Frontiers in Bioscience Landmark

Front. Biosci. (Landmark Ed) **2022**; 27(3): 079
http://doi.org/10.31083/j.fbl2703079

Original Research

Diagnostic assessment of traumatic brain injury by vacuum extraction in newborns: overview on forensic perspectives and proposal of operating procedures

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Academic Editor: Graham Pawelec
Submitted: 4 December 2021 Revised: 14 January 2022 Accepted: 19 January 2022 Published: 4 March 2022

Abstract

Background: Traumatic brain injury (TBI) during birth constitutes one of the most relevant causes of mortality and morbidity in newborns worldwide. Although improvements in obstetrical management and better indications for caesarean section have led to a consistent decrease in the incidence of perinatal mechanical injury, vacuum extraction is still associated with a high complications rate leading to several forensic issues in the evaluation of healthcare professional management. **Methods**: Vacuum-associated lesions may be topographically distinguished as extracranial or intracranial injuries. In order to achieve a correct assessment, diagnostic procedure should include post-mortem computed tomography and magnetic resonance imaging, autopsy examination, brain sampling and histological/immunohistochemical examination. **Results**: Post-mortem imaging represents a valid aid to guarantee preliminary evidence and direct subsequent investigations. An appropriate autopsy sampling must include several areas of cortex and underlying white matter; moreover, any visceral hemorrhages or other lesions should be sampled for the histological and immunohistochemical assessment of vitality and timing. **Conclusions**: This study aimed to promote a validated step-by-step procedure to be adopted in order to standardize and to make easier the post-mortem framing and timing of vacuum-associated pediatric brain injuries.

Keywords: brain injury; birth injury; vacuum; delivery; PMCT; PMMRI; autopsy; immunohistochemistry; paediatric

1. Introduction

1.1 Epidemiology of traumatic brain injury

Traumatic brain injury (TBI) is a clinical condition characterized by lesions caused by blunt or penetrating head trauma and could result in acute and chronic manifestations such as concussion, chronic traumatic encephalopathy and diffuse axonal injury. It is the main cause of death and overall disability in pediatric population worldwide, with an annual incidence of about 475,000 cases in United States in the age group of 0–14 years; its high incidence is primarily due to the anatomical structure of the children head and their partial (or absent) capability to avoid head injuries from falls [1]. The mortality rate is particularly high in the group 0–4 years, partially due to the higher incidence of abuse in that age range [2]. In the newborn, delivery head injury is the most common cause of TBI [3]; obstetric devices such vacuum extractor or forceps represent a risk factor by compressing and tractioning fetal head through vaginal canal [1].

Newborn's head presents unique biomechanical features aimed to minimize physique resistance during natural delivery and, consequently, brain damage. The infant skull is less rigid than adult's one and open sutures allow a higher plasticity and deformity in response to a mechanical stress [4]. Consequently, conditions like hematomas of the scalp are very common, even in uncomplicated deliveries; they can also be clinically silent or determine slightly relevant complications. Major clinical events (i.e., intraventricular hemorrhage) are more frequently seen in presence of risk factors such low birth weight or hypoxemia [5].

Among the possible classification systems of TBI, one of the most frequently used evaluates the trauma extent, identifying:

• focal damage as lacerations, hematomas, hemorrhage, focal secondary lesions due to an increase in intracranial pressure;

• diffuse/multifocal damage as diffuse axonal injury (DAI), global ischaemia, diffuse cerebral oedema.

Moreover, the peculiar action of vacuum extractors mainly leads to compressive lesions; other mechanisms of lesion, such penetration, high or low energy impact or acceleration, should be distinguished in order to hypothesize different causes to delivery.

It is also necessary to distinguish between primary mechanical injuries and secondary changes, including anoxia, oedema, hypoxia, and ischaemia; all the above-mentioned

conditions play a statistically important role in the determinism of death and neurological disabilities [6,7]. However, both macroscopic and microscopic features may be not specific since they are shared between several pathologies. Consequently, an accurate distinction between primary and secondary damage may be complex.

1.2 Traumatic brain injury classification

As previously mentioned, both forceps and vacuum extractors are performing instruments of operative vaginal delivery [8]. They are mostly indicated for cases of complicated vaginal deliveries, including prolonged second-stage labour, cord prolapse, non-reassuring fetal heart rate, intrapartum hemorrhage and exhaustion [9]. Vacuum-assisted vaginal delivery (VAVD) is performed for 1–23% of deliveries [10] and leads to an overall complication rate of about 10% [8] (mortality hasn't never been estimated properly, but some authors report rates <1%) [11].

These events can cause neurologic alterations with a clinical presentation characterized by low Apgar score, convulsions, distress, encephalopathy, jaundice and several aftereffects of different entity; peripheric limb palsy (brachial plexus injury is the most common) can be the direct effect of a fetal malposition or disproportion.

Neonatal cranial complications which can be described after vacuum extraction can be classified as extracranial and intracranial injuries [12,13].

1.2.1 Extracranial injuries

Extracranial injuries include scalp abrasions and lacerations, caput succedaneum, cephalohematoma and subgaleal hemorrhages. The majority of these are transient and related to incorrect placement of the cup, excessive or poorly directed traction, or cephalopelvic disproportion [14].

Their occurrence depends on the site of force application: the scalp is composed of five layers: skin, subcutaneous tissue, galea aponeurotica, loose areolar tissue, and pericranium. The function of the scalp is to protect the skull from fractures via the intrinsic capacity of its components to dissipate mechanical energy [15]; in the newborn it is less represented than adult's one, contains less fat tissue and is more elastic, thus making it more susceptible to blunt impact and tearing forces. Although most of these injuries are transient and asymptomatic [16], they may achieve clinical relevance due to the redistributions of blood which may cause hypovolemia and eventually evolve into life-threatening hemorrhagic shock [17].

Erythema and pressure marks are not frequent [18]; for this reason, external investigation alone cannot always be used to detect lesions produced by blunt forces.

Scalp abrasions and lacerations have an incidence of 10% after vacuum extraction [19] and are both more frequent in infants delivered by metal cup devices. They occur as interruptions of the continuity of tissues in the context of ecchymotic zones; they can be partial or complete and consist of a linear, star-shaped or epsilon-shaped morphology.

"Caput succedaneum" is defined as an extraperiostal serum or blood benign collection which crosses cranial suture lines (consequently it may extend through the midline) and mainly appears shortly after birth. Given by the pressure gradient between the vacuum and the mean arterial pressure, it is caused by scalp vessels rupture which determine the collection of blood between the scalp and subcutaneous tissues [14]. Alternatively, it has a serous aspect and derives from the ex-vacuo extracellular collection of fluids. Even untreated, it usually resolves within 12–18 hours after delivery.

A peculiar kind of lesion that can be documented in newborns is subgaleal (or "sub-aponeurotic") hemorrhage, which can be associated with skull fracture, rupture of interosseus synchondrosis or emissary veins [20,21] as it is located between galea and skull periosteum [22]. Subgaleal hemorrhage occurrence rate is highly variable among authors, spacing from 0.6 to 21% among deliveries by vacuum extraction [23,24]. The amount of collected fluid, potentially as great as 260 mL, may be lethal in 25% of patients due to the previously explained hypovolemia mechanism [25,26], making it one of the most clinically relevant extracranial complications. It presents as a diffuse swelling or fluctuant mass crossing suture lines [22].

Furthermore, vacuum pulling applied to the scalp is capable of separating the peri-cranium from localized areas of the skull; therefore, the resulting empty spaces fill with blood, causing a subperiostal collection called "cephalohematoma".

In most cases, the bleeding overlies the parietal bones, with only occasional involvement of the occipital and frontal bones. A fundamental aspect for differential diagnosis is given by its peculiar localization over the area of a cranial bone, never crossing the midline or the suture lines. Because of the slow nature of subperiosteal bleeding, cephalohematomas are not usually present at birth but develop hours or even days after delivery [27]. The mean incidence of cephalohematoma after vacuum extraction is 6%, varying from 1 to 26% [16,28]; metal cup devices are more likely to determine this complication than silastic cups [29]. Cephalohematomas can be complicated by underlying skull fractures, calcifications or infections [30,31].

1.2.2 Intracranial injuries

This lesional category is mostly relevant for his fatality risk and long-term disability [32]. Intracranial birth injuries occur in 0.5–0.6% of term deliveries, and vacuum extraction represents a strong related risk factor [33].

Among the risk factors, operative delivery deserves particular attention, especially when executed with vacuum extractor or forceps [34].

Epidural hemorrhage is characterized by a blood collection within skull bones and inner periosteum or between

this and the outer fibrous dural layer [35]; meningeal artery is the most common ruptured vessel, especially when secondary to displacement of the skull [36]. This occurs whenever the forces applied to the skull are excessive and result in a stripping of the dura from the bone. However, due to the slight embedding of that artery in cranial bones, this injury is the less represented of the whole category [37].

Conversely, subdural hemorrhages derive from the rupture of bridging veins, characterized by extremely thin walls and particular fragility [38]. Subdural hemorrhage represents 73% of intracranial injuries [33], and its overall occurrence rate is 0.008% of vacuum extractions according to Towner [39]. Two main mechanisms are commonly implied in subdural hematoma pathophysiology. The first consists of a tearing process involving the veins and venous sinus, which bleed into the subdural space. The second one, called osteodiastasis, is the detachment of the squamous portion of the occipital bone, causing cerebellar trauma and brain stem compression [40].

Subarachnoid hemorrhage is caused by a loss of integrity of the small bridging leptomeningeal veins, small subarachnoid arteries or subpial arteries. It usually occurs whether vessels located over the cerebral convexity, especially in the posterior fossa, are involved in the overmentioned injuries. His incidence, according to different authors, spaces between 2.2 and 6 cases for 10,000 vacuum assisted deliveries [34,40].

1.3 Aims of the study

Although improvements in obstetrical management and more appropriate indications for caesarean section have led to a consistent decrease in the incidence of perinatal mechanical injuries, several issues may still arise. The evaluation of vacuum-related lesions has always represented a challenge for the medical examiner in the determination of causes, means of production, timing, and in general in the assessment of medical liability.

According to that, the aim of the following analysis is the establishment of a multidisciplinary forensic methodology focused on the assessment of timing and pathophysiology of injuries, which constitutes an essential question regarding the adequacy of procedures performed by all forensic pathologists [41]. The described procedures will be illustrated through the presentation of the results emerging from inedited multicentric case series and the preliminary evidence deriving from the use of innovative immunohistochemical markers will be discussed.

2. Methodologic approach

The correct instrumental diagnostic procedure should include a preliminary post-mortem computed tomography (PMCT) or postmortem-magnetic resonance imaging (PMMRI), autopsy examination, brain sampling, histological and immunohistochemical examination.

2.1 Post-mortem imaging techniques

Thanks to the scientific achievements of our era, the initial radiological approach to an autopsy is spreading regardless of the single case features (age, sex, race, type of injury, diagnostic or judicial intent). Moreover, such a diagnostic approach allows an objective, standardized, shared and non-perishable datum [42]. Currently, the two main methods used in the forensic field for the purpose of determining cranial injuries are post-mortem computed tomography (PMTC) and post-mortem magnetic resonance imaging (PMMRI) [43].

Regarding the first technique (PMCT), it must be stated that following the brilliant results achieved in the forensic field since its application [44–46], the use of radiographic methods has become a practical standard in many countries, including Switzerland.

In the field of childhood brain trauma, it is estimated that CT is used in up to 70% of cases related to *in vivo* clinical practice [47]. Despite exposure to a radiant source, this data demonstrates its undisputed usefulness for the purpose of detecting such injuries; since in the forensic field the exposure of the body to radiation is not a critical issue, the use of PMCT can be considered the diagnostic gold standard. Direct imaging allows to obtain an accurate visualization in axial or coronal planes, allowing further software reconstructions (e.g., sagittal plane).

Specifically, the sensitivity of this method for the isolated skull fractures detection is higher than MRI [48,49]. Furthermore, PMCT of the brain allows identification of the structure involved in the hemorrhagic event (parenchyma, meningeal layers, bone structures, periosteum, soft tissues) (Fig. 1) and estimation of the pathologic process that spread within tissues in order to assess its causal role in the death event; from the point of view of expansive lesions, this technique is therefore the most suitable.

In fact, in cases of cerebral blood collection a CT scan can identify direct signs such as hyperdensity and indirect signs such as midline shift, hydrocephalus, and fractures [50]. In some specific situations, such as pneumocephalus or the presence of arterial or venous gas emboli, radiographic detection appears to be the only possibility of diagnosis, unless the autopsy investigation is conducted through investigative underwater procedures that are not confirmed in normal forensic practice [51].

In any case, phenomena of a gaseous nature always require an accurate differential diagnosis with respect to the post-mortal genesis of a putrefactive nature which, although slowed down in newborns due to poor visceral bacterial colonization, actually poses the risk of a misdiagnosis. To this end, specific methods such as the one proposed by Eggers *et al.* [52], based on the estimate of the gas content in the right ventricle and hepatic parenchyma to establish the extent of postmortem phenomena.

Obviously, in order to obtain the most accurate anatomical view possible of certain structures (e.g., skull

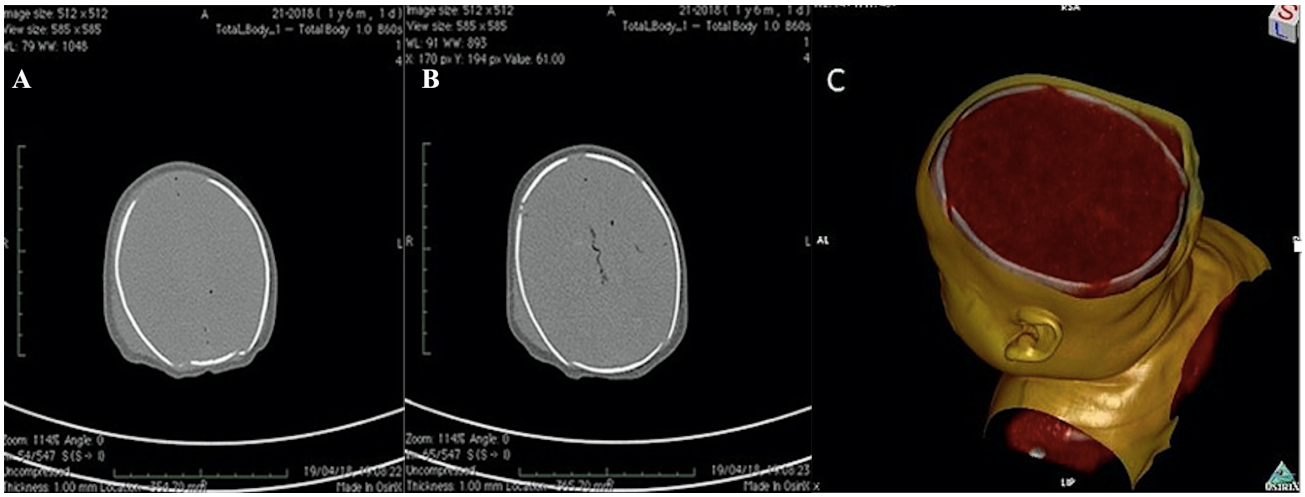

Fig. 1. PMCT Imaging. (A,B) CT axial scans detecting soft tissues swelling attributable to scalp haemorrhage and fractures of the skull. (C) 3D reconstruction of the aforementioned tissues.

base), the use of high-resolution algorithms for soft or bone tissues is useful.

Finally, another possibility offered by the radiographic technique is to proceed with contrasted angiography of the lesional areas (PMCTA). The infusion of oily media such as Angiofil [53,54] through the modified use of heart lung machines to remedy the absence of post-mortem circulatory diffusion allows to obtain the contrast of the vascular lumen in the absence of tissue diffusion, except where permitted by easily highlighted solutions of continuity. For these purposes, PMCT is a valuable aid to correctly address autopsy procedures.

As for PMMRI, it is undeniable that the use of magnetic fields constitutes *in vivo* a widely used method, due to the advantages in terms of absence of emitted radiation and the high sensitivity in distinguishing between areas of different densities. In the forensic field, this technique represents a recent innovation [55]. In fact, among the factors limiting its diffusion there is the prolonged time of diagnostic acquisition, the high cost associated with the procedure, the absence of solid scientific elements in the literature and the poor knowledge of the impact of post-mortal alterations on the final report.

Regarding the last point, the inadequacy of the quantitative reconstructions currently available (T1, T2, diffusion-weighted) was observed especially in the case of hypoxic-ischemic damage and, therefore, the need to develop specific filters for the post-mortem examination [56]; various solutions have been proposed, including a linear correction model based on the post-fatal temperature of the investigated tissues [57]. However, the analysis of post-mortal changes carried out by means of magnetic resonance elastography (MRE) has allowed to estimate a latency of about 24 hours between fatal event and the onset of transformative radiological changes, offering useful insights in terms of timing window of execution [58]. Regarding the time required for the three-dimensional reconstruction of the image, which is why this technique is often used in individual body districts (rather than "panoramic" as in the case of PMTC), it is certainly true that the small size of the bodies of newborns allows a total-body acquisition not possible in adults [59].

However, PMMRI remains an extremely useful and sensitive technique for the study of soft tissues, as in cases characterized by the presence of intra- and extra-cranial hemorrhages, bruises, abrasions and lacerations of the scalp [60], axonal damage and alterations in the relationship between gray matter and white matter [61]. On the basis of a famous pilot study [62], in fact, subsequent studies have shown how this technique allows to appreciate with high sensitivity (100%) and specificity (98–100%) signs attributable to bleeding lesions [63–65] as well as suffering neuronal ischemic. Likewise, it must be underlined the PMMRI suitability to distinguish a hemorrhagic spread into phases.

As stated for PMCT, the post-mortal absence of blood circulation prevents the execution of "clinical" (arterial and venous) sequences; however, special alternative sequences (even without the use of contrast medium) have been proven to allow identification of vascular macro and micro-lesions useful for directing the autopsy procedure. Moreover, we note the existence in the literature of experiences aimed at introducing contrast media for the vascular study of various districts, including the cerebral one (PMMRI angiography); numerous compounds, including mixtures of iodinated compounds and PEG [66], would allow to obtain brilliant results in T1-weighted images through infusion procedures very similar to those foreseen for PMCTA. Ultimately, PMMRI is extremely promising in the forensic field, especially for the neonatal study; the progressive spread of this technique will allow in the immediate future to have more data and protocols for the implementation

Table 1. PMCT versus PMMRI techniques.

Terms of Comparison	PMCT	PMMRI
Current procedural standard	Yes	No
Availability	+	-
Executive quickness	+	-
Cost	+	-
Post-mortem alterations sensibility	-	+
Angiographic technique available	Yes (low availability outside large centers)	Yes (experimental, not always necessary)
Bony tissues	+	-
Soft tissues	+	++
Intra/extra-cranial expansive lesions	+	+
Lesional timing reconstruction suitability	-	+

of post-fatal investigations, in order to equate (or replace) PMCT (Fig. 2, Ref. [43]).

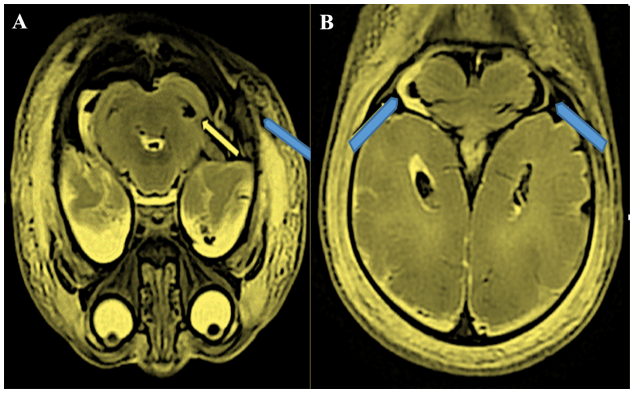

Fig. 2. PMMRI Imaging. (A) evidence of intraparenchymal cerebellar haemorrhage. (B) Blood extravasation in both occipital horns of lateral ventricles. Modified from Vullo *et al.* [43].

The peculiarities related to the two techniques are shown in Table 1.

2.2 Autopsy

Following the execution of post-mortem radiological investigations, it is highly probable that the diagnostic suspicion is already highly oriented on the basis of the lesions found. However, given the large number of factors to be taken into consideration in a neonatal autopsy (gestational age, degree of development, malformations, perinatal ischemic suffering, state of the adnexa, intercurrent infections, maternal intake of teratogenic substances, etc.) it is necessary to use a methodical approach, aimed at investigating all possible factors determining death. Not surprisingly, this phase is the most delicate as operator-dependent: the technique identified, the correct documentation of the findings and the execution of the histological samples require the presence of a pathologist expert in the field. Furthermore, numerous scientific societies in the sector have drawn up specific protocols, such as Guidelines on Autopsy Practice edited by the Royal College of Pathologists [67].

For this purpose, head and brain macroscopic examinations should always be preceded by a scrupulous external examination of the fetus or newborn. Height and weight, crown-rump length and head circumference must be recorded, as well as the state of nutrition [50].

In particular, the following steps should be taken [68]:

(1) Measurement of head circumference and diameter to evaluate fetal growth;

(2) In a prone position, posterior foramen magnum inspection and cerebro-spinal fluid preservation [69–71];

(3) Recording of the color of the sclera, iris and conjunctiva (post-bleeding jaundice) followed by extraction of vitreous fluid. A further microscopic examination of the fundus oculi should be achieved, considering that up to 70% of children with subdural haemorrhage also have retinal haemorrhage [72].

Furthermore, the external inspection phase is of great importance to diagnose stillbirth: a live birth, for example, must be free from degenerative changes from post-mortal stay in the amniotic fluid, such as skin maceration, articular laxity or overlapping cranial bones. On the other hand, the recognition of elements such as head swelling constitute inspective birth-related vital signs. Then, a complete search for eventual malformations should be made and documented.

In this phase, any external lesion should be carefully measured, photographed, described, and sampled. Tissue sampling is particularly important to establish the nature of the lesion and its age. There could be some cases, like abdominal wall defects, in which a true differential diagnosis between malformation and birth trauma is required.

Regarding autoptic techniques, as proceeded in adults, pericranial soft tissues can be dissected by an intermastoid incision. Following this procedure, the scalp must be retracted and an accurate examination of the inner galea and temporal muscles must be assessed. In this phase, the measurement of fontanels is required because of the possible distention caused by internal foreign masses, bleedings, or hydrocephalus.

For newborns, the "butterfly" technique (full reflection of the four bony flaps) should be preferred to perform head tissue dissection [73]. Next, the head is tipped forwards and laterally, and the occipital pole is gently lifted with a finger or scalpel handle to inspect the falx and tentorium for hemorrhage and tears, so that they can be examined in this way; the state of hemispheres can be observed as well, paying attention to slightly evident subdural blood extravasations. Following the midline bone removal, the access to the longitudinal fissure allows an in situ lateral ventricles opening to assess possible bleedings. The extraction procedure shouldn't differ from the adult standard one; in case of very low parenchymal consistence, it could be necessary the help from an assistant. Unlike other circumstances, water suspension of the cerebral parenchyma is highly unrecommended, because hemorrhagic stratifications could be washed away.

Once that skull base has been exposed, dura mater should be adequately removed, and eventual observed fractures should also be documented. It is mandatory to confirm preliminary diagnosis of fractures made during imaging investigation, as radiology reports may often denote fractures of the skull or metaphysis of long bones or ribs that, at autopsy, prove to be anatomic variants of bone formation, aberrant vascular channels, or variant sutures in cases of skull fracture. For this purpose, bone sampling should be executed routinely whenever microscopical confirmation is needed.

With the encephalic mass disposed on the table, a preliminary macroscopic evaluation allows appreciation of the volume, mass and eventual alterations of the brain, whose locations and extensions must be evaluated. Due to his typically poor consistence and the necessity of histological processing, the whole brain should be submerged for at least 2 days in a 20% formalin solution.

Next, the medical examiner will proceed to organ sectioning (frontal, transverse and/or sagittal). This phase is crucial for an appropriate routine sampling, which should include several areas of the cortex and underlying white matter (frontal, parietal, occipital, temporal lobes). It may be appropriate to sample the corpus callosum. A section of the hippocampus should be obtained, as well as a section of each level of the brain stem and cerebellum. For the spinal cord, the dura should be opened, and a crosscut should be made at 1 cm levels throughout its extent. Each major division should be sampled for histological study along with the dura. If lesions are found, more extensive sampling should be performed.

In conclusion, all the areas of histopathological interest should be documented and sampled for further microscopical examination (Fig. 3).

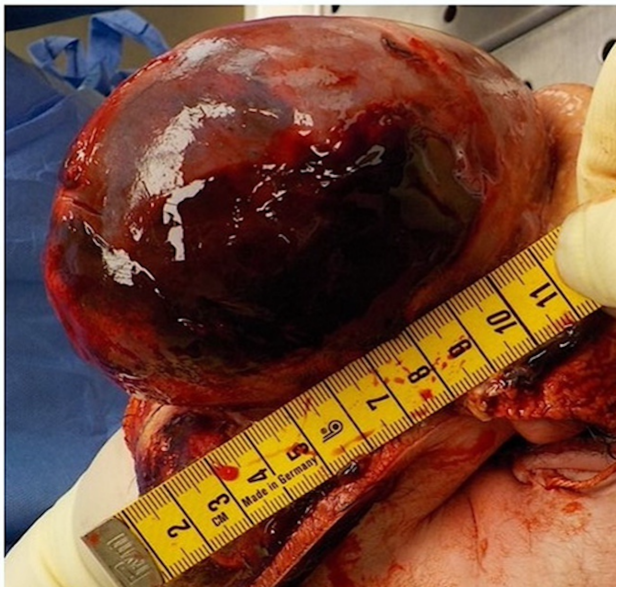

Fig. 3. Autopsy finding of cephalohematoma in the fronto-temporo-parietal-occipital right region.

2.3 Histopathological investigation

An adequate histopathological examination requests at least a basic knowledge of the physical and chemical mechanisms which produce the main alterations seen in such cases. The most evident alteration that a traumatic injury produces on infant brain corresponds to blood extravasation in all layers of the intra and extracranial tissues, which can be adequately assessed by traditional hematoxylin and eosin (H&E) histology or by histochemical techniques further analysed across the present section. However, the primary biomechanical force applied to brain matter contributes partly to parenchymal damage [74]: a consequence of the trauma is cerebral edema, which involves an alteration of the blood circulation and, consequently, a new pathological entity: the hypoxic-ischemic lesion [75].

Although traditional methods don't allow a sufficient estimation of the lesional timing, they must necessarily be performed. In fact, they make it possible to establish an incontrovertible judgment of the vitality of the lesions, as well as to establish their overall extent (judgment based strictly on the number of samples taken) and to identify the underlying areas of suffering worthy of immunohistochemical study.

In order to correctly fix the samples performed, the material must be fixed in 20% formalin for at least 48 hours and then processed and embedded in paraffin. Microtome sections of about 4 μm thickness should be executed and then stained with H&E.

The microscopic observation of the samples strictly depends on the district examined. At the level of the extracranial soft tissues, for example, the evidence of extravasal red blood cells contained between the layers is in itself an evident sign of trauma, determined in this case by the suction produced by the vacuum device used for the extraction of the fetus. Observing the characteristics of this type

of injury allows to arrive at a rough timing from the onset according to criteria already consolidated within the literature, such as those proposed by Janssen [76]. Of equal interest, due to the complex diagnosis required of the forensic pathologist, is the question relating to the viability of the lesion; even in this case, the literature in the sector proposes consolidated methods of differential diagnosis based on the integrity of the red blood cells, the presence of fibrin reaction, the degree of leukocyte infiltration and, finally, the presence of granulation tissue [77].

Conversely, the intracranial side can be affected by hemorrhagic phenomena of a different nature. Epidural hemorrhage, despite being a rare complication in the newborn for the anatomical reasons already mentioned [36], has a high specificity in relation to the use of mechanical devices for operative delivery, as the deformation of the cranial bones, made extremely malleable and scarcely unified with each other by the amplitude and immaturity of the fontanelles, it allows a notable deformation of the arterial and venous meningeal vessels.

Subdural hemorrhage is more likely to happen, and its origin must be properly sought in the dural vessels: scientific evidence shows that the origin of these blood extravasations from the rupture of the bridging veins is typical of adulthood [78]. Consequently, the use of the classification proposed by Di Maio and Dana [79] for dating this type of lesion should be used with caution, as it has not yet been verified in the neonatal setting. Subarachnoid hemorrhage, being typically linked to blunt traumas, is also not a frequent finding in vacuum-assisted delivery [80]; the reconstruction of the time of onset however follows the same principles previously stated for subdural hemorrhage. A finding of absolute importance, given the underlying clinical impact, is certainly that of cerebral contusion.

The discovery of free red blood cells within the brain parenchyma always requires the search for signs of neuronal distress. Therefore, in close relation to the timing of observation, it was observed that cerebral edema represents the most common immediate reaction to the traumatic insult, associated with neuronal vacuolization; apoptosis begins about 45–60 minutes after the insult, resulting incompatible with immediate death; after about 12–24 hours it would be possible to observe signs of nuclear swelling. After about 2 hours, it is possible to observe neutrophils diapedesis [81–83]; further timing characterizations are made possible by the application of immunohistochemical methods which will be further highlighted later. These histological criteria represent a guideline influenced by numerous inter-individual factors, including the persistence of pathological states [84], for which further investigation is necessary. Furthermore, it emerges that histological diagnosis in the case of trauma is not based exclusively on neuronal alterations but also on the integration of changes detected within different tissue components that can reflect the traumatic event (Fig. 4).

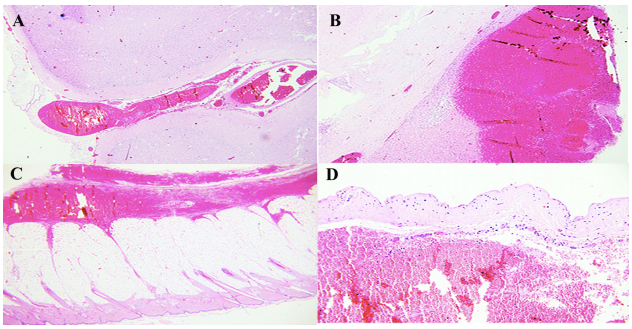

Fig. 4. Histological findings. (A) Sub-arachnoidids hemorrhage. (B) Erythrocyte intraparenchymal collection. (C) Scalp samples showing hemorrhage of the adipose sub-dermal tissue. (D) Erythrocyte collection in the galea capitis.

The immunohistochemical investigation currently represents the method with the greatest specificity for dating a traumatic and hypoxic-ischemic brain injury, also allowing to establish the neonatal survival time following the application of the mechanical insult.

The use of an anti-CD15 reaction (myeloid line marker), for example, reduces the detection latency of neutrophils within the lesion area to just 10 minutes; the CD68 antibody, marker of the macrophage line, is positive a few hours after the application of the traumatic insult, making it much more useful than traditional techniques (which allow to identify Erythrophages and Siderophages with a latency of a few days) [83]. A further highly sensitive cortical marker of traumatic injury, as well as positive just 2–4 hours after the event, appears to be apolipoprotein E (ApoE) expressed by neurons and neuropil affecting the entire injured hemisphere [85]. Furthermore, an important aspect is the detection of neuronal apoptosis using the TUNEL (TdT-mediated dUTP nick end labeling) technique, with a latency time of about 2 hours and a high specificity for TBI [86].

The peculiar traumatic action produced by the vacuum device, however, not causing alterations typical of traumatic injury (i.e., mass acceleration and concussion), requires investigations aimed at studying further pathological mechanisms and the use of specific markers of neuronal hypoxic-ischemic damage (HID).

In this way it is possible to observe a loss in the antibody reaction directed against the Glial Fibrillary Acid Protein (GFAP) molecule starting about 3 hours after the traumatic insult [75].

The positivity to aquaporin 4 (AQP4), the expression of which is directly linked to the development of edema [87], becomes clinically significant (above the basal level) starting from 24 hours after the trauma [75] (Fig. 5).

Based on current scientific knowledge, it is also possible to use additional markers, such as β Amyloid Precursor Protein (β APP), positive starting from the acute phase ($<$3 hours) both at the neuronal and at the glial level [88, 89]. Other markers of hypoxic-ischemic damage, such as

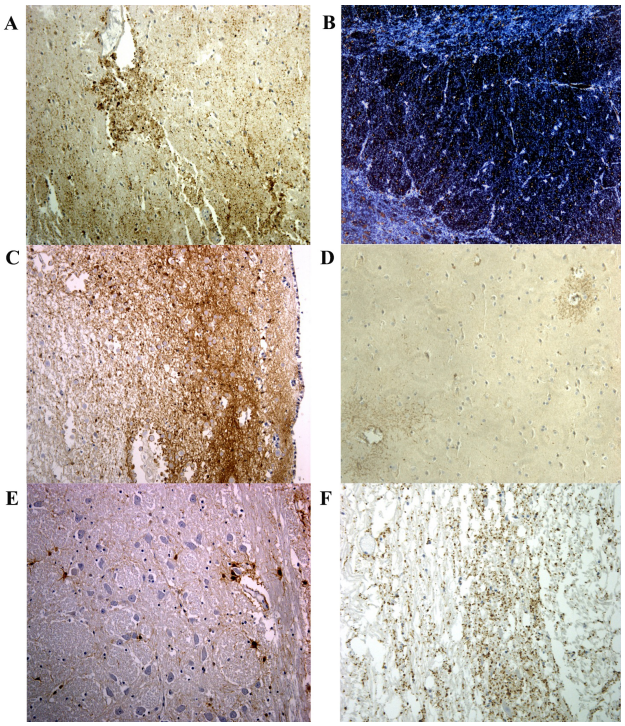

Fig. 5. Immunohistochemical findings. (A) GFAP strong positivity in astrocytes (brown reaction). (B) Confocal microscope: GFAP positivity (white reactions) in astrocytes. (C) Periventricular area: GFAP positivity in astrocytes (brown reaction). (D) Perivasal positivity in cortical region to Aquaporin-4. (E) Selective astrocytic reaction to GFAP. (F) Parietal cerebral cortex: diffuse GFAP positivity.

ORP150 (Oxygen regulated protein 150) and HSP70 (Heat Shock Protein 70), are of help in effectively distinguishing the areas involved in the ischemic process that show positivity already in the acute phase (<48 hours); others, such as HSP90, show late positivization (>48 hours) [90]. A brief synopsis of the immunohistochemical reagents described in this paper is proposed in Table 2.

3. Conclusions

The objective of this paper is to propose an operational methodology to be implemented in the field of neonatal TBI from vacuum-assisted delivery, based on the knowledge made available so far by the scientific evidence reported in the sector literature.

The proposition of illustrations taken from our unpublished experiences testifies how the application of a rigorous forensic diagnostic scheme can lead to the resolution of any case. Furthermore, the concepts expressed above are to be considered applicable even outside the specifically treated topic.

The limitations presented by the use of this multidisciplinary assessment are essentially constituted by the scarce availability of means, by the relatively high cost and by the lack of experience in the field.

Table 2. Immunohistochemical markers and relative timing.

Marker	Pathological mechanism	Latency of positivization
CD15	TBI	10 minutes
ApoE	TBI	2–4 hours
TUNEL	Apoptosis (TBI)	2 hours
β-APP	TBI-HID	<3 hours
GFAP	HID	3 hours
CD68	TBI	Few hours
AQP4	HID	24 hours
ORP150	HID	<48 hours
HSP70	HID	<48 hours
HSP90	HID	>48 hours

These considerations are particularly related to imaging techniques; anyhow, they are spreading very rapidly, and numerous scientific studies are emerging on this issue. A further challenge is represented by the discovery of new immunohistochemical markers, more sensitive and specific than the current ones, capable of finely distinguishing the individual brain damaging mechanisms and able to establish even more finely the time of death of the cases investigated.

Furthermore, in this paper only the main techniques currently available have been described: several studies have been conducted on animal and human subjects to highlight the role of miRNA profiling to characterize TBI-related issues [91–94] and soon such methods will be available in routine practice.

Finally, prior to any pathologic examination, it is fundamental to evaluate the whole clinical history of the subject in order to consider all the neonatal risk factors, pregnancy-related issues, pharmacological administration and even those events that occurred after the trauma, such as vital function status over time; onset of hypoxemia and ischaemia; increases in intracranial pressure; the appearance of hemorrhages that could be located in the subarachnoid space, in the parenchymal tissues or in the intraventricular space; any resuscitation maneuvers; operative interventions; and post-trauma hospitalization with particular attention to the number and types of health care providers that took care of the victim.

Moreover, it should be remembered that the presence of pathological features of brain injury does not necessarily coincide with the cause of death since the presence of cerebral hemorrhage does not imply mortality; meanwhile, the presence of depression fractures of the skull does not always indicate neurological deficiency or patient death. Traumatic brain injury alone should be severe enough to cause death. Then, the link between obstetric management and fetal injuries should be always carefully evaluated.

Thus, our work aims to promote a validated step-by-step procedure that should be adopted whenever a neonatal death occurs after a vacuum assisted device and a TBI is suspected.

Moreover, the results from our review indicate that an autopsy alone is not sufficient to make a diagnosis. In fact, one of either PMCT, PMMRI and histological-immunohistochemical analysis of brain samples should be performed.

There are no guidelines about procedures to follow for post-mortem investigations of cases of pediatric traumatic brain injury. The aim of this perspective is willing to offer a pathway for all medical providers who approach post-mortem injury in both diagnostic and judicial autopsy. These neurobiological insights into the mechanisms of the cellular responses implicated in brain damages, and the characterization of the various mechanisms involved might open new horizons for understanding the time of onset of a brain lesion, the pathophysiological evolution and for effective therapeutic strategies.

Author contributions

RLR and AM designed the research study. LC and NDF performed the research. GD and ADM provided help and advice on imaging and immunohistochemistry, ZDF and FM analyzed the data. PF and VF wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Acknowledgment

We thank Dottoressa Santina Cantatore for her invaluable support as laboratory technician.

Funding

This research received no external funding.

Conflict of interest

The authors declare no conflict of interest. RLR, LC, and VF are serving as Editorial Board members and Guest editors of this journal. We declare that RLR, LC, and VF had no involvement in the peer review of this article and has no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to GP.

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