in high TB burden but resource limited countries. In the present study, we investigated the utility of IHC for detection of mycobacterial antigens in FFPE extrapulmonary biopsies and post-mortem tissue sections using anti-*Mycobacterium tuberculosis* antibody at a tertiary/teaching hospital in Ghana.

2. Materials and methods

2.1. Study design

A retrospective study where archived FFPE biopsies and post-mortem tissues from clinically and histologically suggestive EPTB patients were examined by IHC and ZN staining.

2.2. Study site

The study was performed at the Department of Pathology, University of Ghana Medical School/Korle Bu Teaching Hospital (UGMS/KBTH). The Department of Pathology provides histopathology, cytology, immunohistochemistry, autopsy and other pathological services to patients in KBTH, and other health facilities. KBTH is the largest referral/teaching hospital in Ghana with over 2000-bed capacity.

2.3. Study population

Surgical biopsies and post-mortem tissues from clinically and histologically suggestive EPTB patients received at the Department of Pathology, KBTH, between 2015 and 2020.

2.4. Sample and patient data collection

Clinical and laboratory test records of 62 eligible patients were retrieved from patients' database at the Department of Pathology, KBTH. The patients' archived H&E and ZN-stained slides and corresponding tissue blocks were retrieved but some were deformed or missing, hence excluded. Therefore, 40 tissue blocks (comprising 32 surgical biopsies and eight post-mortem tissues) showing very good histologic features suggestive of TB were used for further laboratory analysis. New tissue sections were made for faded H&E and ZN-stained archived slides. In addition, sections were prepared for IHC staining. Patients' demographic information was obtained from medical records and autopsy files.

2.5. Laboratory methods

2.5.1. Tissue processing (formalin fixing and paraffin embedding)

The archived blocks were prepared as described below. Tissue specimens placed in 10% PBS buffered formalin to prevent autolysis were received in the pathology laboratory, accompanied by a request form that indicated patient information and description of the site of origin. The specimens were registered, and an identification number given that identified each patient's specimen. A Resident Pathologist gross examined the tissues by describing the type, size, colour, and texture among others. Then, whole or portions of tissues were placed into small plastic cassettes to hold the tissues while being processed to be embedded in paraffin. The tissues were initially fixed in formalin to preserve proteins and essential structures and the fixed tissues were dehydrated in varying concentrations of ethanol (70%, 95%, 100%). Next, clearing (removal of ethanol with xylene, a chemical miscible with paraffin) was done. Finally, the cleared tissues were infiltrated with molten paraffin to enable tissue sections to be easily cut to required thickness.

2.5.2. Microtomy, mounting, baking and deparaffinization

Sections (4 μm thickness) were cut from each tissue block on ice using a manual microtome (Leica RM2125 RTS, Leica Biosystems, IL, USA), a specialized precision cutting instrument, which accurately and repeatedly slices sections from a block of embedded tissue. The sections were placed in a water bath to float and subsequently mounted on frosted slides - Fisherbrand™ Frosted Microscope Slides (Fisher Scientific, PA, USA) for H&E. The slides were labelled and dried in a hot air oven at 60

°C for 30 min to remove excess water, increase adherence of the tissue onto the slide and remove surface paraffin (deparaffinization). Further deparaffinization of tissue sections for H&E was achieved by placing the slides in xylene three times, followed by rehydration with serial dilutions of ethanol in descending order (100%, 95%, 70%, 50%) and finally with water.

2.5.3. Haematoxylin and Eosin (H&E) staining

Haematoxylin and Eosin staining was performed with H&E Stain Kit (Atom Scientific, Hyde Cheshire, UK) according to manufacturer's protocol. Tissue sections were stained with filtered Harris' haematoxylin for 10 min, washed under running tap water and decolourized in 0.5% acid alcohol for 10–15 s. Again, the slides were washed under running tap water and stained in 1% Eosin Y for 2 min and followed by washing in running tap water for maximum 5 min, dehydrated and mounted. The stained sections were examined under a light microscope (Olympus, Tokyo, Japan) at 10x and 20x magnification. Sections showing chronic granulomatous inflammation with or without caseous necrosis were suggestive of EPTB and their corresponding blocks were retrieved for new sections to be made for ZN staining, using the same pre-staining processes described above. The new sections were then stained with ZN as outlined below.

2.5.4. Ziehl-Neelsen (ZN) staining

Ziehl-Neelsen staining was performed with AFB Stain Kit (Atom Scientific, Hyde Cheshire, UK) per manufacturer's instructions. Briefly, slides were flooded with filtered Carbol Fuchsin Kinyoun for 10 min and washed well in distilled water. Then, slides were decolourized with TB decolourizer until sections were colourless or pale pink, approximately 3–5 s and washed well in distilled water. The slides were counterstained with methylene blue for 15 s, washed well in distilled water and rapidly placed in absolute alcohol, airdried and mounted. The slides were examined under light microscope (Olympus, Tokyo, Japan) at 10x and 40x magnification and reported as positive or negative.

2.5.5. Immunohistochemistry (IHC) staining

New sections were made from archived blocks and mounted on positively charged glass slides (Leica BOND™ Plus Slides, Leica Biosystems, IL, USA). IHC staining was performed using anti-*Mycobacterium tuberculosis* antibody (ab905, Abcam, Cambridge, UK) with the VENTANA BenchMark GX IHC/ISH automated instrument (Roche Tissue Diagnostics, Tucson, AZ, USA) with slight modifications in the manufacturer's protocol. Positive (ZN and culture positive lung tissue sections) and negative (non-TB tissue section) controls were run simultaneously with the tests under the same conditions. Initially, the antibody (ab905), counterstain (Haematoxylin) and detection kit (OptiView DAB IHC detection kit, Ventana Medical Systems, Tucson, AZ, USA) dispensers were loaded on the reagent tray and placed on the automated Slide Stainer. Slides were loaded on to the automated Slide Stainer and the 'run' button was engaged to start the staining procedure as described below: baking at 60 °C for 30 min followed by deparaffinization using EZ prep concentrate solution at 72 °C for 4 min. After that, sections were subjected to heat-induced antigen retrieval using cell conditioning solution (CC1) at 95 °C for 24 min. The CC1 contains tris-based ethylenediamine tetraacetic acid (EDTA) with pH of 7.8. Pre-primary peroxidase blocking was done to tackle nonspecific antibody binding due to tissue peroxidase by incubating tissue sections with 3% H_2O_2. Primary antibody (ab905) at 1:150 dilution with Tris-buffered saline (TBS) was incubated at 37 °C for 1 h. Next, staining with OptiView DAB IHC Detection Kit comprising OptiView Copper, OptiView H_2O_2, OptiView DAB, OptiView HRP multimer, OptiView Universal Linker and OptiView Peroxidase Inhibitor for 8 min, followed by counterstaining with haematoxylin for 6 min and finally post counterstaining with blueing reagent for 4 min. Slides were removed from the Stainer after the run was complete, washed in warm tap water with detergent and dehydrated in graded ethanol and xylene. The slides were dried in an oven at 65 °C for 5 min and cover slipped in DPX (dibutyl phthalate xylene), a permanent mounting medium. Finally, the mounted slides were examined under light microscope (Olympus, Tokyo, Japan) by a Consultant Pathologist. Any form of brown coloured staining in cellular structures was considered positive according to manufacturer's protocol (ab905 product insert).

2.5.6. Culture and identification

Extrapulmonary tissues placed in Petri dishes with phosphate buffered saline (PBS) were minced using scalpels and scissors and homogenized with a tissue grinder. The homogenate was transferred into 50 ml Falcon tubes and decontaminated by adding equal volumes of 3% oxalic acid for 15 min. PBS was added to the decontaminated samples up to the 50 ml mark on the Falcon tube. The suspension was pelleted by centrifuging at 3000 × *g* for 30 min. The supernatant was decanted, and the pellet was re-suspended in 2 ml PBS and allowed to stand for 5 min. Next, about three drops of the inoculum were directly inoculated in duplicate onto LJ tubes (pyruvate-based and glycerol-based). The LJ tubes were incubated at 37 °C and observed daily for the first week and once a week subsequently for growth. Culture was positive when creamy buffy confluent colonies were seen after 3–4 weeks of incubation. Tubes were considered negative when no growth was observed after 8–12 weeks of incubation.

2.5.7. DNA extraction

Two to three loopfuls of isolates were suspended in 1 ml of sterile distilled water. The isolates were heat-killed on a heating block at 95 °C for 1 h to release DNA from cell wall into suspension. The suspension was centrifuged at 1400 × *g* for 10 min and the supernatant-containing DNA was stored at -20 °C until further use.

2.5.8. Genotyping

Spoligotyping is a PCR-based method which can detect and type MTBC species simultaneously based on the number of spacers within the direct repeat region in the MTBC genome. A commercially available Spoligotyping Kit (Mapmygenome, Hyderabad, India) was used for the experiment as previously described (Addo et al., 2022).

2.5.9. Drug susceptibility testing (DST)

The susceptibility pattern of isolates to rifampicin (RIF) and isoniazid (INH), the most important first line anti-TB drugs were determined using Genotype MTBDR*plus* version 2.0 (Hain Lifescience, Nehren, Germany). The assay involved three major steps: (i) amplification of specific gene targets with biotinylated primers using multiplex PCR, (ii) reverse hybridization of amplicons to immobilized probes to detect resistance and associated mutations (iii) conjugate and substrate reaction with streptavidin and alkaline phosphatase for visualization of bands corresponding to resistance patterns.

2.5.10. Data analysis

Descriptive statistics was used to describe patients' socio-demographic and clinical characteristics and expressed as frequencies and percentages (Microsoft Excel 365, Microsoft Corporation, WA, USA). IHC and ZN positive staining patterns were compared, and respective sensitivity and specificity values were calculated using MedCalc® Statistical Software (https://www.medcalc.org/).

2.5.11. Ethics approval

This study obtained ethical approval from the Noguchi Memorial Institute for Medical Research Institutional Review Board (NMIMR-IRB CPN 093/17-18) and Korle Bu Teaching Hospital Institutional Review Board (KBTH-IRB 000137/2019).

3. Results

3.1. Patients and sample background characteristics

A total of 40 FFPE extrapulmonary tissues were examined, comprising 32 surgical biopsies and eight post-mortem specimens.

Majority (60%; 24/40) of the specimens were from males. The age ranged from 1 year to 67 years old with a median of 30.5 (Table 1). Nine (one biopsy and eight post-mortem) out of 40 samples were from patients with HIV while one specimen was from an EPTB-PTB concurrent patient. Forty percent (16/40) of clinical diagnoses were lymphadenopathies. Specimen sites were mainly lymphoid 40% (16/40) (cervical lymph node 10/16, mesenteric lymph node 2/16, left groin lymph node 1/16, multiple left axillary lymph nodes 1/16, right inguinal lymph node 1/16 and submental lymph node 1/16) and genitourinary 25% (10/40) (kidney 4/10, endometrial tissue 1/10, epididymis 1/10, omentum 1/10, ovaries 1/10, testis 1/10 and uterus/tubes/ovaries 1/10). The rest of the specimen sites were central nervous system (CNS) 15% (brain 2/6, intervertebral disc material 2/6, T5 - T8 vertebrae 1/6 and T8/T9 material 1/6), gastrointestinal 12.5 % (liver 2/5, mesenteric abdominal mass 1/5, peritoneal nodule 1/5 and retroperitoneal mass 1/5) and "other" 7.5% (anterior chest wall mass 1/3, left knee 1/3 and left wrist tendon sheath 1/3). Fifty five percent (22/40) of samples were surgical excision biopsies, while surgical incision biopsies and incision samples from autopsies formed 25% (10/40) and 20% (8/40) respectively. Six cases had more than one (usually 2-3) organ affected including three cases having at least both kidneys and spleen affected. Patients were referred mainly from the KBTH 85% (34/40), Greater Accra Regional Hospital-GARH 7.5% (3/40), La General Hospital-LGH 2.5% (1/40), Nyaho Medical Centre-NMC 2.5% (1/40) and Princess Marie Louise Children's Hospital-PMLCH 2.5% (1/40) (Table 1).

Table 1. Background characteristics of clinically and histologically suggestive extrapulmonary tuberculosis patients whose archived formalin-fixed paraffin-embedded tissue samples were used in the study, N = 40.

Characteristic	Frequency	Percentage (%)
Sex		
Male	24	60
Female	16	40
Age		
<15	6	15
15–24	7	17.5
25–34	12	30
35–44	7	17.5
45–54	5	12.5
55+	3	7.5
Site affected		
CNS	6	15
Gastrointestinal	5	12.5
Genitourinary	10	25
Lymphatic	16	40
Others[a]	3	7.5
Sample types		
Excision biopsy	22	55
Incision at autopsy	8	20
Incision biopsy	10	25
Referral Health facility		
PMLCH	1	2.5
KBTH	34	85
LGH	1	2.5
NMC	1	2.5
GARH (RIDGE)	3	7.5

CNS- Central nervous system; PML CH- Princess Marie Louise Children's Hospital; KBTH- Korle Bu Teaching Hospital; LGH- La General Hospital; NMC- Nyaho Medical Centre; GARH (RIDGE)- Greater Accra Regional Hospital.

[a] Others-a group of affected sites whose frequencies are less than five.

3.2. H&E staining and microscopy

All the 40 tissues sections stained positively for H&E showing chronic granulomatous inflammation with or without caseous necrosis suggestive of EPTB (Figure 1).

3.3. ZN staining and microscopy

Acid fast bacilli (AFB) were not detected in any of the tissues examined by ZN staining. Thus, sensitivity was 0% (95% CI: 0.00%–8.81%) and specificity was 75% (95% CI: 58.80–87.31%) (Figure 2; Figure 3).

3.4. IHC staining and microscopy

Out of 40 tissue sections, 21 (52.5%) stained positive with anti-*Mycobacterium tuberculosis* antibody (ab905) (Figure 4A; Figure 4B) and 19 (47.5%) were negative (Figure 4C). When specimen types were compared, IHC positivity was 43.75% (14/32) in biopsies and 87.5% (7/8) in post-mortem specimens. Based on site of infection, the positivity rate was (50%; 3/6) CNS, (80%; 4/5) gastrointestinal, (50%; 5/10) genitourinary, (43.8%; 7/16) lymphatic and (66.7%; 2/3) "other" which includes anterior chest wall mass, left knee and left wrist tendon sheath. The overall sensitivity was 52.50% (95% CI: 36.13%–68.49%) and specificity was 72.50% (95% CI: 56.11%–85.40%).

3.5. Microbiological and molecular confirmation

Culture, genotyping and DST results were available for the eight post-mortem tissue sections which had been used in our previous study. All tissue samples were culture positive comprising 7 (87.5%) *Mycobacterium tuberculosis* and 1 (12.5%) *Mycobacterium africanum*. Major lineage/sub-lineage were Lineage 4/Cameroon (6, 75%), Lineage 4/Ghana (1, 12.5%) and Lineage 5/West African 1 (1, 12.5%). All the isolates were susceptible to INH and RIF. The concordance between culture and IHC was 87.5% (7/8) (Table 2).

5. Discussion

The study sought to illustrate the utilisation of IHC in detecting MTBC antigens in FFPE tissue sections to establish TB infection. Diagnosing paucibacillary forms of TB such as EPTB remains a challenge. Clinicians mostly rely on high index of clinical suspicion and less sensitive conventional TB diagnostic tests (AFB microscopy and culture). Therefore, using a more specific and sensitive but relatively simple and less expensive method, such as IHC compared to PCR is necessary. Immu-

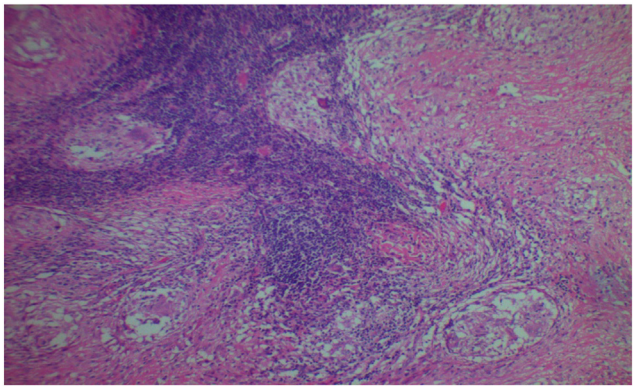

Figure 1. Representative images of haematoxylin and eosin (H&E) staining of lymph node tissues. The haematoxylin stains nuclei blue and the eosin stains cytoplasm in varying shades of pink.

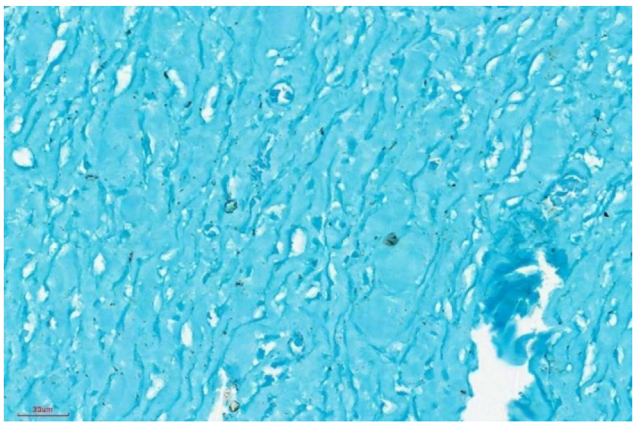

Figure 2. Representative images showing intervertebral disc material that is negative for Ziehl-Neelsen (ZN) (x40 magnification).

nochemistry staining could serve as first line or complement to clinico-pathological examinations for definitive TB diagnosis in areas with high prevalence of TB. In our study, we used polyclonal anti-*Mycobacterium tuberculosis* antibody, anti-purified protein derivative (pAbPPD) for IHC staining on archived FFPE extrapulmonary tissue sections from surgical biopsies and post-mortem tissues for detecting TB. From our study, mycobacterial antigens were detected by IHC in 52.5% (21/40) of histologically suggestive tissue sections and none 0% (0/40) by ZN. This finding is similar to reports from previous studies comparing ZN and IHC staining for TB detection (Karimi et al., 2014; Kohli et al., 2014; Nourein et al., 2014; Purohit et al., 2017). Karimi et al. (2014) and Kohli et al. (2014) used polyclonal anti-Bacillus Calmette-Guérin antibody (pAbBCG) in their work. Karimi et al. (2014) reported 100% (23/23) and 39.1% (9/23) positivity using IHC staining and ZN staining respectively, while Kohli et al. (2014) recorded 72% (72/100) and 23% (23/100) positivity for IHC and ZN respectively. In other studies, Nourein et al. (2014) used monoclonal anti 38-KD antibody (mAb38KD) to detect 78.6% (33/42) TB cases compared to 2.4% (1/42) using ZN while Purohit et al. (2017) used an in-house polyclonal anti-MPT64 (pAbMPT64) with positivity rate of 100 % (51/51) and 10% (5/51) for IHC and ZN respectively. The differences in the detection rates or sensitivities of IHC in our study compared to studies conducted elsewhere could be attributed to factors including sample size differences, specimen variety, specimen condition (fresh, fresh/frozen, fresh/FFPE, archived/FFPE), type and cross reactivity of antibody used (monoclonal, polyclonal, cross reactive, non-cross reactive). IHC is robust and can detect fragmented tubercle bacilli, and is suited for the diagnosis of paucibacillary EPTB (Goel and Budhwar, 2008). Conversely, ZN staining requires an intact cell wall, but due to intensive phagocytotic activity by macrophages in tuberculous granulomas as well as formalin fixation, the morphological characteristics of tubercle bacilli often get distorted. This may account for the very low and suboptimal sensitivity of ZN in our study and most other studies although specificity may be relatively high (Crothers et al., 2021; Goel and Budhwar, 2008; Nourein et al., 2014). Similar to a study by Kohli et al. (2014), our study found less than 100% IHC positivity based on specimen types with values ranging from 43.8% (7/16) in lymph nodes to 80% (4/5) in gastrointestinal organs. On the other hand, a study by Goel and Budhwar (2008) reported 100% positive IHC staining in all specimen types. The finding that most cases presented were lymphadenopathies correlates with reports from other studies showing TB lymphadenitis as one of the most common forms of EPTB (Addo et al., 2021; Arega et al., 2020; Houston and Macallan, 2014; Ohene et al., 2019). Culture and genotyping confirmed all but one of the positive IHC stained post-mortem specimens in our current study. Identification of *M. tuberculosis* and *M. africanum* from the post-mortem specimens confirms the positive IHC staining and suggests the discordant IHC result was false negative. Knowledge of the aetiology of confirmed TB in post-mortem specimens may have important implications on research and epidemiological data and could serve as important feedback for clinicians in the management of such cases in future (Shergill et al., 2017).

The strengths of the present study include the use of tissues from various extrapulmonary sites which reflected routine practice in a hospital setting. Also, to the best of our knowledge, our study used VENTANA BenchMark GX IHC/ISH automated instrument (VENTANA) for detection of mycobacterial antigens by anti-*Mycobacterium tuberculosis* antibodies. The advantage of using VENTANA is that more tissue sections could be auto stained within a short period with increased sensitivity compared to manual staining method. The automated method could be implemented in large referral and research-focussed hospitals where large number of EPTB cases are expected to be sent routinely for accurate diagnosis. Alternatively, the manual IHC method could be used in routine clinical laboratories at the district level. In spite of the strengths, the study had several limitations and interpretations of some of the findings should be seen in the light of these weaknesses. Firstly, the study had

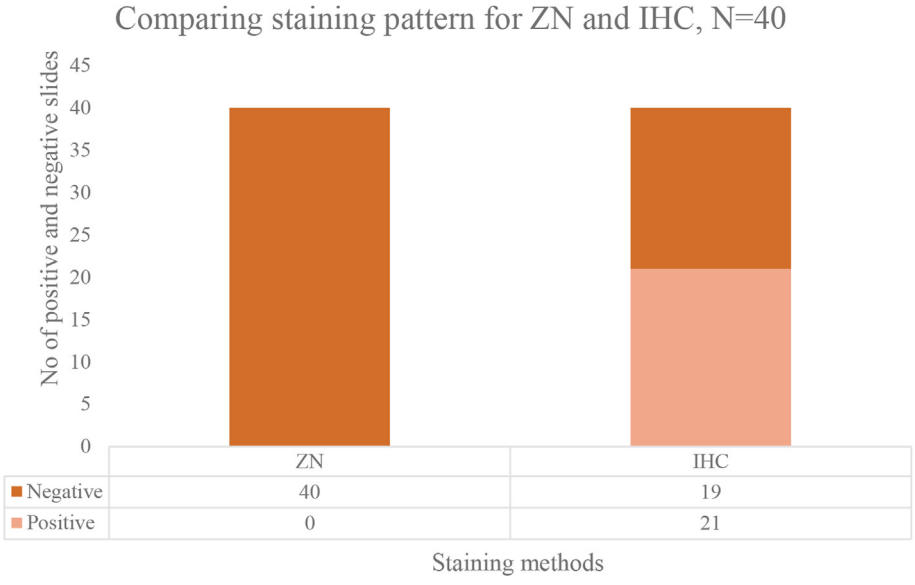

Figure 3. Positive staining pattern for Ziehl Neelsen (ZN) and Immunohistochemistry (IHC).

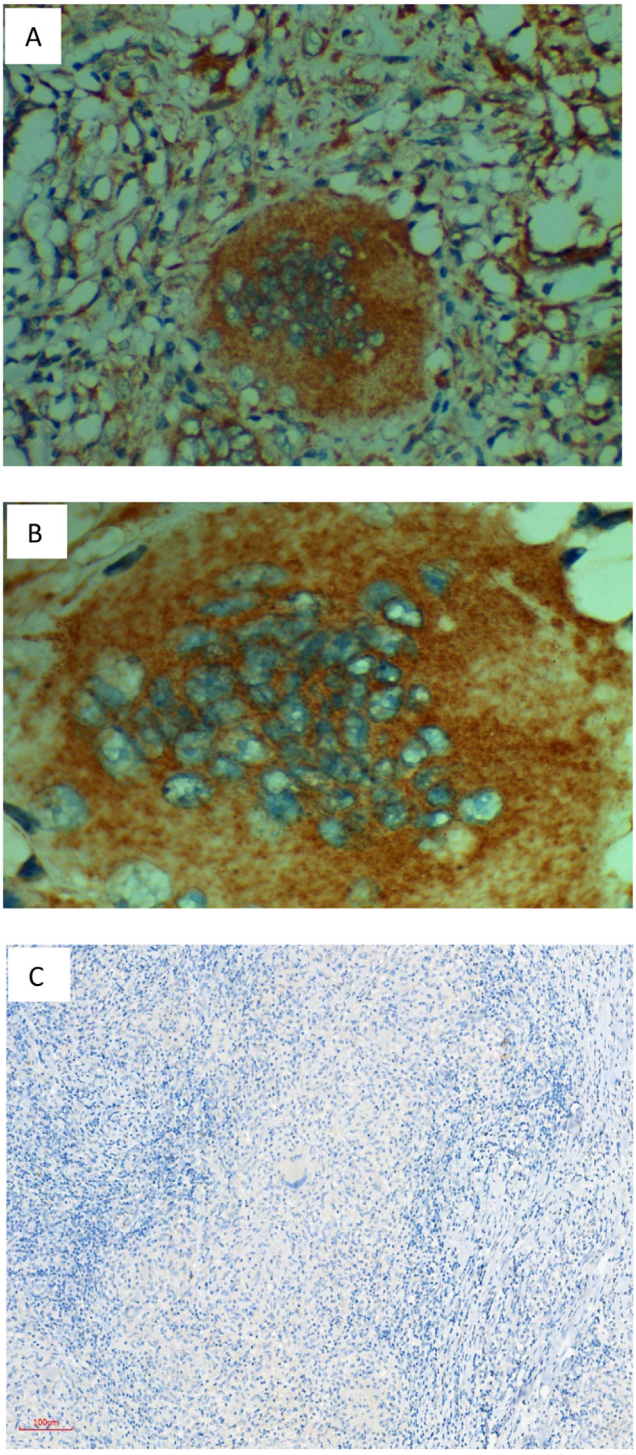

Figure 4. Representative image of lymph node tissues showing strong and granular staining of giant cells. Positive for anti-*Mycobacterium tuberculosis* (ab905) IHC staining any form of brown coloured staining in cellular structures ([A]: x40 magnification; [B]: x100-oil immersion magnification). [C] Negative for anti-*Mycobacteium tuberculosis* (ab905) IHC staining (No brown coloured stain).

relatively small sample size for determining sensitivity and specificity and recommends a study with larger sample size for more realistic results. Also, the study was retrospective and laboratory-based, and hence the amount of demographic and clinical data available was limited. Moreover, we used a polyclonal antibody with known potential cross reaction with three atypical mycobacteria. However, these atypical mycobacteria rarely cause diseases in humans. Also, given that the antibody does not show strong cross-reactivity with *M. bovis* antigens vaccination with BCG would not interfere with the results (Shamaei et al., 2017).

Table 2. Comparing immunohistochemistry findings in post-mortem tissues with culture positive samples (N = 8).

Sample ID	Culture	[a]Species	[b]Lineage/Sub-lineage	IHC	ZN
P/1343/15	Positive	*M. tuberculosis*	Lineage 4/ Cameroon	Positive	Negative
P/1337/15	Positive	*M. tuberculosis*	Lineage 4/ Cameroon	Negative	Negative
P/150/16	Positive	*M. tuberculosis*	Lineage 4/ Cameroon	Positive	Negative
P/478/16	Positive	*M. africanum*	Lineage 5/West African 1	Positive	Negative
P/524/16	Positive	*M. tuberculosis*	Lineage 4/ Cameroon	Positive	Negative
P/625/16	Positive	*M. tuberculosis*	Lineage 4/ Cameroon	Positive	Negative
P/154/16	Positive	*M. tuberculosis*	Lineage 4/Ghana	Positive	Negative
P/588/16	Positive	*M. tuberculosis*	Lineage 4/ Cameroon	Positive	Negative

IHC- Immunohistochemistry; ZN- Ziehl-Neelsen.

[a] Species: *Mycobacterium tuberculosis* complex species was identified with GenoType MTBC (HainLifeScience, Nehren, Germany).

[b] Lineage/Sublineage: Spoligotyping was used to assign lineages/sub-lineages to MTBC species.

6. Conclusion

Immunohistochemistry using anti-*Mycobacterium tuberculosis* antibody (ab905) is a relatively simple, inexpensive and sensitive technique for detecting mycobacterial antigens in FFPE tissue sections. It can be easily incorporated in routine pathology laboratories at the tertiary/regional level health facilities to complement clinicopathological examination for definite EPTB diagnosis. However, for programmatic implementation, more studies are required to be conducted at peripheral level healthcare settings.

Declarations

Author contribution statement

Samuel Ofori Addo: Conceived and designed the experiments; Performed experiments; Analyzed and interpreted data; Wrote the paper.

Afua Owusua Darkwah Abrahams: Conceived and designed the experiments; Analyzed and interpreted data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Gloria Ivy Mensah; Kennedy Kwasi Addo: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Bernice Anane Mawuli: Performed experiments; Wrote the paper.

Lydia Mosi: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Edwin Kwame Wiredu: Analyzed and interpreted data, Wrote the paper.

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Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of interest's statement

The authors declare no competing interests.

Additional information

No additional information is available for this paper.

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