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ORIGINAL ARTICLE

CD117 and HIF-1α as molecular clocks in traumatic brain injury: An autopsy-based study with forensic and clinical implication

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Summary Traumatic brain injury (TBI) is a leading cause of mortality and disability worldwide. Accurate estimation of the post-injury survival interval is essential for reconstructing events, while in clinical neurotraumatology the first 24 h are recognized as a critical window for deterioration. Mast cells (MCs), identifiable by CD117 (c-Kit), and the transcription factor hypoxia-inducible factor 1-alpha (HIF-1α) are key players in neuroinflammation and hypoxic cascades, yet their temporal expression in human TBI remains poorly defined. We investigated 40 forensic autopsy cases with survival intervals ranging from minutes to 16 days. Immunohistochemistry for CD117 and HIF-1α was performed on peri-contusional cortex and white matter. CD117-positive cells exhibited a biphasic perivascular pattern: an early peak within 1–4 h, decline by 19 h, and reappearance at 4–8 days. Intraparenchymal CD117-positive cells, often with glial or neuronal morphology, were abundant in survival $<$ 19 h, declined by day 4, and reappeared at day 16. In contrast, HIF-1α was absent before 19 h (except focal CA1 positivity in a hypoxic–anoxic case), then emerged in endothelial cells at 19 h, extended to neurons by 2 days, peaked in vessels at day 6, shifted to macrophages at 7–8 days, and localized to glial cells at 16 days. These findings demonstrate that CD117 and HIF-1α follow complementary, time-dependent trajectories after TBI, acting as molecular clocks for survival interval estimation. Their dynamics mirror the clinical critical window at 18–24 h, underscoring their translational value in both forensic and clinical settings.

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Introduction

Traumatic brain injury (TBI) is a major cause of mortality and disability worldwide, with an estimated global incidence exceeding 69 million cases annually [1]. TBI is not only a forensic challenge but also a significant clinical burden, with structured acute and subacute management protocols — such as those issued by the Brain Trauma Foundation — shown to reduce mortality by up to 50% and substantially decrease healthcare costs [2]. It represents a crucial issue in forensic pathology, where accurate determination of the post-injury interval can aid in reconstructing events and establishing causality [3—5].

The pathophysiology of TBI involves a primary injury, caused by direct mechanical forces, followed by secondary injury processes such as ischemia, inflammation, and oxidative stress, which may continue for hours to days after the initial trauma [5]. Clinicians recognize the importance of monitoring injury progression over time, since delayed complications — including intracranial hemorrhage expansion and critical rises in intracranial pressure — may occur hours to days after trauma and precipitate sudden deterioration or death. For this reason, repeat neuroimaging within the first 24 h and close surveillance in the following days are considered essential to minimize the risk of fatal outcomes [6—8].

Mast cells (MCs), identified by CD117 (c-Kit) expression, are multifunctional immune cells that participate in neuroinflammation, blood—brain barrier (BBB) disruption, and vascular permeability changes after brain injury. Their recruitment and activation have been observed in experimental TBI models, with biphasic infiltration and mediator release influencing tissue damage and repair [9—11]. Furthermore, a role for MCs in central nervous system disorders, including TBI and ischemia, has been proposed, particularly in initiating and amplifying neuro-inflammatory cascades through the release of proteases, cytokines, and reactive species, ultimately leading to increased BBB permeability [12].

The Stem Cell Factor (SCF) receptor, also known as CD117 or c-Kit, is a class III receptor tyrosine kinase. It is a transmembrane protein composed of an extracellular domain with five immunoglobulin-like motifs, a single transmembrane segment, and a split intracellular kinase domain. Upon SCF binding, the receptor undergoes homo-dimerization and activates major intracellular signaling pathways. CD117 expression has been documented in multiple normal cell types, including hematopoietic progenitor cells, B- and T-lymphocyte precursors, mast cells, germ cells, melanocytes, neurons, glial cells, as well as cells of the placenta, kidney, lung, and gastrointestinal tract. In addition, CD117 is frequently detected in various human tumors, most notably gastrointestinal stromal tumors (GISTs) and germ cell neoplasms [13].

Hypoxia-inducible factor 1-alpha (HIF-1α) is a transcription factor that plays a central role in cellular adaptation to hypoxia. Under normoxic conditions, HIF-1α is rapidly degraded; under hypoxic or ischemic conditions, it accumulates and induces transcription of angiogenic, metabolic, and survival genes [14]. In both experimental and forensic contexts, HIF-1α expression has been proposed as a potential temporal marker of hypoxic—ischemic events, including TBI [15—18].

To date, few studies have investigated the temporal dynamics of mast cells and HIF-1α expression in human brain tissue after TBI, particularly in well-documented forensic cases with known survival intervals. To our knowledge, this is the first systematic autopsy-based study to characterize both markers across a wide range of post-injury survival times, highlighting their potential value as temporal biomarkers in forensic neuropathology. Although conceived as a forensic investigation, our work may also provide clinical insights: the systematic analysis of brain tissue with precisely documented survival intervals offers a unique opportunity to map the temporal dynamics of mast cell activation and HIF-1α expression. Such evidence can complement clinical observations by clarifying the molecular and cellular events that occur at specific post-injury stages, thereby bridging forensic neuropathology with clinical neuro-traumatology.

Materials and methods

Study population

We analyzed 40 forensic autopsy cases with documented post-traumatic cranioencephalic lesions consistent with TBI: the cohort consisted of 33 males and 7 females, with a mean age of 55 years (range: 18—82 years), reconstructed from medical records and emergency reports, ranged from a few minutes to 16 days. All cases consisted of TBI resulting from road traffic accidents. The cause of death was attributed to TBI-related complications. The post-mortem interval (PMI) was not consistently available across cases.

Tissue sampling

For each TBI case, brain samples were collected from the primary lesion site (peri-contusional cortex and adjacent white matter), with at least two tissue blocks obtained from this area. In addition, one tissue block was obtained from the anatomically corresponding contralateral hemisphere, serving as an internal control. In control cases, two tissue blocks from anatomically corresponding cortical and subcortical regions were sampled.

From each block, 2—3 sections were prepared and stained, yielding approximately nine slides per case in TBI cases and six slides in non-TBI cases. All specimens were fixed in 10% neutral buffered formalin for at least 48 h, routinely processed, and embedded in paraffin. Sections 4 μm thick were cut using a rotary microtome.

Immunohistochemistry

Paraffin sections were deparaffinized, rehydrated, and subjected to antigen retrieval by incubation for 30 mins in a thermostatic bath in DAKO Cytomation Target Retrieval Solution (10 $\times$) at high temperature, followed by cooling in DAKO Wash Buffer (10 $\times$). Immunostaining was performed using an automated Dako Autostainer system to ensure standardized protocols and reproducible results.

The CD117 staining protocol included: wash in DAKO Wash Buffer (10 $\times$); incubation for 10 min with 3% hydrogen per-

oxide to block endogenous peroxidase activity; wash in DAKO Wash Buffer (10 ×); incubation for 60 min with polyclonal rabbit anti-human CD117 antibody (Dako, Glostrup, Denmark); wash in DAKO Wash Buffer (10 ×); incubation for 20 min with DAKO EnVision System peroxidase/DAB+, rabbit/mouse secondary antibody; wash in DAKO Wash Buffer (10 ×); incubation for 5 min with DAKO DAB-AWAY; wash in distilled water; counterstaining with Harris hematoxylin for 1 min; final wash in DAKO Wash Buffer (10 ×).

The HIF-1α staining protocol included: wash in DAKO Wash Buffer (10 ×); incubation for 10 min with 3% hydrogen peroxide; wash in DAKO Wash Buffer (10 ×); incubation for 30 min with mouse monoclonal anti-human HIF-1α antibody, clone ESEE122 (Novus Biologicals, Centennial, CO, USA), diluted 1:250; wash in DAKO Wash Buffer (10 ×); incubation for 30 min with DAKO EnVision Detection System peroxidase/DAB+, rabbit/mouse secondary antibody; wash in DAKO Wash Buffer (10 ×); incubation for 5 min with DAKO DAB-AWAY; wash in DAKO Wash Buffer (10 ×); counterstaining with Harris hematoxylin for 1 min; double wash in DAKO Wash Buffer (10 ×).

Morphological and quantitative evaluation

Immunohistochemically stained sections were examined under a light microscope at multiple magnifications. Cases were grouped according to post-injury survival time, based on the interval between trauma and death.

For CD117-positive cells, we assessed their presence, number, cell type (MSs, glial cells, neurons), and localization (perivascular compartment, intraparenchymal compartment). Quantitative evaluation was performed at high power field (HPF, 400 × magnification; field area approximately 0.2 mm^2). For each case, at least 10 non-overlapping fields in the peri-contusional area were examined. Positive cells were manually counted and the mean number of positive cells per HPF was calculated.

For HIF-1α, evaluation focused on vascular activation as well as all cellular elements involved in the expression of this transcription factor, including endothelial cells, neurons, glial cells, and macrophages. HIF-1α expression was assessed qualitatively as present or absent, with additional characterization of its cellular localization.

Results

CD117-positive cells

In the acute survival interval (few minutes to 40 mins), TBI cases with hypoxic/anoxic injury demonstrated marked perivascular CD117 immunoreactivity — morphologically consistent with MCs (mean: 25 cells/HPF — High Power Field) (Fig. 1a), with sparse intraparenchymal positivity (mean: 12 cells/HPF) corresponding mainly to glial- or neuron-like cytotypes, and occasional intravascular positivity (mean: 3

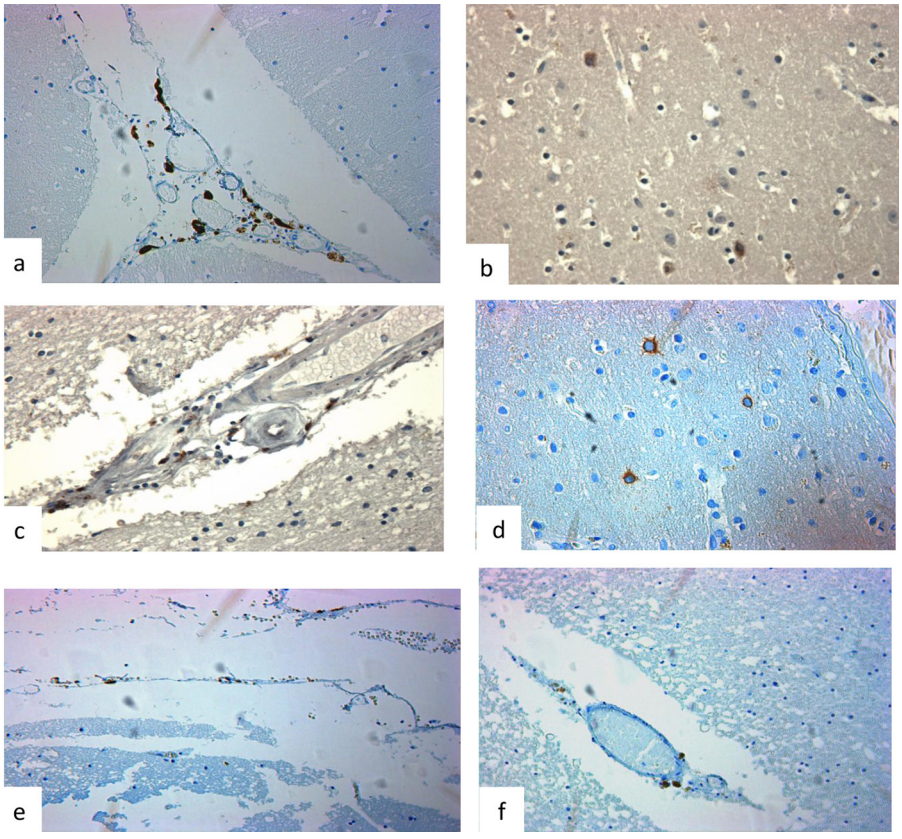

Figure 1 CD117-positive cells in different compartments at various post-injury intervals: (a) 40 min, hypoxic/anoxic injury — perivascular compartment (40 ×); (b) 40 min, no hypoxic/anoxic injury — intraparenchymal compartment (20 ×); (c) 1—4 h — perivascular (40 ×); (d) 1—4 h — intraparenchymal (20 ×); (e) 4 days — perivascular (20 ×); (f) 7 days — perivascular (20 ×).

cells/HPF). In contrast, TBI cases without hypoxic/anoxic injury showed a CD117 staining predominantly intraparenchymal (mean: 75 cells/HPF) (Fig. 1b), involving primarily non—MC morphologies, with only sporadic perivascular MCs. Between one-and four-hours post-injury, a redistribution pattern was observed, with mean counts of 50 intraparenchymal CD117-positive cells/HPF (mainly glial/neuronal morphology) and 30 perivascular MCs/HPF (Fig. 1c, Fig. 1d).

Between five- and eighteen-hours post-injury, both perivascular and intraparenchymal CD117-positive cell counts declined markedly. At 19 h, intraparenchymal CD117 positivity further decreased (mean: 27 cells/HPF, predominantly glial/neuronal morphology), and perivascular MCs were rare.

By 2 days, only sporadic intraparenchymal CD117-positive cells were detected, with no perivascular MS staining. Perivascular MC positivity reappeared at day 4 (mean: 19 cells/HPF) (Fig. 1e) but was absent by day 6. At 7—8 days, perivascular MCs persisted (mean: 15 cells/HPF) (Fig. 1f) with no intraparenchymal positivity. At 16 days, intraparenchymal CD117 positivity predominated again (mean: 38 cells/HPF), mostly involving glial/neuronal morphologies, while perivascular MCs were absent. These findings are summarized in Fig. 2.

HIF-1α expression

HIF-1α immunoreactivity was absent in all cases with survival times shorter than 19 h, except for focal neuronal positivity observed in the Sommer's sector CA1 of the hippocampus in a hypoxic—anoxic case surviving 40 mins, indicating the high vulnerability of this region to hypoxia (Fig. 3a).

At 19 h, HIF-1α expression was detected in endothelial cells of capillaries, arterial vessels, and leptomeningeal vessels (Fig. 3b—d). Initial parenchymal cell positivity was also observed at this stage.

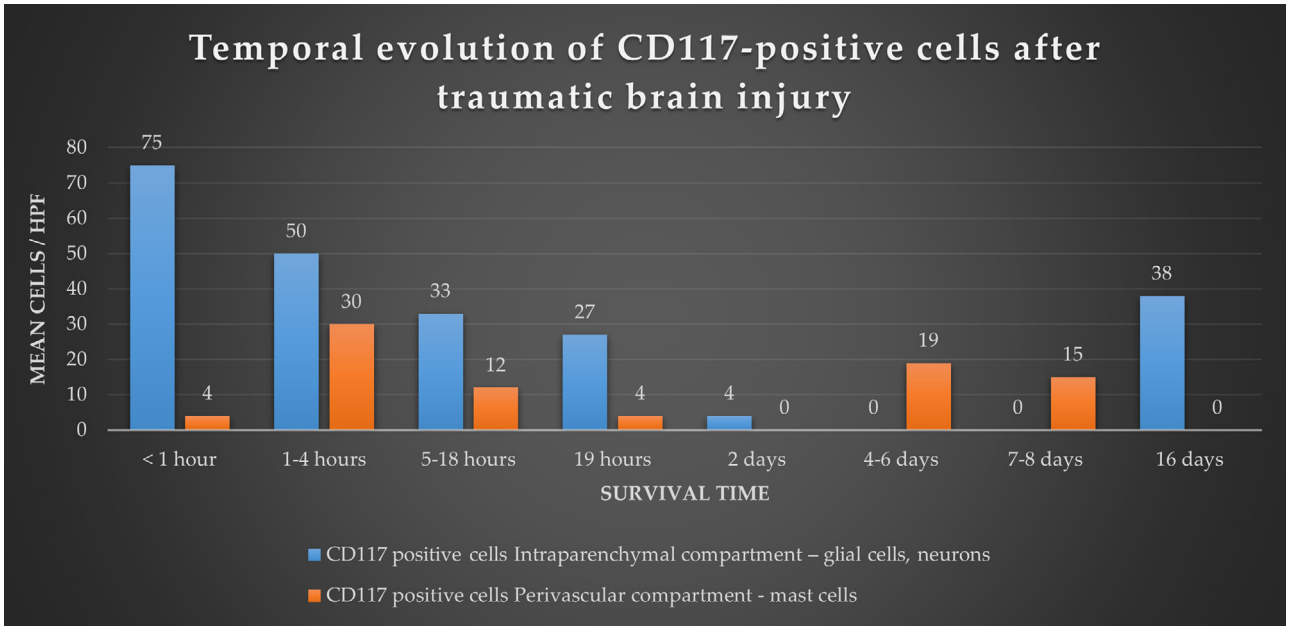

Figure 2 Temporal evolution of CD117-positive cells after traumatic brain injury.

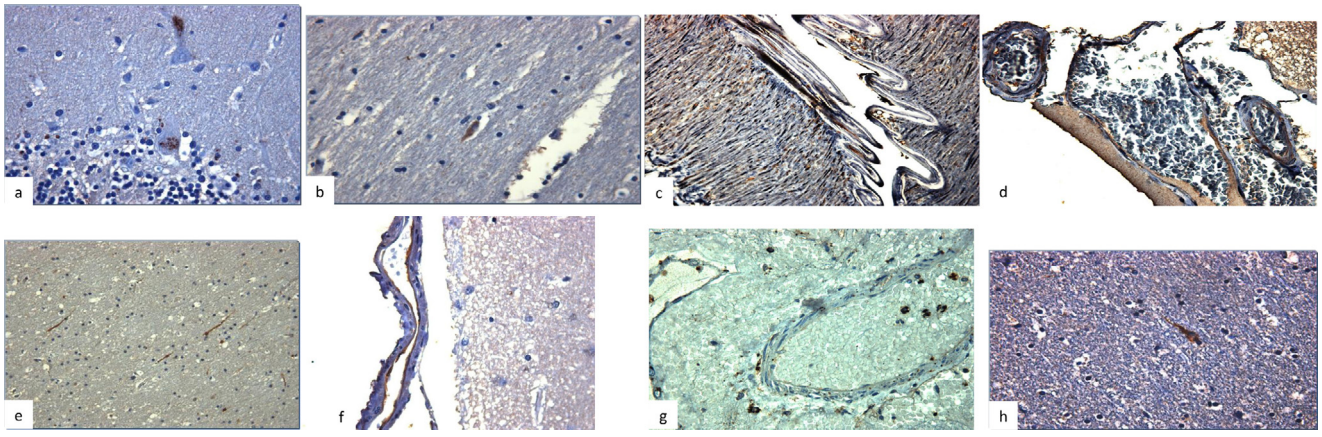

Figure 3 HIF-1α-positive cells at different post-injury intervals: (a) 40 min — neurons (40 ×); (b) 19 h — parenchymal cells (40 ×); (c, d) 19 h — endothelial cells (40 ×); (e) 2 days — axons (10 ×); (f) 6 days — leptomeningeal vessels (40 ×); (g) 8 days — macrophages in intraventricular hemorrhage (20 ×); (h) 16 days — glial cells (20 ×).

Table 1 Temporal evolution of CD117 and HIF-1α expression after TBI.

Time after injury	CD117 (intraparenchymal)	CD117 (perivascular)	HIF-1α
< 1 h	High	Present (hypoxic)	Absent[a]
1–4 h	High	Peak	Absent
5–18 h	Decreasing	Decreasing	Absent
19 h	Low	Rare	Endothelial onset
2 days	Minimal	Absent	Neuronal
4–6 days	Absent	Reappearance	Vascular peak
7–8 days	Absent	Present	Macrophages
16 days	Reappearance (glial)	Absent	Glial

[a] Except for focal CA1 neuronal positivity in a hypoxic–anoxic case.

By 2 days post-injury, HIF-1α was expressed in neurons and axons (Fig. 3e). A second major expression peak occurred at day 6, involving leptomeningeal and parenchymal vessels (Fig. 3f). At 7–8 days, HIF-1α was present in macrophages within intraventricular hemorrhage (Fig. 3g). At 16 days, HIF-1α positivity was localized to glial cells within non-lesioned parenchyma (Fig. 3h).

Table 1 provides a summary of the temporal evolution of CD117 and HIF-1α expression following TBI.

Internal controls

Control brain tissue from regions remote from the injury was consistently negative for HIF-1α. CD117 positivity was sporadic and intraparenchymal, except in one case with survival under 1 h, which exhibited numerous intraparenchymal positive cells (50 cells/slide) in association with a subarachnoid hemorrhage involving lobes not initially affected by trauma.

Discussion

CD117-positive cells: perivascular and intraparenchymal patterns

In line with evidence implicating MCs in both traumatic and hypoxic–ischemic brain injury [10–12], our data show that CD117-positive cells appear in distinct temporal phases and anatomical compartments. Perivascular CD117-positive cells – morphologically consistent with MCs – were detected as early as 40 mins post-injury in hypoxic–anoxic cases and within 1 h in post-traumatic cases, indicating rapid recruitment to the BBB region. This is consistent with Hu et al. (2004), who reported early MC activation following acute hypoxia [19]. Experimental studies have also demonstrated that MCs are among the earliest responders after hypoxic–ischemic brain injury and contribute to secondary damage if not stabilized [20,21].

A biphasic perivascular pattern was observed in our cases: an initial peak within the first 1–4 h, followed by a decline by 19 h, and a second, lower-intensity peak between days 4–8. While this biphasic response has not previously been demonstrated in human TBI, similar dual-phase dynamics have been described in experimental models. For instance, Lindsberg et al. (2010) highlighted both an immediate and a delayed phase of MC activation after brain ischemia and hemorrhage [22]. Several experimental studies have also confirmed mast cell involvement in post-traumatic neuroinflammation. Kempuraj et al. reported mast cell activation within 24–72 h after experimental TBI, associated with BBB disruption and upregulation of inflammatory mediators such as CCL2 and VEGFR2 [9]. Similarly, Lozada et al. described mast cell infiltration in contused cortical areas in rat models [23]. Recent reviews have reinforced the concept of mast cells as 'first responders' to brain injury, modulating both acute and delayed vascular responses [24]. However, the temporal pattern we observed in human autopsy cases – characterized by an early perivascular peak within the first hours, a decline by 19 h, and a delayed reappearance between days 4–8 – differs from these experimental models, underscoring the novelty and translational relevance of our findings.

The rapid recruitment of MCs to perivascular regions after TBI is biologically plausible, as trauma-induced damage triggers release of danger-associated molecular patterns (DAMPs), disruption of the BBB, and secretion of chemoattractant cytokines (e.g., CCL2, CXCL8) by endothelial cells, astrocytes, and microglia. In addition, hypoxia-driven HIF-1α activation promotes VEGF release, further enhancing MC chemotaxis and vascular permeability [9,11,12,19,22]. These mechanisms explain why MCs act as early responders in the neuroinflammatory cascade after brain injury.

The delayed perivascular reappearance of mast cells between 4–8 days post-injury may reflect secondary injury processes. During this subacute phase, vascular remodeling and neoangiogenesis are prominent, with maximal expression of endothelial and extracellular matrix markers such as tenascin and thrombomodulin around one week after trauma [25]. Hypoxia-driven VEGF release and ongoing glial–macrophage cross-talk may further contribute to mast cell chemotaxis and activation, accounting for the biphasic pattern observed in our series. These observations are in line with Zwirner et al. (2022), who reviewed histological markers of TBI and highlighted the potential of MCs as temporal indicators, while also stressing that further validation is required before routine forensic application [26].

Intraparenchymal CD117-positive cells – most often displaying glial or neuronal morphology – followed a different pattern, with high counts immediately after injury, gradual decline to negativity by day 4, and reappearance at day 16. While mast cells can occasionally migrate into the parenchyma, CD117 is also expressed by other cell types, including neural progenitors and certain glial populations, suggesting

that the parenchymal expression observed here may reflect a broader cellular stress response rather than MC-specific infiltration. This interpretation is consistent with previous forensic and experimental studies documenting temporally distinct patterns of neuroinflammation and vascular response after TBI [25,27,28].

CD117 expression has been documented in glial tumors, suggesting its non-specificity to mast cells [29]. Moreover, CD117 marks progenitor-like populations with regenerative potential, as shown by studies in ischemic neonatal brain models [30] and developing retinal progenitors [31]. These data support the interpretation that late-stage CD117 positivity (e.g., at 16 days) may reflect activation of neural progenitor or immature glial cells involved in tissue repair.

Interestingly, glial fibrillary acidic protein (GFAP) — an intermediate filament protein of astrocytes widely used as a plasma biomarker in clinical neurotraumatology — shows a peak around 20 h post-injury, reflecting acute astroglial damage [32]. In our series, intraparenchymal CD117-positive cells, often with glial morphology, were abundant in survival intervals shorter than 19 h and declined thereafter, suggesting that CD117 parenchymal expression may parallel GFAP release into the circulation. This raises the possibility of a direct link between tissue-based and plasma-based markers of astroglial activation, reinforcing the translational value of our findings in bridging forensic and clinical perspectives. Such parallelism suggests that CD117 intraparenchymal expression may serve as a tissue correlate of astroglial injury measurable in plasma.

Notably, the delayed perivascular reappearance of CD117-positive cells between 4 and 8 days post-injury overlaps with the subacute phase of TBI, during which secondary injury mechanisms are most prominent. In particular, the marked vascular expression observed around days 6–7 is consistent with processes such as endothelial remodeling, VEGF-driven angiogenesis, and glial–macrophage cross-talk, which define the pathophysiology of secondary brain injury [9,11,12,19,22,25]. This temporal overlap reinforces the translational value of CD117 as a potential histological marker of subacute secondary damage in human TBI. Indeed, current evidence indicates that during the first week after moderate TBI, up to one third of patients may experience secondary neurologic deterioration, characterized by an abrupt worsening of consciousness or the need for intensive interventions. This phenomenon is strongly associated with poor outcome and highlights the importance of close clinical and radiological monitoring throughout the early post-traumatic period [33].

HIF-1α expression: delayed and biphasic dynamics

HIF-1α was undetectable in post-traumatic cases with survival < 19 h, except for focal neuronal positivity in the CA1 sector of the hippocampus in a hypoxic — anoxic case — confirming the exceptional vulnerability of this region to hypoxia. The delayed onset of HIF-1α expression in our autopsy findings (≥ 19 h) is physiologically plausible. Under normoxic conditions, HIF-1α is constitutively degraded via the prolyl-hydroxylase/VHL pathway [13]. Only when oxygen tension drops below a critical threshold does stabilization occur, leading to nuclear translocation and activation of hypoxia-responsive genes such as VEGF and PGK1 [34]. This mechanism explains why HIF-1α is absent in the earliest survival intervals: the cascade of secondary hypoxic–ischemic events — edema, BBB disruption, and microvascular compromise — typically evolves over several hours rather than minutes [15,16,35].

The biphasic dynamics of HIF-1α expression observed in our autopsy series — early vascular and neuronal activation (19 h–2 days), a vascular peak at day 6, and glial expression at 16 days — are in line with experimental evidence suggesting time-dependent, dual roles of HIF-1α. In a mouse model of transient focal ischemia, Baranova et al. (2007) demonstrated that neuron-specific inactivation of HIF-1α aggravated brain injury, underscoring its early neuroprotective role, while persistent activation at later stages has been associated with detrimental effects, including promotion of apoptosis and neuroinflammation [35]. Conversely, Ding et al. (2009) reported that HIF-1α signaling promoted aquaporin upregulation and cerebral edema after TBI, highlighting its detrimental impact in later phases [15]. Together, these findings support the interpretation that HIF-1α acts as a double-edged sword in TBI, initially adaptive but potentially harmful when sustained over time.

Forensic studies corroborate these observations: Barranco et al. (2021) validated HIF-1α immunohistochemistry as a reliable marker of acute hypoxia in autopsy tissues [36], while Cecchi et al. (2024) demonstrated post-mortem expression in cardiac tissue, reinforcing its value as a general marker of hypoxic–ischemic damage [18]. Neri et al. (2018) showed HIF-1α upregulation in fatal TBI cases alongside aquaporin-4 and glial activation markers, further supporting its role in secondary injury mechanisms [16]. In addition, Umschweif et al. (2013) demonstrated that HIF-1α is essential for spontaneous recovery after TBI and mediates protective effects of heat acclimation [37], whereas Yuan et al. (2021) and Xu et al. (2023) highlighted detrimental aspects, showing that persistent HIF-1α activation can aggravate microglial NLRP3-mediated pyroptosis and neuroinflammation [38,39].

Taken together, these data support the interpretation that the absence of HIF-1α within the first 19 h reflects the latency of secondary hypoxic–ischemic cascades, while its subsequent biphasic expression mirrors the evolving vascular, neuronal, inflammatory, and glial responses to TBI. The marked expression of HIF-1α observed at 6–7 days falls within the sub-acute phase of TBI and is consistent with the well-described phenomena of secondary brain injury, including vascular dysfunction, oedema, and neuro-inflammation [15,16,38,39].

Forensic implications

From a forensic perspective, the combined analysis of the two markers suggests that the co-occurrence of CD117-positive cells in both intraparenchymal and perivascular compartments is indicative of a post-injury survival interval of less than approximately 19 h. Conversely, positivity for HIF-1α serves as a marker of survival exceeding approximately 19 h, thus providing a useful temporal threshold for post-traumatic interval estimation. CD117 and HIF-1α may

serve as complementary *molecular clocks*, providing useful tools in autopsy practice for estimating the post-injury interval.

Future studies should investigate the same molecular markers in pediatric TBI, particularly in the context of subdural hematoma in abusive head trauma (shaken baby syndrome), where accurate dating is of critical clinical and forensic importance [40,41].

Clinical implications

From a clinical perspective, the interval around 18—24 h after TBI represents a well-recognized critical window for neurological deterioration. Several studies have shown that patients may experience a secondary rise in intracranial pressure, diffuse cerebral edema, or delayed hematoma expansion within this timeframe, often leading to sudden worsening of neurological status or even death if not promptly identified [6—8]. For this reason, current clinical guidelines recommend repeat neuroimaging within the first 24 h and intensive monitoring during this period. The convergence of our autopsy data — showing abundant intraparenchymal CD117 positivity before 19 h and its decline thereafter — with clinical observations such as the plasma GFAP peak at ~20 h [32] and the heightened risk of secondary complications, underscores the translational value of our findings. These results suggest that the < 20-hour window may represent a pathophysiological tipping point in both forensic and clinical settings, where early glial and MC responses give way to subsequent secondary injury processes.

In addition, the delayed onset of HIF-1α expression after 19 h further aligns with this clinical ''critical window.'' The absence of detectable HIF-1α before this threshold suggests that molecular hypoxic responses have not yet stabilized, whereas its emergence coincides with the period in which patients are at highest risk of cerebral edema, vascular dysfunction, and neurological decline. This parallelism supports the hypothesis that HIF-1α may contribute to the cascade of secondary injury processes clinically observed after the first day post-trauma, and may represent a promising target for therapeutic modulation.

Strengths and limitations

The sample size was limited, and cause-of-death heterogeneity in controls may have influenced baseline staining. Moreover, CD117 is not MC—specific, and its interpretation requires careful morphological correlation. However, a major strength of this study is the availability of cases with precisely documented post-injury survival intervals, which allowed us to map temporal expression patterns with high specificity. At the same time, the requirement for accurate survival data represented the main limiting factor, as it substantially reduced the number of eligible cases and thus explains the relatively small cohort size.

An additional limitation is represented by the lack of consistent PMI data, which may influence antigen preservation and immunohistochemical staining intensity, potentially affecting the reliability of the results.

Conclusions

This study provides the first systematic autopsy-based characterization of CD117-positive cells and HIF-1α expression across a wide range of post-injury survival intervals in human TBI. Our findings highlight that the two markers follow complementary temporal trajectories, offering potential value both for forensic neuropathology and for clinical neuro-traumatology. Our results show that CD117 and HIF-1α display complementary, time-dependent expression dynamics after TBI. CD117 positivity in perivascular and intraparenchymal compartments marks survival < 19 h, while HIF-1α emerges beyond this threshold, with a delayed biphasic pattern. Both HIF-1α and CD117 showed marked expression in the sub-acute phase (days 6—7), consistent with secondary injury mechanisms in TBI. These findings provide a novel molecular framework for post-injury interval estimation in forensic settings and parallel the clinical ''critical window'' of deterioration at 18—24 h. CD117 and HIF-1α may thus serve as translational molecular clocks, bridging pathology, clinical monitoring, and therapeutic timing in TBI.

Clinical Trial number

Clinical trial number: not applicable.

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Author contributions

All authors contributed equally to the conception, drafting, and revision of the manuscript.

Disclosure of interest

The authors declare that they have no competing interest.

References

[1] Dewan MC, Rattani A, Gupta S, Baticulon RE, Hung YC, Punchak M, et al. Estimating the global incidence of traumatic brain injury. J Neurosurg 2018;130:1080—7. http://dx.doi.org/10.3171/2017.10.JNS17352.

[2] Carney N, Totten AM, O'Reilly C, Ullman JS, Hawryluk GWJ, Bell MJ, et al. Guidelines for the management of severe traumatic brain injury, fourth edition. Neurosurgery 2017;80:6—15. http://dx.doi.org/10.1227/NEU.0000000000001432.

[3] Bertozzi G, Sessa F, Maglietta F, Cipolloni L, Salerno M, Zotti F, et al. Traumatic brain injury: a forensic approach: a literature review. Forensic Sci Int 2020;315:110456. http://dx.doi.org/10.1016/j.forsciint.2020.110456.

[4] Cecchi R, Camatti J, Schirripa ML, Ragona M, Pinelli S, Cucurachi N. Postmortem biochemistry of GFAP, NSE and S100B in cerebrospinal fluid and in vitreous humor for estimation of

postmortem interval: a pilot study. Forensic Sci Med Pathol 2025;21:589–98. http://dx.doi.org/10.1007/s12024-024-00874-9.
[5] Ng SY, Lee AYW. Traumatic brain injuries: pathophysiology and potential therapeutic targets. Front Cell Neurosci 2019;13:528. http://dx.doi.org/10.3389/fncel.2019.00528.
[6] American College of Surgeons. Best practices guidelines: traumatic brain injury. American College of Surgeons, Chicago; 2023. https://www.facs.org/media/vgfgjpfk/best-practices-guidelines-traumatic-brain-injury.pdf. Accessed 24 Sep 2025.
[7] O'Phelan KH, McArthur DL, Chang CWJ, Green D, Hovda DA. Patterns of intracranial pressure elevation after severe traumatic brain injury. Neurocrit Care 2009;10:280–6. http://dx.doi.org/10.1007/s12028-008-9140-0.
[8] Cucciolini G, Motroni V, Czosnyka M. Intracranial pressure for clinicians: it is not just a number. J Anesth Analg Crit Care 2023;3:31. http://dx.doi.org/10.1186/s44158-023-00115-5.
[9] Kempuraj D, Mentor S, Thangavel R, Ahmed ME, Selvakumar GP, Raikwar SP, et al. Mast cell activation, neuroinflammation, and tight junction alteration following TBI. Front Cell Neurosci 2020;14:612169. http://dx.doi.org/10.3389/fncel.2020.612169.
[10] Skaper SD, Facci L, Giusti P. Mast cells, glia and neuroinflammation: partners in crime? Immunology 2014;141:314–27. http://dx.doi.org/10.1111/imm.12170.
[11] Hendriksen E, van Bergeijk D, Oosting RS, Redegeld FA. Mast cells in neuroinflammation and brain disorders. Neurosci Biobehav Rev 2017;79:119–33. http://dx.doi.org/10.1016/j.neubiorev.2017.05.001.
[12] Theoharides TC, Alysandratos KD, Angelidou A, Delivanis DA, Sismanopoulos N, Zhang B, et al. Mast cells and inflammation. Biochim Biophys Acta 2012;1822:21–33. http://dx.doi.org/10.1016/j.bbadis.2010.12.014.
[13] Bio-Techne. Human CD117/c-kit antibody. 2025. https://www.bio-techne.com/p/antibodies/human-cd117-c-kit-antibody_af332. Accessed 24 Sep 2025.
[14] Marxsen JH, Stengel P, Doege K, Heikkinen P, Jokilehto T, Wagner T, et al. Hypoxia-inducible factor-1 (HIF-1) promotes its degradation by induction of HIF-alpha-prolyl-4-hydroxylases. Biochem J 2004;381:761–7. http://dx.doi.org/10.1042/BJ20040620.
[15] Ding JY, Kreipke CW, Speirs SL, Schafer P, Schafer S, Rafols JA. Hypoxia-inducible factor-1alpha signaling in aquaporin upregulation after traumatic brain injury. Neurosci Lett 2009;453:68–72. http://dx.doi.org/10.1016/j.neulet.2009.01.077.
[16] Neri M, Frati A, Turillazzi E, Cantatore S, Cipolloni L, Di Paolo M, et al. Immunohistochemical evaluation of aquaporin-4 and its correlation with CD68, IBA-1, HIF-1a, GFAP, and CD15 expressions in fatal traumatic brain injury. Int J Mol Sci 2018;19:3544. http://dx.doi.org/10.3390/ijms19113544.
[17] Choi YK. Detrimental roles of hypoxia-inducible factor-1a in severe hypoxic brain diseases. Int J Mol Sci 2024;25:4465. http://dx.doi.org/10.3390/ijms25084465.
[18] Cecchi R, Camatti J, Bonasoni MP, Clemente GM, Nicolì S, Campanini N, et al. HIF-1a expression by immunohistochemistry and mRNA-210 levels by real time polymerase chain reaction in post-mortem cardiac tissues: a pilot study. Leg Med (Tokyo) 2024;71:102508. http://dx.doi.org/10.1016/j.legalmed.2024.102508.
[19] Hu W, Xu L, Pan J, Zheng J, Li L. Effect of cerebral ischemia on brain mast cells in rats. Brain Res 2004;1019:275–80. http://dx.doi.org/10.1016/j.brainres.2004.05.107.
[20] Jin Y, Silverman AJ, Vannucci SJ. Mast cell stabilization limits hypoxic-ischemic brain damage in the immature rat. Dev Neurosci 2007;29:373–84. http://dx.doi.org/10.1159/000105478.
[21] Jin Y, Silverman AJ, Vannucci SJ. Mast cells are early responders after hypoxia-ischemia in immature rat brain. Stroke 2009;40:3107–12. http://dx.doi.org/10.1161/STROKEAHA.109.553883.
[22] Lindsberg PJ, Strbian D, Karjalainen-Lindsberg ML. Mast cells as early responders in the regulation of acute blood-brain barrier changes after cerebral ischemia and hemorrhage. J Cereb Blood Flow Metab 2010;30:689–702. http://dx.doi.org/10.1038/jcbfm.2009.282.
[23] Lozada A, Maegele M, Stark H, Neugebauer EM, Panula P. Traumatic brain injury results in mast cell increase and changes in regulation of central histamine receptors. Neuropathol Appl Neurobiol 2005;31:150–62. http://dx.doi.org/10.1111/j.1365-2990.2004.00622.x.
[24] Strbian D, Kovanen PT, Karjalainen-Lindsberg ML, Tatlisumak T, Lindsberg PJ. An emerging role of mast cells in cerebral ischemia and hemorrhage. Ann Med 2009;41:438–50. http://dx.doi.org/10.1080/07853890902887303.
[25] Hausmann R, Betz P. The time course of the vascular response to human brain injury—an immunohistochemical study. Int J Legal Med 2000;113:288–92. http://dx.doi.org/10.1007/s004140000143.
[26] Zwirner J, Ritz-Timme S, Hartwig S, Heinemann A, Kernbach-Wighton G, Madea B, et al. Forensic biomarkers of lethal traumatic brain injury. Int J Legal Med 2022;136:1639–47. http://dx.doi.org/10.1007/s00414-022-02889-7.
[27] Dell'Aquila M, Maiese A, De Matteis A, Viola RV, Arcangeli M, La Russa R, et al. Traumatic brain injury: estimation of injury age based on neuroinflammation, endothelial activation markers and adhesion molecules. Histol Histopathol 2022;37:433–44. http://dx.doi.org/10.14670/HH-18-430.
[28] Poblete RA, Zhong C, Patel A, Kuo G, Sun PY, Xiao J, et al. Post-traumatic cerebral infarction: a narrative review of pathophysiology, diagnosis, and treatment. Int J Mol Sci 2023;24:11574. http://dx.doi.org/10.3390/ijms241411574.
[29] Cetin N, Dienel G, Gokden M. CD117 expression in glial tumors. J Neurooncol 2005;75:195–202. http://dx.doi.org/10.1007/s11060-005-2318-1.
[30] Corcelli M, Hawkins K, Vlahova F, Dellepiane M, Kenny A, Kendall GS, et al. Neuroprotection of the hypoxic-ischemic mouse brain by human CD117+CD90+CD105+ amniotic fluid stem cells. Sci Rep 2018;8:2425. http://dx.doi.org/10.1038/s41598-018-20710-9.
[31] Zou T, Gao L, Zeng Y, Li Q, Li Y, Cheng J, et al. Organoid-derived C-Kit+/SSEA4– human retinal progenitor cells promote a protective retinal microenvironment during transplantation in rodents. Nat Commun 2019;10:1205. http://dx.doi.org/10.1038/s41467-019-08961-0.
[32] Abdelhak A, Foschi M, Abu-Rumeileh S, Yue JK, D'Anna L, Gaetani L, et al. Blood GFAP as an emerging biomarker in brain and spinal cord disorders. Nat Rev Neurol 2022;18:158–72. http://dx.doi.org/10.1038/s41582-021-00616-3.
[33] Aries P, Ognard J, Cadieu A, Degos V, Huet O. Secondary neurologic deterioration after moderate traumatic brain injury: development of a multivariable prediction model and proposition of a simple triage score. Anesth Analg 2024;138:171–9. http://dx.doi.org/10.1213/ANE.0000000000006460.
[34] Wang Z, Tian J, Wang L, Yan H, Feng S, Zhang Y. PGK1 is involved in the HIF-1 signaling pathway as a hub gene for ferroptosis after traumatic brain injury. Mol Neurobiol 2025;62:233–45. http://dx.doi.org/10.1007/s12035-024-04170-z.
[35] Baranova O, Miranda LF, Pichiule P, Dragatsis I, Johnson RS, Chavez JC. Neuron-specific inactivation of the hypoxia inducible factor 1 alpha increases brain injury in a mouse model of transient focal cerebral ischemia. J Neurosci 2007;27:6320–7. http://dx.doi.org/10.1523/JNEUROSCI.0449-07.2007.
[36] Barranco R, Bonsignore A, Ventura F. Immunohistochemistry in postmortem diagnosis of acute hypoxia. Med Sci Law

2021;61:118—26. http://dx.doi.org/10.1177/0025802420959272.

[37] Umschweif G, Alexandrovich AG, Trembovler V, Horowitz M, Shohami E. Hypoxia-inducible factor 1 is essential for spontaneous recovery from traumatic brain injury and is a key mediator of heat acclimation induced neuroprotection. J Cereb Blood Flow Metab 2013;33:524—31. http://dx.doi.org/10.1038/jcbfm.2012.193.

[38] Yuan D, Guan S, Wang Z, Ni H, Ding D, Xu W, et al. HIF-1a aggravated traumatic brain injury by NLRP3 inflammasome-mediated pyroptosis and activation of microglia. J Chem Neuroanat 2021;116:101994. http://dx.doi.org/10.1016/j.jchemneu.2021.101994.

[39] Xu X, Yang M, Zhang B, Dong J, Zhuang Y, Ge Q, et al. HIF-1a participates in secondary brain injury through regulating neuroinflammation. Transl Neurosci 2023;14:20220272. http://dx.doi.org/10.1515/tnsci-2022-0272.

[40] Adamsbaum C, Morel B, Ducot B, Antoni G, Rey-Salmon C. Dating the abusive head trauma episode and perpetrator statements: key points for imaging. Pediatr Radiol 2014;44(Suppl. 4):S578—88. http://dx.doi.org/10.1007/s00247-014-3171-1.

[41] Delteil C, Humez S, Boucekine M, Jouvet A, Hedouin V, Fanton L, et al. Histological dating of subdural hematoma in infants. Int J Legal Med 2019;133(2):539—46. http://dx.doi.org/10.1007/s00414-018-1980-8.