Histology of Brain Trauma and Hypoxia-Ischemia

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ABSTRACT

Forensic pathologists encounter hypoxic-ischemic (HI) brain damage or traumatic brain injuries (TBI) on an almost daily basis. Evaluation of the findings guides decisions regarding cause and manner of death. When there are gross findings of brain trauma, the cause of death is often obvious. However, microscopic evaluation should be used to augment the macroscopic diagnoses. Histology can be used to seek evidence for TBI in the absence of gross findings, e.g., in the context of reported or suspected TBI. Estimating the survival interval after an insult is often of medicolegal interest; this requires targeted tissue sampling and careful histologic evaluation. Retained tissue blocks serve as forensic evidence and also provide invaluable teaching and research material. In certain contexts, histology can be used to demonstrate nontraumatic causes of seemingly traumatic lesions. Macroscopic and histologic findings of brain trauma can be confounded by concomitant HI brain injury when an individual survives temporarily after TBI. Here we review the histologic approaches for evaluating TBI, hemorrhage, and HI brain injury. Amyloid precursor protein (APP) immunohistochemistry is helpful for identifying damaged axons, but patterns of damage cannot unambiguously distinguish TBI from HI. The evolution of hemorrhagic lesions will be discussed in detail; however, timing of any lesion is at best approximate. It is important to recognize artifactual changes (e.g., dark neurons) that can resemble HI damage. Despite the shortcomings, histology is a critical adjunct to the gross examination of brains. *Acad Forensic Pathol. 2018 8(3): 539-554*

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ETHICAL APPROVAL

As per Journal Policies, ethical approval was not required for this manuscript

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No identifiable personal data were presented in this manuscript

DISCLOSURES & DECLARATION OF CONFLICTS OF INTEREST

The authors, reviewers, editors, and publication staff do not report any relevant conflicts of interest

FINANCIAL DISCLOSURE

The authors have indicated that they do not have financial relationships to disclose that are relevant to this manuscript

KEYWORDS

Forensic pathology, Axon injury, Hemorrhage, Hypoxia, Ischemia, Traumatic brain injury, Neuropathology

INFORMATION

ACADEMIC FORENSIC PATHOLOGY: THE OFFICIAL PUBLICATION OF THE NATIONAL ASSOCIATION OF MEDICAL EXAMINERS

 • (ISSN: 1925-3621) • https://doi.org/10.1177/1925362118797728
Submitted for consideration on 22 May 2018. Accepted for publication on 2 Jul 2018

INTRODUCTION

Traumatic brain injury (TBI) can be categorized in several ways; physical mechanism (e.g., penetrating versus nonpenetrating; direction of acceleration), magnitude of force, single versus multiple, and temporal spectrum from acute to chronic. Relatively mild injuries such as concussions, by definition, have no gross or histologic findings, but may have occurred in individuals who died as a consequence of trauma to another part of the body or drug/alcohol toxicity. More severe brain injuries, which can cause death, usually present with intracranial hemorrhages (e.g., epidural, subdural, subarachnoid, and intraventricular) and parenchymal lesions (e.g., contusions, lacerations, or diffuse axonal injury [DAI]) (1, 2). If the victim survives, the evolving lesion can develop cerebral edema and swelling, anatomical herniations, secondary hypoxic-ischemic (HI) damage, inflammation, and (with penetrating injury) infection. Vascular lesions frequently accompany trauma, or they can occur in the clinical situation of stroke. These are categorized as ischemic (focal interruption of blood flow) or hemorrhagic (rupture of a subarachnoid or parenchymal blood vessel). Diffuse HI brain injury can follow arterial hypotension, hypoxia, cardiac arrest, or respiratory arrest.

This manuscript is written for forensic pathologists and novice neuropathologists. Both groups are frequently tasked with examination of brains from traumatized or suddenly deceased individuals. Traumatic brain injury, stroke, and HI brain damage following cardiorespiratory arrest are common problems to be addressed (3, 4). The focus is on changes in the mature nervous system. The developing nervous system (i.e., neonatal and pediatric) exhibits a different spectrum of morphological and reactive changes, with differences in the cellular vulnerability. Details of these are beyond the scope of this review and pathologists encountering such situations should consult with an expert in the field.

DISCUSSION

Tissue Sampling

Imaging studies of brain obtained during life should always be reviewed prior to sectioning the fresh or fixed brain. The location, size, and appearance of grossly identifiable lesions should be documented with photographs and detailed diagrams, which may include correlated mapping of lesions and tissues sampled for histologic evaluation. Sections of macroscopic lesions, including hematomas, should be taken from the periphery to show transition to the surrounding parenchyma.

A systematic approach is useful when submitting tissue for evaluation of TBI. A minimum set of blocks includes sections from: the body of the corpus callosum with parasagittal white matter, splenium of the corpus callosum, posterior limb of the internal capsule with adjacent thalamus, rostral pons (including middle cerebellar peduncles), and cerebellum (including the dentate nucleus). Note that these focus on deep white matter lesions. They are less important in the situation of epidural or subdural hematoma associated with low acceleration trauma. In the case of subdural hematoma, evidence of chronic subdural hemorrhage should be sought. Additional brain sections should include hippocampus, dorsal frontal interarterial border zone, midbrain (decussation of the superior cerebellar peduncles), medulla, and spinal cord. Blocks should include sections from both sides but bilaterally paired sections are not necessary (5-7).

When evaluating for HI injury, the regional susceptibility of the brain in association with vascular anatomy (i.e., in the interarterial boundary zones) and in relation to different neuron types (e.g., relative sensitivity of the cornu ammonis/CA1 hippocampus) must be considered (8). A minimum of the interarterial ("watershed") zones, basal nuclei (putamen and globus pallidus), thalamus, hippocampus (ideally at the level of the lateral geniculate body), midbrain, pons, medulla oblongata, and cerebellum should be sampled (9). Palpably soft regions of the brain might indicate focal edema.

In cases where an intracerebral hematoma is present and evidence for TBI is questionable (e.g., decedent found at the bottom of a stairwell with minimal extracranial head injury), several sections should be submitted from the periphery of the hematoma to search for nontraumatic sources of hemorrhage including vascular malformation, amyloid angiopathy, neoplasm, or abscess. Slides stained with hematoxylin and eosin (H&E) can be scrutinized for evidence of cerebral atherosclerosis or arteriolosclerosis, which would be indicators of chronic arterial disease. Elastic stains can highlight splitting of the elastic lamina in small vessel disease and saccular aneurysms. Diagnosis of cerebral amyloid angiopathy requires demonstration of amyloid beta protein deposition in cerebral and leptomeningeal vessel walls using Congo red stain or appropriate immunostain (10). Martius scarlet blue (MSB) stain is useful for evaluating sites of hemorrhage (bright red staining of fibrin and yellow-orange staining of recently extravasated red blood cells) (11, 12). Neutrophil aggregates are associated with blood clotting; Gram stain can aid in distinguishing a bacterial abscess with secondary hemorrhage. Close attention to blood cell morphology can raise the possibility of sickle cell disease, malaria, or leukemia as a cause of intracranial hemorrhage.

Neuron Changes Following Hypoxia-Ischemia

Owing to their high metabolic needs and vulnerability to excitotoxicity, neurons are generally more sensitive to HI than macroglia (i.e., astrocytes and oligodendrocytes), microglia, or endothelia (8). In the mature brain, the most sensitive populations (or at least the most obvious victims) are the long-projection large neurons of the third and fifth neocortical layers, the neurons of the hippocampal CA1 regions, and the Purkinje neurons of the cerebellum. Histologic changes following HI are determined by the pathobiology (13). Early subtle changes can be difficult to define in autopsy samples. Also, not all cellular processes are energy dependent and morphology is not immediately frozen after death; passive water movements can continue after death. Swelling with cytoplasmic pallor of neurons and astrocytes occurs within 15-30 minutes of HI because the Na^+-K^+ exchange pumps are dependent on ATP; water passively follows the altered ion gradients. This can give the tissue a spongy or pale appearance at low magnification as early as one hour after the insult (**Image 1**). Occasionally, swelling of specific organelles including Golgi apparatus and endoplasmic reticulum can be identified in compromised neurons (**Image 1**) (14). Experimental studies indicate that shrinkage and scalloping (pyknosis) of dying neurons is apparent as early as 30 minutes after HI and persists for up to one day. Initially, the nucleus is collapsed and dark and the nucleolus is poorly defined. Note that it can be difficult to distinguish these early changes from "dark neuron" artifact (see below). Calcium influx activates a variety of enzyme systems, including calpains, that digest cytoplasmic proteins. Protein homogenization, combined with the loss of ribosomes, renders the cytoplasm of dead neurons brightly eosinophilic. Although typically not identifiable in large quantities earlier than six hours after HI, eosinophilic neurons have been reported as early as one hour (15, 16). The same population of dead neurons can be easily identified with the Fluoro-Jade fluorescent label (17). Loss of cytoskeletal integrity, demonstrated for example by loss of immunoreactivity for the protein MAP2, can help in the early detection of damaged neurons (18). Accompanying cytoplasmic eosinophilia, the nucleus degrades with the chromatin and fragments into fine basophilic particles before disappearing, leaving only the outlines of neurons (**Image 1**). Large globules of homogenized DNA, characteristic of classic apoptosis, are seen only in fetal and perinatal brains. Dead neurons are phagocytosed by microglia (in circumstances of selective neuron death) or macrophages (in pan-necrosis) within seven to nine days (19, 20). It is important to note that neurons can survive temporarily in a compromised state following a moderately severe HI insult. Neuron death, with all of the associated morphologic changes, can then be delayed for several days (21, 22). Purkinje neurons of the human cerebellum undergo changes similar to those in the cerebrum. However, the disappearance of these may occur more rapidly, with reduced quantity evident at one and a half to two days after HI (23).

The pathogenesis of neuronal damage in the context of severe hypoglycemia partially overlaps with that of

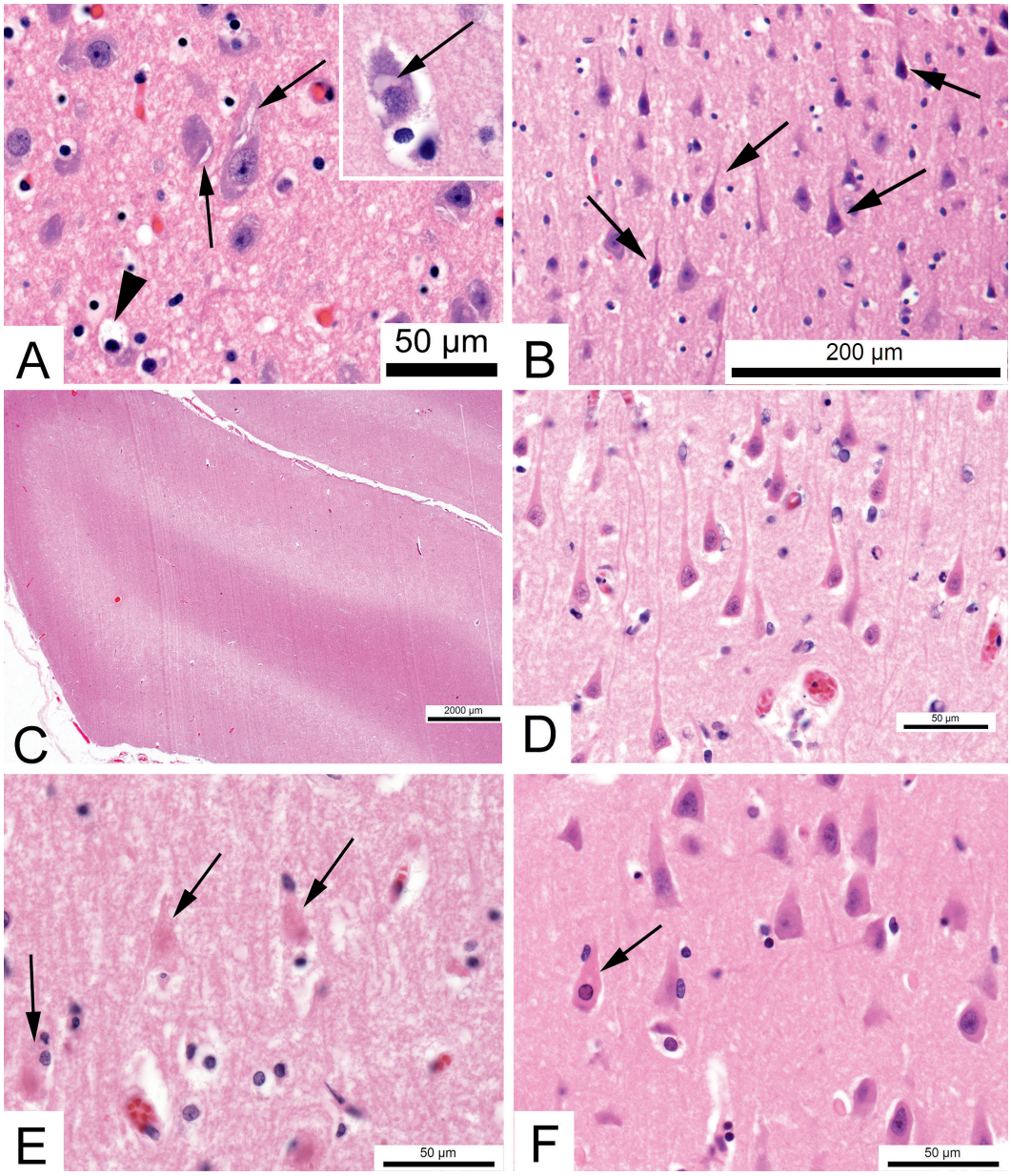

Image 1: Neuron changes in hypoxia-ischemia. The earliest changes reflect shifts in water accompanying alterations in ion gradients. **A)** Swelling of the endoplasmic reticulum appears as clefts at the periphery of neurons (arrows). These should not be mistaken for crystalline inclusions. Astroglial cells take up K^+ released from neurons and the cytoplasm becomes pale due to water accumulation (arrowhead) (H&E, x600). Inset - Swelling of the Golgi apparatus (arrow) can also reflect early neuron damage. **B)** In the vicinity of a recent contusion, neurons can appear dark and shrunken (pyknotic, arrows) (H&E, x200). **C)** If the concentration of swollen cells is sufficient (i.e., cellular edema), regions of the brain may appear pale. This example shows laminar edema of the deep cortex three days after resuscitation following respiratory arrest in a 50-year-old (H&E, x12.5). **D)** Dead neurons have uniformly eosinophilic cytoplasm. This example shows the hippocampal CA1 sector of a 22-year-old who was in a coma for three days after circulatory arrest (H&E, x600). **E)** After six days coma following respiratory arrest in a 48-year-old, hippocampal neurons are reduced to ghost cells, with no nuclear staining and a barely visible cell outline (arrows) (H&E, x600). **F)** However, not all cases are the same. In the hippocampus of this 34-year-old, who was in coma six days following respiratory arrest, only rare neurons are eosinophilic (arrow) (H&E, x600).

HI. However, there are significant differences including local alkalosis (rather than acidosis). The distribution of hippocampal neuron damage differs from HI, with relatively greater dentate neuron loss (24, 25). It should be noted, though, that this assertion is based on a small number of autopsy cases.

Axon Changes Following Traumatic and Hypoxic-Ischemic Brain Injury

Nonpenetrating head injuries associated with acceleration forces can cause distortion and shearing injuries in deep parts of the brain. Axons in the white matter structures must be evaluated. Axon damage is also a common consequence of HI.

The study of fatalities with nonpenetrating "diffuse brain damage of immediate impact type" (26, 27) led to the term "diffuse axonal injury" (DAI), proposed in 1982 (28). This was followed by a grading scheme where grade 1 consists of microscopic changes in white matter of the hemispheres, corpus callosum, and rostral brainstem, grade 2 has an additional focal lesion in the corpus callosum, and grade 3 has a focal lesion in the brainstem (7). One problem with the system is that the "focal lesion" was not clearly defined; "*grades 2 and 3 can be said to be severe if the focal lesions are apparent macroscopically*" (i.e., with accompanying vascular damage and hemorrhage) (7). The coexistence of axonal and vascular injury is well established (29). It is likely that most changes reported as DAI on computed tomography (CT) or magnetic resonance (MR) imaging studies are influenced more by the vascular pathology (hemorrhage or edema) than actual axon changes. To encompass the axonal and vascular damage, the senior author prefers the more generic term "distortional brain injury." The concept of DAI is undoubtedly useful in explaining brain dysfunction or sudden traumatic deaths in the absence of extensive contusions or hematomas (30). However, the presence of diffusely damaged axons in a victim who survives hours to days and who does not have hemorrhagic lesions is not necessarily proof of head/brain trauma.

Understanding some axon biology is necessary for understanding the histological features of axon damage. In addition to conducting the electrical action potential from the neuron soma to the synapse, axons physically transport synaptic proteins, neurotransmitter-filled vesicles, and organelles toward the synapse (anterograde); to a lesser extent, they transport materials (including lysosomes) back to the cell body (retrograde) (31). This transport is dependent on physical integrity of the axoskeleton (which consists of microtubules and intermediate filaments, particularly the neurofilament family) and a constant supply of energy. Thus, mitochondria are present throughout axons through which they move and in which they provide ATP locally (32). Physical or metabolic disruption of axons prevents movement of the organelles at that site, leading to accumulation of organelles proximal to the point of damage where axons remain viable. Eventually (6-24 hours), this is microscopically apparent as swelling of the axon ("bulbs") near the site of damage (**Image 2**).

Because the recognition of axonal damage may be important in the explanation of clinical findings, tools to detect damage earlier have been applied. Immunohistochemical markers of structural degeneration are of value. For example, accumulation of neurofilament (33-36) and proteolytic fragments of alpha spectrin, one of the proteins at the interface between the axoskeleton and the membrane (37), are reliable indicators of focal damage. Many proteins that undergo anterograde transport to the synapse accumulate can be detected by immunohistochemistry at the site of damage (38, 39).

By far, the most practical and widely used approach is immunohistochemical detection of the amyloid precursor protein (APP; sometimes called beta APP), an important synaptic membrane protein (**Image 2**). Amyloid precursor protein moves by fast anterograde axonal transport (40). Detection of APP in acutely damaged axons was first reported around cerebral infarcts (41). Amyloid beta (also referred to as Aβ, Abeta), is a cleavage product of APP that is important in the pathogenesis of Alzheimer disease. Early studies suggested that Abeta did not accumulate in damaged

axons (42), while more recent studies suggested that both APP and Abeta can be detected (34, 43, 44). Amyloid precursor protein accumulation after TBI was also recognized decades ago (45) and its immunoreactivity is definitely increased within two hours (38, 46), and likely within less than 30 minutes of axonal damage (47-49). There is a positive relationship between the size of axonal swelling and survival time, which was shown to plateau at ~85 hours in one study (50). One report suggested that abnormal immunoreactivity could persist for up to 99 days after mild head injury (51), but that was based on a single subject. Marked immunoreactivity for APP is present for one month after spinal cord injury, while less intense immunoreactivity has been reported for up to 14 months (52). More consistently, axonal bulbs begin to lose APP immunostaining one week after TBI and may not be detectable after 30 days (5). Amyloid precursor protein

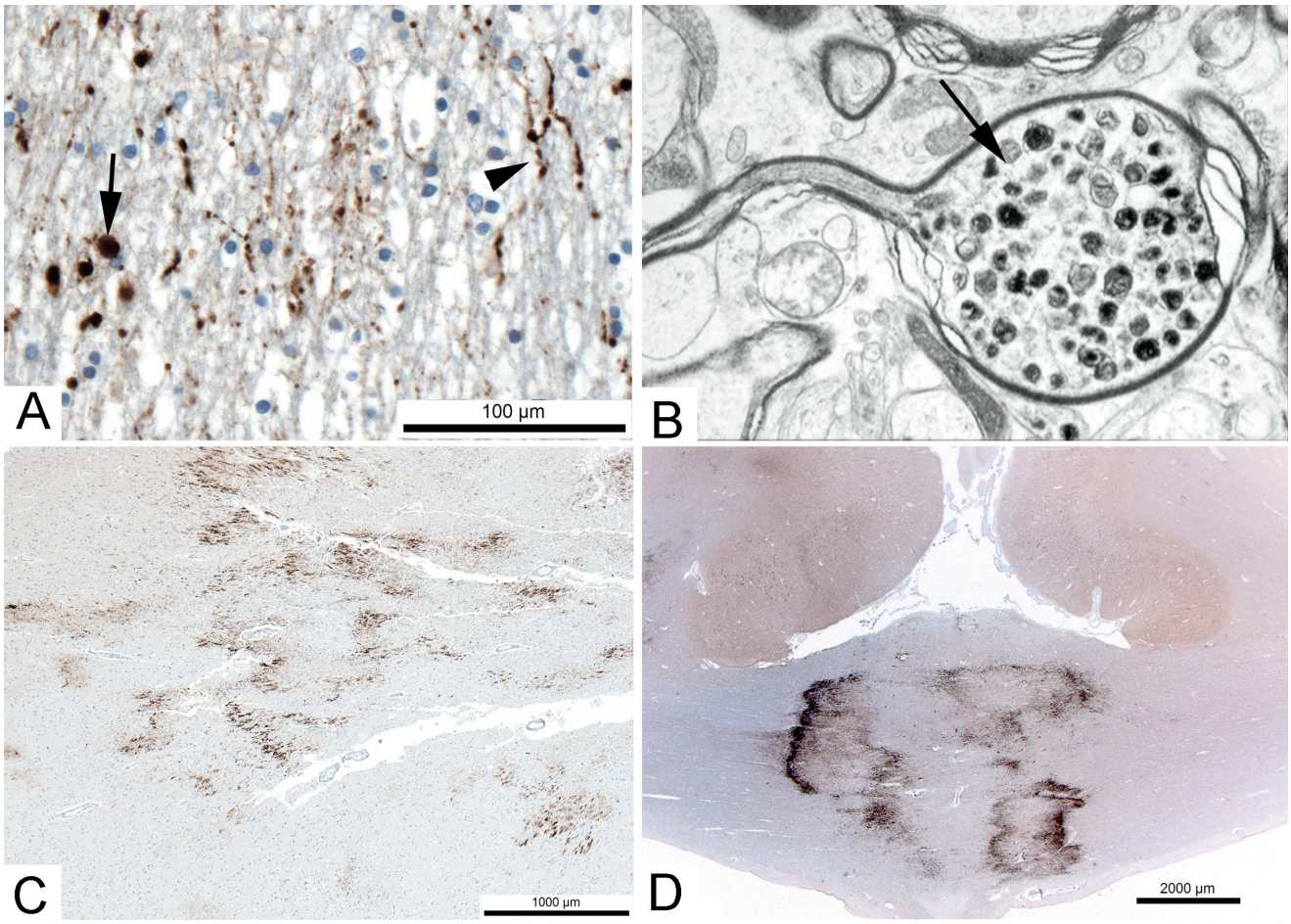

Image 2: Axon damage. **A)** Immunostain for amyloid precursor protein (APP) showing damaged axons in the corpus callosum of a person who died 18 hours after a fall. Varicosities (arrowhead) and swollen axons (bulbs; arrow) are evident (x400). **B)** Electron micrograph showing the structure of an axon bulb on the cell body side of the injury site. Note the accumulation of organelles (x20000). **C)** APP immunostain showing clusters of damaged axons in the frontal white matter adjacent to an acute infarct in a 58-year-old (x40). **D)** APP immunostain showing dense collections of damaged axons in the corpus callosum of a 37-year-old who died ~24 hours after a severe head injury that was associated with a large subdural hematoma and subfalcine herniation (x12.5). In this example, ischemia is likely a greater contributor to the damage.

immunostaining is useful for evaluating optic nerve damage in infants (53).

Axons are more vulnerable to injury than blood vessels (54); therefore, the absence of hemorrhage is not necessarily an indicator of the absence of trauma. The absence of APP immunostaining can be useful in ruling out traumatic brain injury in individuals with survival times greater than one hour after an event that renders them unconscious. However, in individuals with survival intervals less than 30 minutes it would be difficult to completely exclude TBI as a cause of death due to known limits of APP staining in very short survival intervals (5, 55, 56).

The pattern and distribution of APP immunostaining has been proposed to distinguish TBI from HI. In general, TBI is described as having single or small clusters of APP positive axons arranged in linear or bundled patterns along white matter tracts with fusiform or beaded patterns ("varicosities") (**Image 2**). Axon injury associated with HI is described as having granular APP immunostaining of axon groups resulting in circumscribed, irregular foci with a "zigzag" pattern, often at the edge of ischemic tissue or around blood vessels (5, 57-64). However, there are overlaps in the patterns and correlation with the anatomical distribution and gross findings is necessary. Victims of TBI who survive a few days usually have accompanying HI brain damage as a consequence of respiratory compromise or raised intracranial pressure. Graham et al. concluded:

> *…that the proper interpretation of cases requires the examination of a sufficient number of blocks (n =15), processing using standardized protocols including βAPP immunohistochemistry and in some cases the mapping of any [immunoreactive] on anatomical line diagrams. βAPP carried out on a small number of randomly taken blocks is likely to lead to misinterpretation of the clinico-pathological correlations and possibly to a miscarriage of justice (55, 65).*

Even among victims of known falls, the extent of axonal injury was not correlated with the height of the fall (66). Some authors, including the senior author of this paper, contest the practical utility of pattern distinction in individual cases (67-70). In summary, interpretation of APP immunostaining must not be performed in isolation. Its presence, no matter the distribution, should not be the sole evidence used to conclude that TBI has occurred.

Increased Permeability of Blood Vessels

The cerebral vasculature possesses a blood-brain barrier (BBB) that is necessary for homeostasis of the neuronal extracellular environment. So-called "vasogenic edema" is the result of a disrupted BBB and subsequent movement of plasma proteins into the brain. Histologically, enlarged extracellular spaces are expanded by acellular, faintly eosinophilic material. Macroglia can ingest plasma proteins, presumably by pinocytosis, whereupon they become engorged and uniformly eosinophilic (**Image 3**). Albumin and immunoglobulins can be detected by immunohistochemistry in swollen astrocytes and oligodendrocytes (71, 72). Damaged neurons may be immunoreactive for fibrinogen (73); however, albumin and other proteins that leak from blood vessels postmortem can enter otherwise normal neurons (74, 75).

Inflammation in Association with Cell Death and Hemorrhage

Microglia are the brain's resident macrophages. They are activated in a range of systemic and local brain disorders. Detection of the activated state may be the only evidence of early neuronal damage. Activation is associated with transformation from a bipolar cell with delicate processes to one with shorter thicker cell projections. These cells often wrap themselves around dead neurons (**Image 4**). As phagocytosis progresses, the microglia acquire a spherical macrophage morphology. Shape changes are associated with expression of new cell surface markers that are demonstrable by lectin histochemistry or, more practically, with immunostaining (e.g., with anti-CD68 or anti-HLA-DR) (76). Circulating leukocytes are minimally involved in the response to selective HI neuron damage (77). In contrast, neutrophils, monocytes, and, to a lesser extent, T

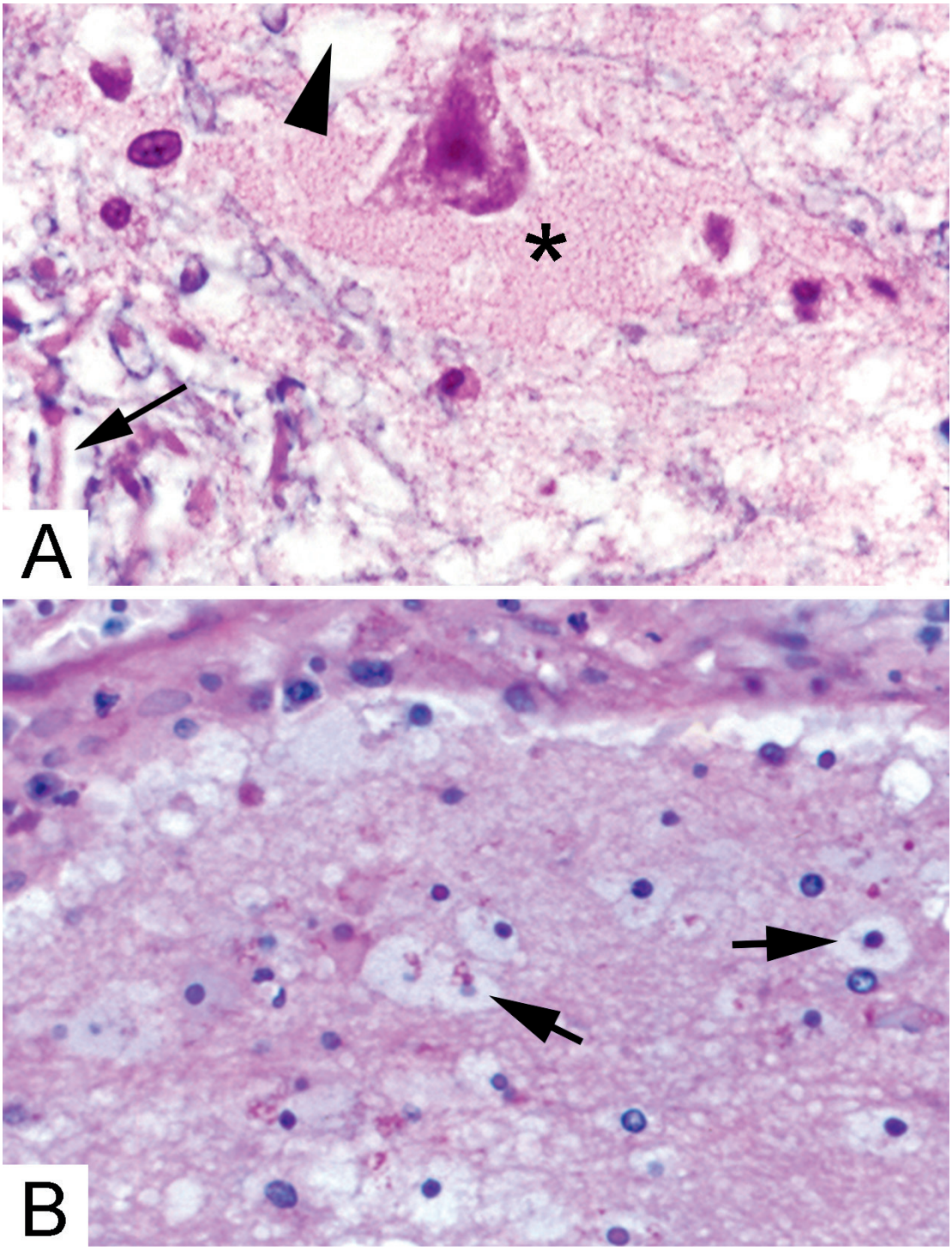

Image 3: Histologic features of brain edema. **A)** Near sites of hemorrhage or blood-brain barrier disruption, plasma proteins fill the expanded extracellular space surrounding neurons (*). Intracellular edema can manifest as empty appearing glial cell cytoplasm (arrowhead) or separation of myelin (blue) from the surrounding axons (arrow) (Solochrome cyanin & eosin, x400). **B)** Plasma proteins are ingested by astrocytes and oligodendrocytes leading to a swollen, eosinophilic appearance (arrows) (H&E, x200).

lymphocytes are prominent in the reaction to pan-infarction and hemorrhagic brain lesions (78) (**Image 4**).

Inflammatory reactions are somewhat helpful in estimating the stage of intracranial lesions. Histologic aging can help determine if an insult occurred within an alleged time frame. This involves estimating the time between when an insult occurred and death (the posttraumatic interval or survival interval). The general tempo of changes is similar in intracerebral and meningeal (i.e., subdural and epidural) hematomas, but there are major cytologic differences. The meninges lack the microglial reaction but exhibit a much more exuberant fibrovascular proliferation in the resolution phase. The phases of healing after traumatic tissue injury can be categorized by distinct, chronologic, partially overlapping morphological changes that have been established by numerous experimental investigations (79).

Degradation of Hemoglobin After Hemorrhage

Rupture of blood vessels permits extravasation of blood cells and plasma into intracranial compartments

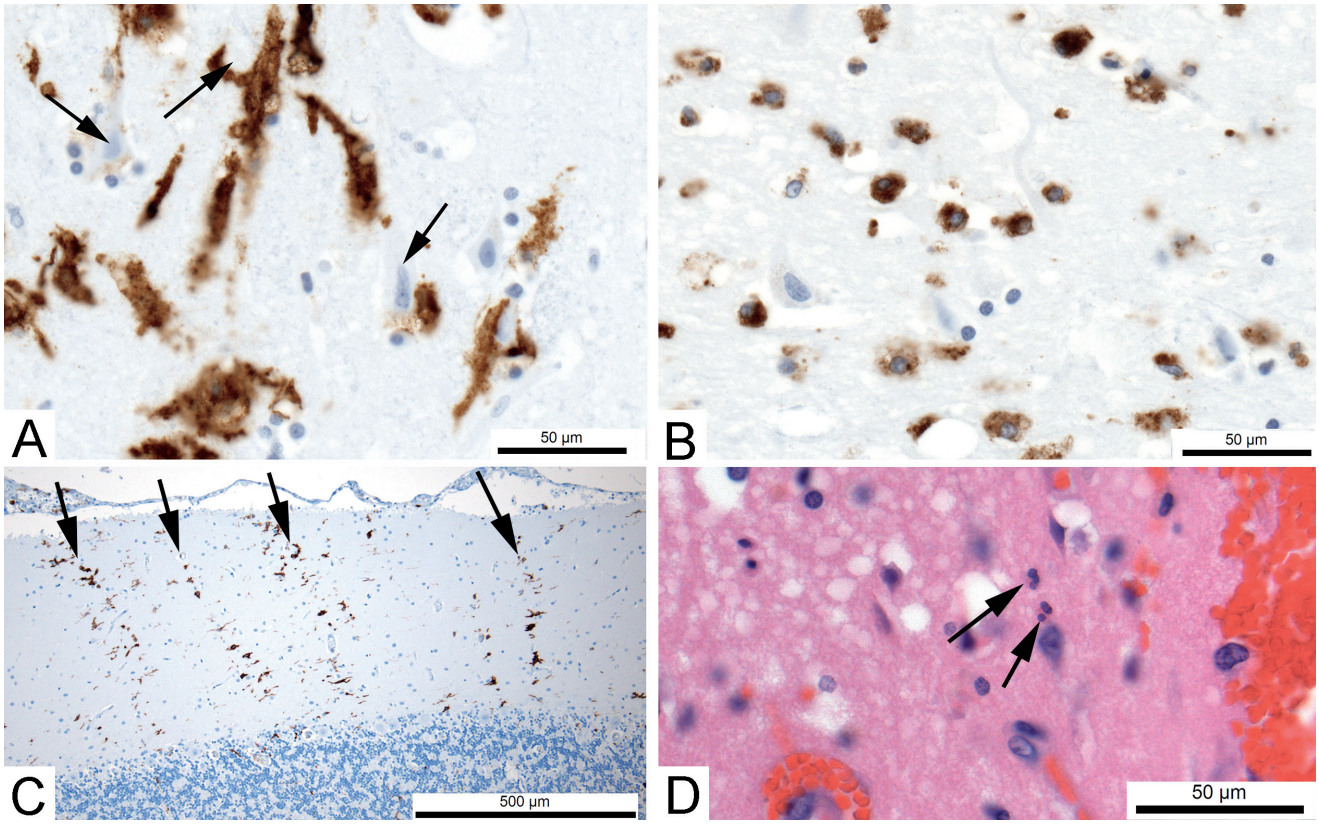

Image 4: Inflammation associated with brain damage. **A)** Immunostain for HLA-DR showing activated microglia (brown); many are in contact with dead neurons (arrows) in the hippocampus of a 22-year-old with severe hypoxic-ischemic brain damage and in deep coma for three days after resuscitation following cardiac arrest (x600). **B)** Immunostain for HLA-DR showing round macrophages (brown) in the same case (x600). **C)** After neurons have been phagocytosed and are no longer visible, microglial activation can persist in patterns that indicate the loss of neurons. In this example, immunostain for HLA-DR shows columns (arrows) corresponding to the dendrites of lost Purkinje neurons in the cerebellum of a 60-year-old who had been in coma for three weeks after severe hypoxic brain injury (x100). **D)** Circulating leukocytes more commonly enter brain in hemorrhagic lesions. In this example, rare neutrophils (arrow) have moved into the brain parenchyma from the site of hemorrhage in frontal lobe of a 42-year-old who died three days after brain trauma with contusions (H&E, x600).

and extracellular spaces. Acutely extravasated erythrocytes are easily detected on routine H&E stains immediately after injury. In a large hematoma, they can remain intact for months because their ultimate degradation is dependent on macrophages delivered via the vasculature of live tissue (15, 79, 80). Martius scarlet blue stain can provide better contrast than H&E; the cytoplasm of fresh red blood cells stains yellow-orange, fibrin stains red, and collagen stains blue. As erythrocytes begin to degenerate, the intensity of yellow staining diminishes and the cells appear as empty "ghost" cells (11). The chemistry and exact timing of this change is unclear. Martius scarlet blue stain cannot distinguish between antemortem hemorrhage and postmortem blood leakage. Note that blood vessels are disrupted during the autopsy and organ removal and it is possible that some seemingly hemorrhagic lesions (e.g., intradural blood near the superior sagittal sinus) simply reflect the forces necessary for blunt separation of the tissues after death.

Hemosiderin, an iron-storage complex that results from hemoglobin degradation, is often used in estimating the timing of hemorrhage (**Image 5**). After hemoglobin is released from lysed red blood cells, the heme molecule is converted to hematin by oxidization of the ferrous iron ($Fe2^{+}$) to ferric iron ($Fe3^{+}$). Both hemoglobin and hematin are bound by proteins that facilitate phagocytosis by macrophages. When in sufficient concentration and in the right environment, hematin aggregates into yellow-orange triclinic (tilted rectangular) crystals (81). Hematin is converted by heme oxygenase 1 to biliverdin by release of ferric iron. Biliverdin is converted to bilirubin (also known as hematoidin) by the action of biliverdin reductase. Bilirubin can form radiating needle-like crystals (82, 83). Ferric iron is sequestered in ferritin whose denaturation forms the ferrihydrite compounds that make up hemosiderin, which appears as coarse brown granules histologically. Perls' Prussian blue method can be used to stain for non-heme ferric iron in hemosiderin. The appearance of detectable hemosiderin at the site of cortical contusions takes at least 48 hours (15, 79). Hemosiderin in macrophages (siderophages) can persist for months and in glial cells for years. The time course for the appearance and resolution of Perls' Prussian blue stainable material has been reviewed in detail recently (11). Typically, macrophages stain with Perls' method no earlier than two days. Note that the Prussian blue stain can fail in tissue that has been decalcified in acidic solution (84). Also, it can give a positive result (typically a smeared appearance) unrelated to hemoglobin breakdown in putrefied tissue where cyanide has been generated (85).

Subdural Hemorrhage/Hematoma

The histologic evolution of subdural hemorrhage/hematoma is well documented (86-88). Briefly, a layer of fibrin forms on the dural and arachnoid surfaces early during clotting. Neutrophils in the extravasated blood tend to aggregate during clotting; as a reactive phenomenon, they likely peak along the interfaces with viable tissue at two to three days. Although rare macrophages may be seen as early as 12-17 hours, hemosiderin is typically not seen until three to five days at the dural surface (3). Because the arachnoid mater itself has few blood vessels, delivery of macrophages to this surface is slower. Reactive fibroblasts appear at the dural interface within three to five days and neocapillary formation (granulation tissue) is evident along the dural surface by five to ten days (3). By three to four weeks, the fibrovascular membrane can equal the dural thickness. If the hematoma is thick, clotted blood persists between the dura and arachnoid for weeks to months. In this situation, a thin fibrovascular membrane forms at the arachnoid surface beginning at 10-20 days and gradually thickens (89). As a consequence of repeated hemorrhage, chronic subdural hematoma membranes often have a mixed inflammatory infiltrate with lymphocytes and eosinophils (90). The histologic evolution of subarachnoid hemorrhage follows a similar time course (91, 92).

Cortical Contusions and Hematomas

Cortical contusions are more complex lesions than meningeal hematomas because the blood collections are interspersed with viable and damaged brain parenchyma. Immediately after trauma, ruptured blood vessels release blood cells and plasma into the extracellular spaces. Some neurons are subject to HI damage

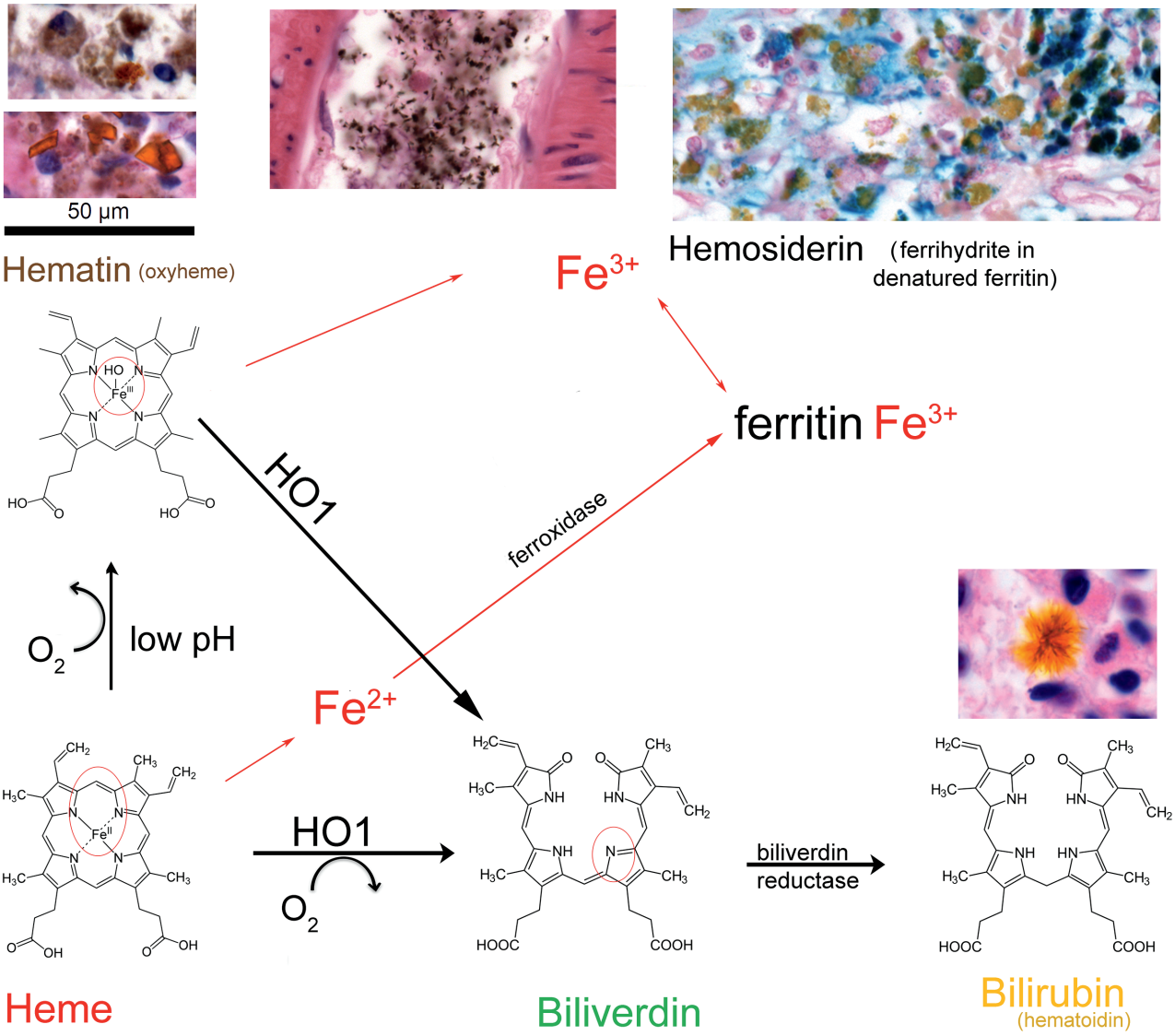

Image 5: Heme degradation following hemorrhage. After hemorrhage, the heme component (bottom left) of hemoglobin is released from erythrocytes. In low extracellular pH conditions, the iron atom becomes oxidized to form hematin (oxyheme), which is bright orange and has a rhomboid crystal structure (top left lower; hemosiderin is brown and globular). Following formalin fixation and paraffin embedding, the crystals usually lose their distinct structure, but remain orange (top left upper). In some circumstances, the action of formaldehyde on heme that is not associated with a hemorrhagic process can create hematin. In this situation, the coarse brown granules are called "formalin pigment", which can appear in blood vessel lumens (top middle) or within cells. Phagocytosis of heme or hematin, usually by macrophages but also potentially by astrocytes, allows heme oxygenase 1 (HO1) to remove the iron ion, yielding biliverdin. Biliverdin, in turn, is converted to bilirubin (also called hematoidin) by biliverdin reductase. Bilirubin is usually removed from the tissue into blood but can form radiating needle-like extracellular crystals (lower right). Ferrous iron ($Fe2^{+}$) from heme is complexed with intracellular ferritin, which has ferroxidase activity that converts iron to the ferric form ($Fe3^{+}$). Hemosiderin is a ferrihydrite (hydrous ferric oxyhydroxide) compound bound to denatured ferritin. It gives the yellow color to sites of old hemorrhage. Ferric iron can be detected with the Perls' Prussian blue stain (top right; note that the hematin does not stain because the ferric iron remains chelated).

because the vascular supply is interrupted while others are compromised by plasma enzymes (e.g., thrombin and plasmin) (93). The temporal course of histologic changes has been described in several detailed studies and reviews (15, 79, 80, 94-98). Note that there is some variability in the observations; this could reflect the patient populations studied or the criteria used for reporting cellular details. Eosinophilic macroglia become engorged by plasma proteins within hours (see above) (71). Pyknotic neurons can be seen less than one hour post-trauma. Neuron eosinophilia follows the same time course seen in HI (see above). Neutrophils begin to enter the tissue in one to three hours, while lymphocytes appear at 18 hours. Swollen axons are typically obvious at 10-24 hours (although note detailed consideration of APP immunostaining above). Mitotic figures among astrocytes and endothelial cells can be seen at two to three days and hypertrophic astrocytes are obvious by five to seven days. Hemosiderin-laden macrophages begin to appear at two days and increase gradually. Hemosiderin-containing astrocytes appear at five days. All of these features can appear over a protracted time course because of delayed HI from compromised blood flow due to herniation or raised intracranial pressure, or the toxicity of plasma proteins.

Special histochemical stains and immunohistochemistry can be used to highlight the evolution of cortical contusions. Anti-HLA-DR can show early microglial reaction and anti-CD68 can highlight macrophages (99). Anti-GFAP and anti-vimentin can be used to evaluate astrocyte changes. Anti-Ki67 shows cell proliferation from 3-15 days after contusion (100). Modified phosphotungstic acid-hematoxylin (PTAH) stain is very useful for demonstration of chronic, persistent alterations of the astrocyte cytoskeleton, even years after the hypertrophic forms have resolved (101).

Artifacts

Decomposition can make the evaluation of TBI or HI challenging (102, 103). Autolysis (i.e., digestion and degradation by unregulated enzymes from local cells) and putrefaction (i.e., decomposition as a consequence of bacterial growth) can create gross and cytologic changes (104). The brain should be carefully examined *in situ* to ascertain macroscopic changes that might not be obvious once the softened brain is removed from the skull. Many anaerobic bacteria produce endonucleases that can digest the DNA of brain cells (105), leading to a loss of nuclear staining that can be mistaken for ischemic damage. Careful microscopic inspection of blood vessel lumina or parenchyma for bacterial forms (usually bacilli) must be done to help interpret degenerative cell changes.

Dark neurons are commonly identified in histologic sections of the brain. They are characterized by shrunken cell bodies with hyperbasophilic staining of the nuclei and convoluted dendrites. Their cause and significance have been debated for over a century (106, 107). Dark neurons can be produced by mechanical handling of tissue prior to fixation (108). Physiologic changes prior to death can also cause them. In surgically excised tissue, prior depolarization seems to be important (109). Dark neurons appear at sites of contusion and areas of secondary compression in cases with short survival intervals (15). Damaged dark neurons may be indistinguishable from artifactual dark neurons (110). If the nucleoli are of normal size despite the shrunken cell, the change is more likely an artifact (111).

CONCLUSION

Forensic pathologists and neuropathologists predominantly rely on gross findings to diagnose TBI. However, histologic slides and paraffin blocks can serve as forensic evidence even in the absence of gross photographs. Histology should be used to augment macroscopic diagnoses, particularly in the absence of gross evidence of intracranial trauma. Histology can detect nontraumatic causes of seemingly traumatic lesions. Histology can help estimate the survival interval after an insult. However, even detailed documentation of cytologic, inflammatory, and immunohistochemical (including APP) changes is at best an approximation. Note that the legal time of death might not be the same as the biological time of death. In individuals who have been declared brain dead and whose bodies are kept alive until transplant harvesting occurs, the pathologist should consider the time of cardiac

cessation. Regardless, it is crucial to be aware of the limitations of such an evaluation. An integrated consideration of the macroscopic and microscopic features, along with historical and medical evidence, are necessary to distinguish TBI from HI from artifact. Even if TBI is definite, examination of the brain in isolation cannot help distinguish between accidental and nonaccidental etiologies.

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