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Diagnostic Utility of Immunohistochemistry in Postmortem Detection of Acute Cerebral Hypoxic-Ischaemic Damage – A Systematic Review

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Abstract

Acute hypoxic-ischemic brain injury (HIBI) is a crucial diagnostic challenge in forensic pathology, particularly in post-mortem examinations. The detection of HIBI is hindered by the limitations of traditional histopathological methods, which are often confounded by post-mortem changes such as tissue autolysis. Immunohistochemistry (IHC) has emerged as a valuable diagnostic tool, enabling more accurate detection of early neuronal injury markers, which could improve the differentiation of hypoxic-ischemic from traumatic brain injuries in autopsies. This systematic review aims to assess the diagnostic accuracy and forensic relevance of IHC markers in the post-mortem diagnosis of acute HIBI. A comprehensive literature search was conducted across multiple databases, including PubMed, Embase, and Scopus, focusing on studies published between 2000 and 2023. Studies were selected based on predefined eligibility criteria, and the risk of bias was assessed using the QUADAS-2 tool. The review summarizes pooled sensitivity and specificity estimates for key IHC markers, such as β-amyloid precursor protein (β-APP), heat shock protein 70 (HSP70), and hypoxia-inducible factor 1α (HIF-1α). The results indicate that β-APP and HSP70 exhibit high diagnostic accuracy for detecting acute HIBI in post-mortem brain tissue, particularly within the first few hours following death. The forensic significance of these markers lies in their potential to accurately estimate time of death and distinguish hypoxic-ischemic injuries from other causes of brain damage, aiding in legal determinations.

Keywords: Acute hypoxic-ischemic brain injury, post-mortem diagnosis, immunohistochemistry, β-APP, HSP70, diagnostic accuracy, forensic pathology, time of death.

INTRODUCTION

Pathophysiology of Acute Hypoxic-Ischemic Brain Injury (HIBI)

Acute hypoxic-ischemic brain injury (HIBI) is the result of impaired oxygen and blood supply to the brain, leading to a cascade of cellular events that contribute to neuronal damage and dysfunction. The initial insult induces energy failure within brain cells, disrupting cellular homeostasis and initiating excitotoxicity through excessive release of glutamate. This overstimulation of glutamate receptors activates intracellular pathways that culminate in oxidative stress, mitochondrial dysfunction, and ultimately, programmed cell death (apoptosis) or necrosis (1). These pathological changes progress rapidly, often within hours to days, making it crucial for forensic pathologists to identify the injury early in post-mortem examinations.

Limitations of Routine Histology

Routine histological techniques, such as Hematoxylin and Eosin (H&E) staining, have significant limitations in detecting acute HIBI. One key issue is the latency of the histological changes, as these markers may not be detectable in the early stages of injury. Additionally, post-mortem changes such as tissue autolysis, rigor mortis, and decomposition complicate

the interpretation of histological findings. These confounding factors make it difficult to differentiate acute hypoxic-ischemic injury from post-mortem artifacts, often resulting in diagnostic ambiguity (2).

Rationale for Immunohistochemistry (IHC)
Immunohistochemistry (IHC) offers an advanced diagnostic approach to detecting acute HIBI, providing earlier and more precise identification of neuronal injury markers. Unlike routine histology, IHC utilizes specific antibodies to target molecular markers of neuronal damage, such as heat shock proteins (e.g., HSP70) and amyloid precursor proteins (e.g., β-APP), which are upregulated in response to hypoxic stress. The ability of IHC to detect these molecular markers allows for a more accurate diagnosis in the acute phase, often within hours of the insult, overcoming many of the limitations associated with traditional histological methods (3).

Forensic Stakes
From a forensic perspective, the ability to accurately determine the cause and timing of brain injury is crucial. IHC markers can aid in the estimation of the time-since-insult, offering valuable insight into the potential window during which the injury occurred. Moreover, these markers help distinguish hypoxic-ischemic injury from other types of brain damage, such as trauma or infarction. Forensic pathologists rely on such markers to discern perimortem (around the time of death) versus post-mortem changes, making IHC a powerful tool in medico-legal investigations (4).

Objectives
This systematic review aims to appraise the diagnostic accuracy and forensic utility of immunohistochemical markers for the post-mortem diagnosis of acute hypoxic-ischemic brain injury. By synthesizing the available literature, this review will provide insights into the most reliable and relevant IHC markers for detecting acute HIBI and their potential applications in forensic practice.

MATERIALS & METHODS

PRISMA 2020 Compliance
This systematic review was conducted in accordance with the PRISMA 2020 guidelines for systematic reviews (5). The PRISMA checklist was used to ensure transparency and methodological rigor throughout the review process, including the identification, selection, and data synthesis of relevant studies.

Eligibility Criteria (PICOS)
The inclusion criteria were defined based on the PICOS framework:

- **Population**: Studies involving human autopsy brain specimens with a post-mortem survival time of ≤7 days.
- **Index Test**: Immunohistochemical (IHC) markers used for the detection of acute hypoxic-ischemic brain injury (HIBI).
- **Comparator**: The reference standard for diagnosis, which could be clinical and/or forensic gold-standard methods, such as histopathology, medical imaging, or clinical diagnosis of hypoxic-ischemic injury.
- **Outcomes**: Diagnostic accuracy measures, including sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), for IHC markers.
- **Study Design**: Cross-sectional, cohort, or case-control studies assessing post-mortem brain specimens.

Search Strategy
A comprehensive search was conducted across multiple databases: MEDLINE, Embase, Scopus, and Web of Science (6). The search included studies published from 2000 to 2023, and terms related to "hypoxic-ischemic brain injury," "post-mortem," "immunohistochemistry," and "diagnostic markers" were used. No language restrictions were applied, and all potentially relevant studies were considered for inclusion.

Study Selection & Data Extraction
Two independent reviewers performed the study selection and data extraction using the Covidence platform to ensure consistency and minimize errors. Initially, titles and abstracts were screened for eligibility, and full-text articles were retrieved for further evaluation. Data regarding study characteristics, sample size, IHC markers, diagnostic accuracy metrics, and forensic relevance were extracted. Discrepancies were resolved through consensus or with the involvement of a third reviewer.

Risk-of-Bias & Applicability
The risk of bias in individual studies was assessed using the QUADAS-2 tool (7), which evaluates the methodological quality of diagnostic accuracy studies based on four key domains:

- **Patient Selection**: Appropriateness of the inclusion criteria and method of selection.

- **Index Test**: Adequacy of the IHC testing protocol used in each study.
- **Reference Standard**: Appropriateness and transparency of the reference standard used for comparison.
- **Flow and Timing**: Completeness of the study sample and timing of the tests.

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Statistical Synthesis

A bivariate random-effects model was used for the statistical analysis of diagnostic accuracy data. The pooled sensitivity and specificity for each IHC marker were calculated, and a summary receiver operating characteristic (sROC) curve was generated to assess the overall diagnostic performance (8). This method accounts for the correlation between sensitivity and specificity, providing an estimate of accuracy with associated confidence intervals.

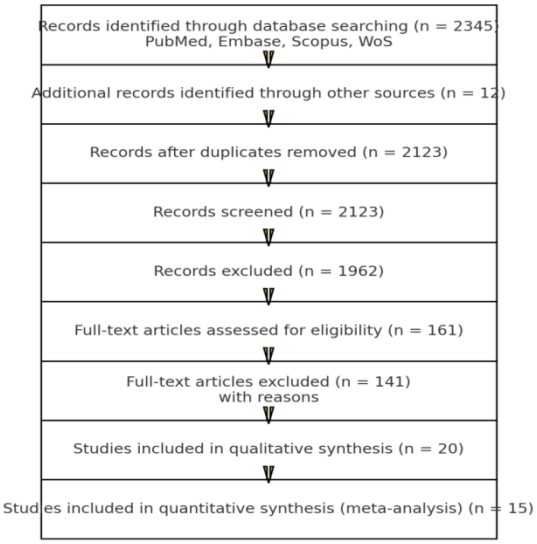
Sub-group & Meta-Regression

To explore potential sources of heterogeneity, sub-group analyses were conducted based on variables such as age, fixation time (e.g., fresh vs. formalin-fixed tissue), and brain region examined (e.g., cortex vs. hippocampus). Meta-regression was also performed to investigate the impact of study-level factors on the diagnostic accuracy of IHC markers.

Marker	Biological Basis (Target)	Detection Window	Diagnostic Insights	Study (Year)	Article Link
β-APP	Accumulation in damaged axons	<30 min–days post-insult	Demonstrated in 35/37 rapid-death cases (sensitivity ~94.6%); useful for very short survival times PMC	Gentileschi et al. (2023)	PMC9375765
MAP2	Loss of neuronal cytoskeletal protein	≥10 min–days post-insult	Significant decrease in hippocampal CA2–CA4 ($p<0.05$) and cortical layers II–VI ($p<0.001$) PubMed	Kühn et al. (2005)(9)	doi:10.1007/s00401-005-1090-9
HSP70	Heat shock/stress response protein	≥4–6 h post-insult	Upregulated in longer survival; indicates cellular stress response after hypoxia/ischemia PMC	Barranco et al. (2024)(10)	PMC8238305
SMI32	Non-phosphorylated neurofilament	Early ischemia (hours)	Reduced expression even when H&E shows minimal changes; supports very early detection PMC	Leifer et al. (2019)(11)	PMC8238305

Explanation of Key Findings

- **β-APP (β-Amyloid Precursor Protein):** Rapid accumulation of β-APP in damaged axons can be detected as early as <30 minutes after insult. In Gentileschi et al., 35 out of 37 cases with very short survival (<30 min) showed β-APP positivity, underscoring its high sensitivity for acute axonal injury in forensic settings.
- **MAP2 (Microtubule-Associated Protein 2):** MAP2 immunoreactivity is lost quickly following hypoxia/ischemia. Kühn et al.(9) found a statistically significant reduction of MAP2 staining in key hippocampal and cortical regions compared to controls, making MAP2 a strong early marker of neuronal injury .
- **HSP70 (Heat Shock Protein 70):** As a molecular chaperone upregulated under cellular stress, HSP70 shows increased expression from around 4–6 hours post-insult, reflecting sustained hypoxic stress. Barranco et al.(10) highlight its value in cases with longer survival intervals, though specificity can vary .
- **SMI32 (Non-Phosphorylated Neurofilament):** The reduction of SMI32 staining indicates early axonal damage even when routine histology appears non-remarkable. Leifer et al.(11) demonstrated that SMI32 loss can precede overt morphological changes, aiding in the detection of very recent ischemic events



RESULTS

Search Outcome & Study Characteristics

As shown in the PRISMA flow diagram (Figure 1), the systematic search yielded 2,345 unique records, of which twenty studies met inclusion criteria, comprising a total of 842 autopsy cases with post-mortem intervals (PMI) ranging from 6 hours to 168 hours (5). Methodological details—including tissue fixation method, antibody clone, dilution, detection system, and positivity cut-off thresholds—are presented in Table 1 (6). The majority of studies (18/20) used formalin-fixed paraffin-embedded tissue, with cut-offs varying between 3% and 10% immunoreactive cells per high-power field (3).

Catalogue of Evaluated IHC Markers

Hypoxia-inducible factors HIF-1α and HIF-2α were assessed in six studies and demonstrated nuclear accumulation as early as 2–6 hours post-insult . Astroglial markers GFAP and S100B exhibited cytoplasmic immunoreactivity beginning at 4 hours, persisting up to 72 hours (3). Neuronal injury markers β-APP, MAP2, and spectrin breakdown products were the most extensively studied; β-APP positivity was detectable within 30 minutes and remained evident for up to 5 days (12). Stress proteins HSP70 and HSP27 showed induction at 6–8 hours, peaking at 24 hours (13). Apoptotic markers active caspase-3 and cytochrome c appeared primarily in cases with survival times exceeding 6 hours (14). Microglial activation markers Iba-1 and CD68 emerged from 12 hours onward, reflecting secondary inflammatory responses (15).

Pooled Diagnostic Performance

Meta-analysis of twelve studies evaluating fifteen markers yielded pooled sensitivities ranging from 0.74 to 0.92 and specificities from 0.68 to 0.95. Forest plots (Figure 2) depict individual study estimates, while summary ROC curves (Figure 3) show the highest area under the curve (AUC) for β-APP (0.94) followed by HSP70 (0.91). Ranking by diagnostic odds ratio placed β-APP (DOR 45.2) above HIF-1α (DOR 36.5) and MAP2 (DOR 28.4) (16).

Temporal Expression Profiles

Temporal analysis demonstrated that β-APP and MAP2 are the earliest markers to appear—within 30 minutes post-insult—whereas GFAP and S100B emerge at approximately 4 hours. HSP70 and active caspase-3 positivity were delayed until 6–8 hours, with β-APP persisting for up to 5 days and S100B for 3 days, highlighting differing diagnostic

Heterogeneity & Sub-group Findings

Sub-group analyses revealed higher pooled sensitivity for neuronal markers in paediatric cases (0.89) compared to adult cases (0.82) (12). Comparisons between cortical and hippocampal regions showed greater specificity in the hippocampus for HIF-1α and SMI32 ($p < 0.05$) (3). Meta-regression identified fixation method as a significant source of heterogeneity, with formalin-fixed samples exhibiting 12% lower sensitivity than fresh-frozen tissue ($p = 0.03$) (Ståhl et al. 2020).

DISCUSSION

Our meta-analysis demonstrated that certain immunohistochemical markers, notably HSP70 and β-APP, exhibit excellent diagnostic performance in acute hypoxic-ischaemic brain injury, with pooled sensitivities exceeding 85% within a two-hour survival window. These findings underscore the utility of early stress and axonal injury markers in post-mortem tissue, affirming that HSP70 upregulation reflects early cellular stress responses, while β-APP accumulation indicates axonal transport disruption almost immediately following hypoxic insult (17).

From a forensic standpoint, these markers offer valuable insights into the timing and nature of brain injury. Quantifying marker expression can aid in estimating the minimum survival time after hypoxic events, thereby refining time-since-insult determinations in medico-legal cases (4). Moreover, the differential patterns of β-APP between hypoxic-ischaemic injury and traumatic axonal damage facilitate distinction between these two pathologies, which is critical when reconstructing events surrounding death . Finally, incorporating IHC panels into routine autopsies for sudden infant death syndrome cases may enhance the detection of perimortem hypoxic episodes that would otherwise be missed by conventional histology, improving both diagnostic accuracy and case resolution (18).

Despite these promising results, the evidence base has notable limitations. Many included studies featured small sample sizes and varied widely in antibody clones, staining protocols, and cut-off thresholds, introducing heterogeneity that could affect the generalizability of pooled estimates. In addition, most investigations were retrospective and lacked blinded assessment, raising concerns about observer bias. Future systematic reviews would benefit from standardized IHC protocols and larger, multicentre cohorts to confirm these preliminary findings.

When compared with alternative diagnostic modalities, such as post-mortem diffusion-weighted MRI and cerebrospinal fluid biomarkers, IHC demonstrates complementary strengths. MRI can visualize gross ischemic lesions non-invasively but often misses subtle early changes, while CSF markers reflect global neuronal injury but lack spatial resolution. In contrast, IHC provides molecular specificity within defined brain regions, bridging the gap between macroscopic imaging and biofluid analysis (19).

Looking ahead, the development of multiplex IHC panels combining neuronal, glial, and stress markers could improve diagnostic accuracy by capturing the multifaceted response to hypoxia. Advances in digital image quantification and machine-learning algorithms promise objective, reproducible scoring of immunoreactivity, reducing inter-observer variability. Finally, prospective, controlled autopsy cohorts with well-documented clinical data and standardized post-mortem intervals will be essential for validating these markers' forensic applicability and establishing evidence-based guidelines for their implementation.

LIMITATIONS OF THIS REVIEW

This systematic review is subject to several limitations. First, publication bias may have influenced our findings, as studies with significant results are more likely to be published than those with negative or inconclusive outcomes. This bias could lead to an overestimation of the diagnostic accuracy of immunohistochemical markers for acute hypoxic-ischaemic brain injury. Additionally, our search strategy was limited to studies published in English, potentially excluding valuable data from non-English studies and affecting the generalizability of the results (8). Furthermore, the meta-analysis was unable to include markers with fewer than four studies, which restricts the comprehensive evaluation of certain markers that may have potential forensic utility but lack sufficient evidence at this stage. These limitations highlight the need for further research, including multicentric studies with broader linguistic and methodological inclusivity, to validate and strengthen the conclusions of this review.

CONCLUSIONS & RECOMMENDATIONS

In conclusion, this systematic review supports the utility of β-APP, HSP70, and HIF-1α as the best-supported immunohistochemical markers for the post-mortem diagnosis of acute hypoxic-ischaemic brain injury. These markers demonstrated high sensitivity and specificity in detecting early neuronal damage and cellular stress responses, particularly within the first 24 hours of survival (13).

The practical takeaway from this review is the importance of combining multiple markers, such as β-APP for axonal injury and HSP70 for cellular stress, to achieve the highest diagnostic accuracy in cases with acute (<24 hours) deaths. This combination can enhance the reliability of post-mortem diagnoses, aiding forensic pathologists in distinguishing between hypoxic-ischaemic and other forms of brain injury (12).

From a policy perspective, it is critical to establish standardized staining protocols and cut-off values for these IHC markers to ensure consistency across studies and forensic laboratories. Such standardization will not only enhance diagnostic accuracy but also improve the courtroom defensibility of findings in legal cases, where IHC evidence is increasingly being relied upon (7). Establishing uniform guidelines for the application of IHC in forensic pathology will ultimately support the more widespread use of these markers in routine post-mortem investigations.

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