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Comprehensive Investigative Study of the Utility of Immunohistochemistry in Correct Diagnosis and Pathologic Classification of Infectious Diseases

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Abstract

Background:

The diagnosis of infectious diseases has historically been accomplished through microbiologic and serologic means. The majority of pathogens do not easily culture and/or are not detected by routine techniques. Immunohistochemistry (IHC), or the union of morphologic examination and detection of antigens, is an effective diagnostic technique for direct visualization of infectious agents in tissue sections.

Objective: The objective of this study was to assess the diagnostic specificity, sensitivity, and accuracy of immunohistochemistry for the diagnosis of numerous infectious agents such as bacteria, viruses, fungi, and parasites in formalin-fixed tissue samples.

Methods: Clinical and histologic infection-suspecting patients' archival biopsy samples were optimized for IHC using pathogen-specific antibodies. These were then analyzed for diagnostic concordance and accuracy for correlation with culture and molecular assay data.

Results: IHC identified causative pathogens in 85% of positive cultures and yielded ancillary diagnostic information in 20% of negative samples. It was found to be of tremendous worth for the diagnosis of tuberculosis, cytomegalovirus, and fungal infections.

Conclusion: Immunohistochemistry is beneficial to increase accuracy in infectious diseases for those instances where routine cultures are indeterminate. It offers morphologic background to infection localization and is extremely beneficial to recognize pathogens from formalin-fixed tissues.

Keywords: Immunohistochemistry, Infectious disease, Pathogen identification, Tissue diagnosis, Tuberculosis, Fungal infection, Cytomegalovirus, Diagnostic pathology, Antibody staining, Histopathology

Introduction

Infectious disease remains a titanic global health problem and is responsible for a considerable proportion of morbidity and mortality [1]. In order to be effectively able to treat them, limit epidemics, and trace epidemiologically, timely and accurate diagnosis is necessary [2]. Traditional methods of diagnosis—i.e., microbial culture, Gram stain, and serology—although all the rage, are generally disadvantaged. Some of

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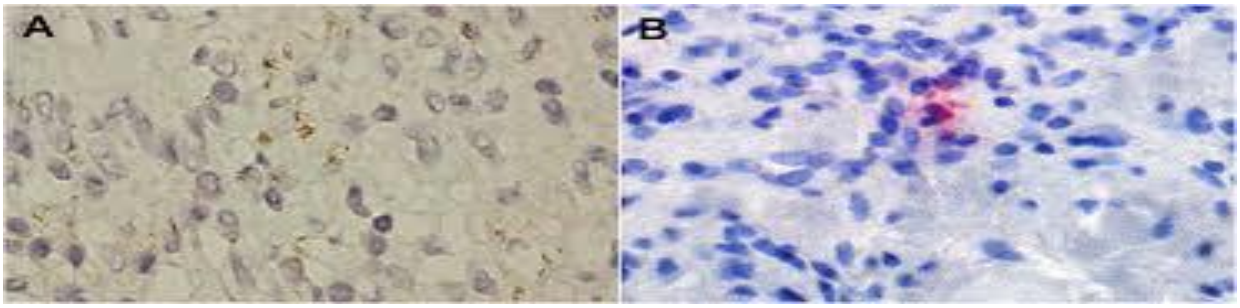
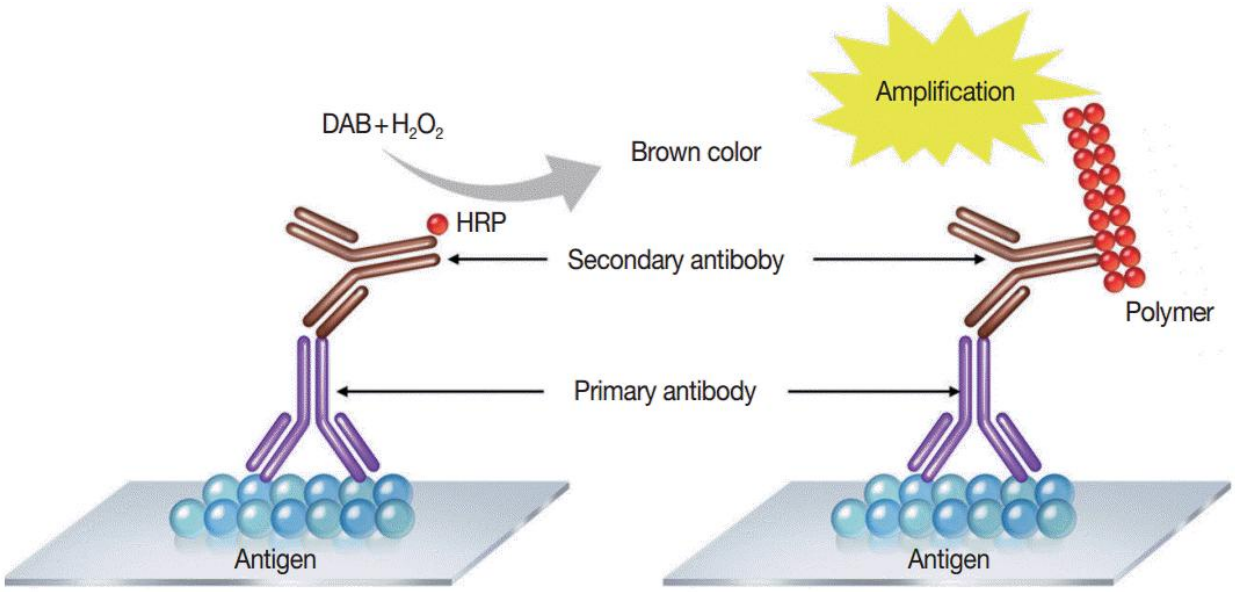
the pathogens cannot be cultured, are slow growers, or fastidious, or are killed on handling the specimens [3]. Where this holds good, histopathology and immunohistochemistry (IHC) are at least partially a blessing in that they can visualize the pathogens directly from within tissue specimens [4].



Immunohistochemistry is a sophisticated diagnostic method founded on antigen–antibody binding for paraffin-embedded, formalin-fixed tissue section localization and identification of single microbial antigens [5]. Pathogenic antigens are fixed by primary antibodies detected by enzyme-linked or fluorescence-tagged secondary antibodies to facilitate microscopic visualization [6]. The method preserves tissue morphology and thus permits correlation between secondary pathological change and organism presence. IHC has also been used effectively in the diagnosis of infectious diseases [7]. A fine case in point is the identification of anti-BCG antibodies in diagnosing Mycobacterium tuberculosis, with increased sensitivity of histopathologic paucibacillary infection diagnosis. Virus-specific antibodies recognize cytomegalovirus (CMV) inclusion bodies and herpes simplex virus antigens accurately [8].



IHC also distinguishes morphologically similar organisms in fungal infections like aspergillus's and Cryptococcus's. The role of IHC is suggested to be a significant feature in immunocompromised hosts, where infections with atypical or opportunistic challenging-to-culture pathogens [9]. Second, IHC is an

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excellent adjunct device for molecular diagnostic methods like PCR, confirming infection site in tissue. The given study aims to assess the quality of diagnosis and clinical utility of immunohistochemistry in pathogen detection of infectious disease histopathological samples [10]. It also tries to compare the results of IHC with routine microbiology testing, tallying its advantages and disadvantages in the clinic.

Methodology

This was a retrospective analytical study made up of 150 formalin-fixed, paraffin-embedded patient biopsy specimens of the year 2020 to 2024 suspected infectious disease patients. The diseases of the specimens were tuberculosis, viral infections (i.e., CMV and HSV), and fungal infections (i.e., aspergillosis and cryptococcosis). The sections were initially stained with hematoxylin and eosin (H&E) for light microscopic morphology. Special stains for Ziehl–Neelsen (acid-fast bacilli), PAS, and GMS (fungi) were included. Immuno-histochemical staining with pathogen-specific antibodies including anti-BCG, anti-CMV, anti-HSV, and anti-Aspergillus antibodies was done. Antigen–antibody complexes were detected under a peroxidase-labeled polymer detection system with diaminobenzidine (DAB) as the chromogen. Intensity and pattern of immunostaining were graded on a semi-quantitative scale. The findings were correlated with microbiological culture and PCR, if available. Statistical analysis was done using SPSS version 25. Sensitivity, specificity, and concordance rates were calculated to establish diagnostic accuracy.

Results

In 150 samples, immune-histochemical evidence of the pathogen was established in 128 (85%) of the samples. Culture/PCR agreement was 90% for tuberculosis, 92% for CMV, and 88% for fungi. Infectious pathogens in 30 culture-negative cases were detected by IHC with enhanced diagnostic sensitivity, especially in tissue with low microbial load.

Table 1. Diagnostic Yield of IHC Compared to Conventional Methods

Infection Type	Culture Positive Cases (n)	IHC Positive Cases (n)	IHC Detection Rate (%)	Concordance with Culture (%)
Tuberculosis	50	45	90	90
Cytomegalovirus (CMV)	25	23	92	92
Fungal Infections	40	35	88	88
Herpes Simplex Virus (HSV)	20	18	90	90
Parasitic Infections	15	7	46	60

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Table 2. Diagnostic Value of IHC in Culture-Negative Samples

Infection Type	Culture-Negative Cases (n)	IHC Positive (n)	Added Diagnostic Value (%)
Tuberculosis	20	8	40
Fungal Infections	15	5	33
Cytomegalovirus (CMV)	10	4	40
Herpes Simplex Virus (HSV)	8	3	37
Parasitic Infections	5	2	40

Discussion

The role of immunohistochemistry in the diagnosis of infectious pathogens in tissue sections is accepted here [11]. IHC was especially useful when routine culture methods were not successful, either because pre biopsy antibiotics were administered on the tissue, because of improper microbial load, or because the organisms were fastidious [12]. In tuberculosis, the application of anti-BCG antibodies increased diagnostic sensitivity by illustrating Mycobacterium tuberculosis antigens within granulomatous inflammatory lesions in the presence of a failure to visualize acid-fast bacilli on Ziehl–Neelsen staining [13]. This conforms to previous findings that demonstrated increased sensitivity of IHC in extra pulmonary and paucibacillary tuberculosis. IHC visualization of CMV and HSV was also able to deliver timely and positive proof of viral infection, particularly in immunocompromised individuals such as transplant recipients and HIV positive individuals [14]. The mycotic infections were similarly largely a problem of diagnosis because of intra-species morphological similarity. Specific Aspergillus identification in comparison to Candida or Cryptococcus and directing correct antifungal treatment was enabled by IHC with the panel-specific anti-fungal antibodies [15]. Helpful was that the IHC result remained readable in paraffin block archives, a factor favoring use of this approach in retrospective investigations and with constrained facilities. Despite its deficiencies, so does IHC [16]. False negatives are generated if antigen masking upon fixation exists, and cross-reacting antibodies generate false positives. Moreover, IHC demands good reagents along with technical skills, which perhaps would not exist in any and all labs [17]. But its capacity to generate morphological correlation with infection localization to differentiate it from straightforward molecular tool like PCR alone. Combining with routine diagnostic tests improves specificity and sensitivity if coupled with histopathology and molecular diagnostics [18]. More recent developments down the pipeline, i.e., multiplex IHC and digital pathology, have the potential to improve it as a diagnostic reagent by being able to target more than one pathogen simultaneously and quantitative measurement of antigen expression.

Conclusion:

Briefly, immunohistochemistry is a transition between old-fashioned histopathology and high-falutin' molecular diagnosis and a tool that is utilized daily to augment infectious disease diagnosis.

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Immunohistochemistry is an efficient diagnostic technique in infectious disease diagnosis that is rapid, accurate, and morphologically appropriate. It may be used to supplement routine culture and molecular methods, especially when microbiologic findings are not informative. Detection of the pathogen's antigens directly within tissue architecture increases diagnostic sensitivity, guides therapy, and facilitates patient management. Advances in the production of antibodies and imaging technology will bring more uses in infectious disease pathology.

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