

## Editorial

# Commentary – Severe IVH: Time for newer, earlier interventions to prevent brain injury?

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## 1. Introduction

Severe germinal matrix-intraventricular hemorrhage (IVH) is a major problem in preterm infants [1]. “Severe IVH” generally refers to lesions with large amounts of intraventricular blood, distending the lateral ventricular (Grade III), or a comparable degree of IVH with an associated periventricular hemorrhagic infarction (Grade IV) [2]. The incidence of severe IVH among very preterm infants (<32 weeks’ gestation) currently is approximately 6–10% [1, 3, 4]. However, the incidence is related strikingly to gestational age and birthweight, with incidence values at 24 weeks’ gestation exceeding 20%. Unfavorable outcomes include mortality rates of approximately 25–35% and subsequent neurological deficits in approximately 50–75% [1, 5].

A major complication of severe IVH is *progressive* ventricular dilation (PVD) which ultimately requires

ventriculo-peritoneal shunting (VPS) in 25–40% [1]. Indeed, the vast majority of PVD in premature infants occurs as a consequence of severe vs milder IVH. In one large study, only 1 of 160 (<1%) of infants with Grade I/II IVH developed PVD requiring a VPS, whereas approximately 30% of 61 infants with Grade III/IV IVH did so; 95% of infants requiring a VPS shunt had Grade III/IV IVH [6]. A very large and controversial literature has addressed the appropriate timing and types of interventions for PVD to prevent brain injury. The many approaches can be divided broadly into those in which intervention is based on (1) quantitative measures of ventricular size or (2) onset of clinical signs of increased intracranial pressure or both [7, 8].

The purpose of this Commentary is to suggest that waiting for the development of PVD to intervene misses an opportunity for earlier and perhaps more effective intervention. Recent experimental studies suggest that the large amount of intraventricular (and intraparenchymal) blood per se causes brain injury, that this injury may be ameliorated, and that the focus of targeted management of severe IVH should not be delayed until PVD develops.

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## 2. Intraventricular blood and brain injury

A variety of studies in experimental models and, to a limited degree, in human infants indicate that intraventricular blood is deleterious to developing brain. These deleterious effects do not require the presence of severe PVD.

### 2.1. Experimental studies

An excellent series of studies, primarily performed in neonatal rats, have demonstrated *deleterious effects of intraventricular blood and blood components* on developing brain [9–13]. The injury scenario begins with erythrocyte lysis and *hemoglobin release* into the ventricular system. The ependymal injury related to the IVH likely facilitates penetration of hemoglobin and heme into brain parenchyma, especially cerebral white matter but also neuronal structures (see below). Heme released from hemoglobin is taken up especially by microglial cells and then degraded by heme oxygenase to iron, carbon monoxide and biliverdin. *Iron facilitates the formation of injurious reactive oxygen species*. The latter are injurious to both pre-myelinating oligodendrocytes (pre-OLs) and axons in cerebral white matter. In human premature brain, pre-OLs and developing axons are abundant in cerebral white matter [14]. Injury to these components would lead to subsequent hypomyelination and related neuro-axonal deficits, as reviewed elsewhere [14]. Heme can also be taken up by contiguous neurons, which can accumulate iron by the action of heme oxygenase. Moreover, because neurons lack iron-sequestering ferritin, they are especially vulnerable. Such neurons could include those adjacent to blood in the ventricles (e.g., hippocampus, subventricular zone, thalamus) or in the subarachnoid space (e.g., external granule cells of cerebellum). Hippocampal neurons have been shown to be especially affected. Heme is involved also in the subsequent development of hydrocephalus, although the mechanisms are not entirely clear.

The particular role of free iron in the scenario leading to injury is supported by the *beneficial effects of systemically administered deferoxamine* in an animal model of IVH [11]. The latter agent led to reduced hippocampal neuronal loss and also reduced the occurrence of posthemorrhagic hydrocephalus.

*Additional blood products* are important in leading to *neuroinflammation and parenchymal injury* after IVH [12]. Thus, after vessel rupture prothrombin and fibrinogen enter brain and

activate the coagulation cascade. The resulting thrombin promotes neuroinflammation via activation of microglia and astrocytes and thereby may lead to cellular injury. Thrombin specifically suppresses differentiation of pre-OLs into myelin-producing mature OLs [9]. Fibrin also leads to microglial activation. These neuroinflammatory responses would be expected to injure vulnerable pre-OLs, axons and contiguous neurons.

An additional mechanism involves *hyaluronan*, generated from reactive astrocytes and damaged extracellular matrix. IVH leads to an increase in hyaluronan receptors which activate microglia and directly inhibit pre-OL maturation in cerebral white matter [10, 15]. Hyaluronidase, injected intraventricularly, degrades hyaluronan, represses the neuroinflammation, promotes pre-OL maturation and restores myelination and neurological function in a preterm rabbit model of IVH [10].

The *potential benefit of therapies designed to counteract the deleterious neuroinflammatory responses is suggested by recent research*. Thus, in one neonatal model of IVH, systemically administered minocycline, an anti-microglial, anti-inflammatory, iron-chelating agent reduced brain injury and development of hydrocephalus [16]. In another model, combined, extended treatment with systemically administered erythropoietin and melatonin was similarly beneficial, perhaps also via anti-inflammatory effects [17]. In a particularly informative, recent experimental study, intraventricular instillation of blood into the neonatal rat at postnatal day 4 (P4) led to neuroinflammation, posthemorrhagic hydrocephalus and brain injury over the ensuing weeks [18]. Notably, these deleterious effects could be prevented by intraventricular administration of *human umbilical cord blood-derived mesenchymal stem cells (MSCs)* two days after the instillation of blood but not seven days after the instillation. These observations emphasized the *importance of intervention early after the onset of IVH*. In related work, in the same model, similar therapeutic benefits were obtained after *systemic* administration of MSCs two days following the experimental IVH [19].

### 2.2. Human studies

Studies of IVH in human premature brain postmortem and *in vivo*, albeit relatively few, appear consistent with the experimental data. Elegant studies of postmortem human brain with preterm IVH have shown (1) in the proliferative ventricular

zone, adjacent to the lateral ventricles, and the subventricular zone of the ganglionic eminence (germinal matrix), impairment of proliferation of oligodendroglial precursor cells and of neuronal cells, and in the cerebral white matter, (2) impairment of pre-OL maturation, (3) axonal injury, and (4) microglial activation [20–22]. The pre-OL disturbance would be expected to lead to impaired myelination. The neuronal disturbance in the ventral ganglionic eminence likely involves late proliferating GABAergic neurons destined especially for thalamus. Notably, two MRI studies of relatively mild GMH-IVH in premature infants have shown subsequently impaired cortical and deep nuclear development [23–25]. Whether the cortical disturbance could represent a dysmaturational effect caused by an impairment of axonal ensheathment and by direct axonal injury, as in nonhemorrhagic cerebral white matter injury [14, 26] is unclear.

Concerning pathophysiology, it is noteworthy that large amounts of non-protein-bound iron are present in CSF of infants after large GMH-IVH (with PVD) [27], and quantitative susceptibility map analysis by MRI at term equivalent age in premature infants with severe GMH-IVH shows *changes consistent with accumulation of hemosiderin/ferritin iron throughout cerebral white matter* [28]. The latter observations suggest that diffusion of extracellular hemoglobin and ultimately iron is widespread after IVH. The temporal aspects of this iron accumulation, after severe IVH require clarification. The mechanistic implications are clear and likely involve the free radical-mediated disturbances described in experimental models, as described earlier.

### 3. Intraventricular blood and development of hydrocephalus

The occurrence of PVD in the weeks after severe IVH is a clear deleterious consequence of intraventricular blood. The mechanisms involved include disturbances in CSF flow and absorption, as discussed elsewhere [12]. Hypersecretion of CSF flow by inflammatory mechanisms also may be important [29]. A central role for intraventricular blood products, i.e., especially hemoglobin and iron, is suggested by experimental studies [16, 19, 30, 31]. Intraventricular injection of hemoglobin or iron in neonatal rats led acutely to hydrocephalus, pronounced gliosis with increased inflammatory cytokines in cerebral white matter,

impaired myelination and “cell death” in the hippocampus and “cortex”. The importance of iron in the deleterious effects was shown by the lack of effects of intraventricular injection of iron-deficient protoporphyrin, the correlation of injury to hippocampus and cortex with the enhanced levels of heme oxygenase in those structures (heme oxygenase releases iron from heme), and the amelioration of the hydrocephalus, inflammation and tissue injury by the *systemically administered* iron chelator deferoxamine [31]. Additionally *intravenous* or intraventricular administration of MSCs also prevented or blunted the deleterious effects as described earlier [18, 19, 30].

### 4. Intraventricular blood, PVD, and brain injury

Although the data outlined above strongly suggest that intraventricular blood can cause brain injury and PVD, the *combination* of IVH and PVD enhances the likelihood of brain injury. The largest systematic study of premature infants with severe IVH ( $n = 173$ ) showed that increasing ventricular dilation correlated with more adverse motor, cognitive and language outcomes [32]. Multiple MRI studies suggest that the deficits are related to a disturbance in development of cerebral white matter, deep nuclear structures — especially thalamus, cerebral cortex — especially hippocampus, and cerebellum.

The mechanisms of these effects in the human infant with PVD are likely multifactorial. However, it appears likely that in addition to cerebral ischemia (related to periventricular vascular compression), mechanical distortion of axons, and neuroinflammation, factors related to the presence of blood, leading ultimately to iron release and free radical formation are pathogenic (see earlier). Cerebral white matter is contiguous and readily accessed by intraventricular blood in the lateral ventricle, and thalamus (third ventricle) and hippocampus (temporal horn) are similarly contiguous to intraventricular blood. The disturbance of the actively proliferating external granule cell layer of the developing cerebellum by blood, hemosiderin, and iron in the overlying, contiguous subarachnoid space, likely underlies the cerebellar deficit, as reviewed in detail elsewhere [33]. Thus, disturbances that occur with severe IVH per se, as described earlier, are accentuated with PVD because the disturbance in CSF flow causes greater exposure of these vulnerable structures to blood, hemosiderin and ultimately, iron.

## 5. Interventions to prevent brain injury with severe IVH

Many studies of PVD address the relative value and optimal timing of interventions, i.e., lumbar punctures, ventricular access devices, VPS, etc., and the relevant large and somewhat controversial literature is beyond the scope of this presentation. Moreover, the purpose of this Commentary is to suggest that brain injury with severe IVH occurs *before and not long after the onset of PVD*. The challenges may be to prevent the diffusion of blood products into contiguous brain parenchyma that may occur *before PVD* and to counter the effects of parenchymal iron and inflammation. Attempts to accomplish these goals after development of PVD have included interventions that involve insertion of a ventricular catheter to drain and irrigate ventricular blood/CSF with or without addition of fibrinolytic therapy [34, 35]. This so-called DRIFT trial resulted in improved cognition at 2 and 10-year follow-up. More recently, neuro-endoscopic lavage has been utilized and may reduce the need for VPS [36, 37]. However, these approaches have been instituted *after* the onset of severe PVD.

The question raised in this Commentary is whether institution of intervention *before* pronounced PVD develops and focused on preventing injury from blood products, especially iron, would be optimal. *Systemic* deferoxamine is effective in animal models (see earlier). Moreover, *systemic* minocycline has been effective in reducing IVH-associated neuro-inflammation in an animal model (see earlier). A related mechanism may underlie apparent benefit for *systemic* erythropoietin/melatonin (see earlier). A potential role for *systemically* administered mesenchymal stem cells also has been shown experimentally (see earlier). Will any or all of these agents prove to be safe and effective in larger studies? A recent study in nine premature infants with severe IVH supports the safety and feasibility of intraventricular instillation of UCB-derived MSCs [38]. Still more provocatively, *intranasal* instillation of *breast milk* (known to contain stem cells and neurotrophins) was shown in 16 premature infants to lead to lower incidences of PVD (71% vs. 91%) and need for surgery for hydrocephalus (50% vs. 67%), although differences did not achieve statistical significance in this underpowered study [39]. Would ventricular lavage approaches be safe and effective if utilized soon after severe IVH but before the wait of many weeks for institution of the usual therapies for

PVD? Would methodologies that can provide serial quantitative assessments of iron penetration into brain parenchyma (quantitative susceptibility map analysis by MRI, as described earlier) be valuable in determining when brain parenchyma is under threat for iron-related, free radical-mediated injury? More data clearly are needed, and studies would require careful design. However, it may be time for newer and earlier approaches to the infant with severe IVH.

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