

Cerebral hypoxia and ischemia in preterm infants

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The role of the clinical pathological dialogue in problem solving

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Abstract

Premature birth is a major public health issue internationally affecting 13 million babies worldwide. Hypoxia and ischemia is probably the commonest type of acquired brain damage in preterm infants. The clinical manifestations of hypoxic-ischemic injury in survivors of premature birth include a spectrum of cerebral palsy and intellectual disabilities. Until recently, the extensive brain abnormalities in preterm neonates appeared to be related mostly to destructive processes that lead to substantial deletion of neurons, axons, and glia from necrotic lesions in the developing brain. Advances in neonatal care coincide with a growing body of evidence that the preterm gray and white matter frequently sustain less severe insults, where tissue destruction is the minor component. Periventricular leukomalacia (PVL) is the major form of white matter injury and consists classically of focal necrotic lesions, with subsequent cyst formation, and a less severe but more diffuse injury to cerebral white matter, with prominent astrogliosis and microgliosis but without overt necrosis. With PVL a concomitant injury occurs to subplate neurons, located in the subcortical white matter. Severe hypoxic-ischemic insults that trigger significant white matter necrosis are accompanied by neuronal degeneration in cerebral gray and white matter.

This review aims to illustrate signs of cerebral embryology of the second half of fetal life and correlate hypoxic-ischemic brain injury in the premature

infant. This should help us better understand the symptoms early and late and facilitate new therapeutic strategies.

Keywords

Hypoxic-ischemic brain injury, preterm infants, white matter injury, periventricular leukomalacia, subplate zone, connectome.

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Introduction

Premature birth is a major public health issue internationally affecting 13 million babies worldwide. In the United States, the rate of preterm birth continues to rise, with prematurity now complicating 1 in 8 deliveries [1]. Among children born very preterm, 5% to 10% have major motor deficits, including cerebral palsy (CP), and more than half have significant cognitive, behavioral, or sensory deficits; for these reasons, prematurity is a leading cause of neurodevelopmental disability in North America [2, 3]. The extraordinary importance of brain injury in the premature infant relates in part to the fact that 1.5% of the more than 4 million live births in the United States (approximately 63,000 infants) are born yearly with a very low birth weight (VLBW; $\leq 1,500$ g) [4]. Of the approximately 90% of VLBW infants who survive, the resulting brain abnormalities account for the subsequent occurrence of cognitive, behavioral, attentional, and socialization defects in 25-50% of infants, and of major motor deficits (e.g., CP) in 5-10% of infants [5, 6]. Notably, therefore, cognitive deficits without major motor deficits are now the dominant neurodevelopmental sequelae in the survivors of early preterm birth.

Hypoxia and ischemia is probably the commonest type of acquired brain damage in preterm infants. The pattern of injury is to some extent age dependent but also dependent on the nature, intensity, and duration of the insult.

Essentials of brain embryology in preterm infants

Development of the CNS occurs in successive epochs, each with multiple phases and different mechanisms of morphogenesis. The organization of cerebral connections in the preterm infant is substantially different from that in newborns [7].

The second half of gestation and the neonatal period are the most important developmental periods for the formation of cerebral pathways: their path-finding, target selection, and growth into the cortical plate. During this period the major growing afferents from the thalamus (thalamocortical fibres, Th-Cx) spread within the transient subplate zone, relocate in the cortical plate, and form a significant number of functional synapses with both transient and permanent neuronal populations [8-13]. Knowledge of the organization of cortical pathways during this period is necessary for the study of normal development of preterm and newborn infants, as well as for analysis of the consequences of perinatal lesions which cause cognitive, mental and behavioural disorders in children. It's also important to know the changing organization of cerebral connectivity, in particular during the period between the 23-38 weeks' gestation period (estimated as the post-conceptual weeks, PCW), at birth and post-natal life. This period was divided into four phases: (1) between 23-32 PCW; (2) between 33-38 PCW; (3) term newborn; (4) post-natal life.

23-32 post-conceptual weeks

The cortical periventricular neuroepithelial cell layer is thick and produces neurons and glia in all the lobes. The basic six layers of the cortex are observed from PCW 26 to PCW 29. The subpial granular layer increases in thickness in some areas up to PCW 26, while it disappears almost entirely in other areas. The cells of the subpial granular layer give rise to neurons and glia while a significant number undergoes developmental cell death. The major event in the development of cerebral connections is ingrowth of thalamocortical axons in the cortical plate of the frontal, somatosensory, visual and auditory cortex. For the first time in prenatal development there is a formation of synapses in the deep cortical plate [14]. The arrival of thalamocortical fibres in the cortical plate, with continuation of synaptogenesis below the cortical plate in subplate zone, gives the framework of coexistence of the transient

endogenous (in subplate) and permanent sensory-driven circuitry of the developing preterm cortex [11, 13]. The endogenous transient subplate circuitry coexists with thalamocortical circuitry (permanent) in preterm infants for a prolonged period [13]. During this period the subplate zone is four to five times thicker and larger than the cortical plate [10]. Glial hypercellularity can be observed by PCW 24 in the hemispheric white matter (WM). It continues to increase during late gestation into the early postnatal period. Myelination is mostly absent in the WM at this gestational age [15].

33-38 post-conceptual weeks

The subpial granular layer of the cerebral cortex is scant or absent over most of the lobes. There is a persistent neuroepithelial layer in all the lobes generating neurons and glial cells. There are migrating and sojourning neurons in the stratified transitional fields. The subplate is highly developed in the somatosensory cortex and is small in the primary visual cortex. After PCW 35 the subplate decreases in thickness [16]. The decrease in the thickness of the subplate occurs parallel to the ingrowth of the callosal and long cortico-cortical pathways into the cortex. Thalamocortical fibres, together with other projection fibres, build up the corona radiata and adapt their course to the process of gyration. Intracortical elaboration of thalamocortical axons promotes the development of intracortical circuitry [11]. The WM shows increased cellularity but myelination has not begun.

Term newborn

The cortical layers are well established. A reduction in the thickness of the subplate begins in the depth of sulci and later in the crown of gyri [17-18]. The hemispheric WM increases in thickness but myelination has not begun.

Synaptogenesis is a crucial event in the neonatal cortex. In quantitative terms, the first postnatal month shows a rapid increase in the formation of synapses [19-20].

Post-natal life

Neurons in the cortical layers are maturing and migrating neurons/glial cells are observed in the stratified transitional fields for the first few months postnatally. The subplate disappears by the first postnatal month and subplate neurons persist

in adult brain WM as interstitial neurons. Active neurogenesis persists in adults in the subventricular zone around the lateral ventricles [17]. WM cellularity seems to decrease with the onset of myelination. Myelination in the hemispheric WM proceeds frontally and occipitally from the areas subjacent to the central sulcus. Myelination in the temporal cortex may be observed near the end of the first postnatal year and is similar to that observed in adults by the end of the second year of life.

Vascular supply to the cerebral white matter in preterm infants

The supply consists principally of the long and short penetrating arteries. The distal fields of the long penetrating arteries are not fully developed in the premature infant, and, thus, with decreases in cerebral blood flow (CBF) these areas would be subjected to severe ischemia [21]. The short penetrating arteries do not fully develop until the cerebral cortex fully develops in the postterm period. The periventricular WM in preterm infants is a vascular end zone, particularly vulnerable to ischaemia [22]. A physiologic correlate of these vascular anatomic factors appears to be the extremely low blood flow to cerebral WM in the human premature newborn, first shown clearly by work with positron emission tomography [23]. The finding of extremely low WM flows is consistent with measurements of mean global CBF in ventilated human premature infants [24, 25]. The premature neonate exhibit a pressure-passive circulation related to disturbances of cerebral autoregulation [26, 27]. The basal CBF in healthy preterm neonates is markedly lower than in term infants or adults [24, 28]. Basal flow to cerebral WM was estimated to be < 20% of gray matter [29].

Brain damage due to hypoxia and ischemia in preterm infants

The pattern of injury depends primarily on gestational age, nature, intensity and duration of the insult. Many other factors may contribute to the extent of damage, including carbon dioxide retention, acidosis, circulatory arrest, hypotension, and hypoglycemia. Infants who have recently suffered severe asphyxia are extremely ill with coma, seizures, hypotonia, poor respiratory effort, and various other neurological signs. The clinical term used to describe this syndrome is hypoxic-ischemic encephalopathy (HIE), which is usually clinically graded as an aid to assessing prognosis; 35% have

a history of adverse intrapartum events, 35% have additional predisposing antepartum events, and in 20% there is evidence that antepartum events alone are responsible [30]. The chronic outcome is usually some form of CP. This term covers a wide range of neurological impairment resulting from non progressive damage to the developing brain spanning minor learning disorders as well as severe mental and motor impairment.

The neuropathological correlates of brain damage due to hypoxia and ischemia include various lesions, most notably periventricular leukomalacia (PVL), and accompanying neuronal/axonal deficits that involve the cerebral WM, thalamus, basal ganglia, cerebral cortex, brainstem, and cerebellum. PVL refers to injury to cerebral WM, classically with two components: focal and diffuse [31]. In cases of severe HIE we can observe the most severe form of damage to the WM: multicystic leukoencephalopathy.

Focal periventricular leukomalacia (cystic periventricular leukomalacia)

The focal component consists of localised necrosis deep in periventricular WM, with loss of all cellular elements. These focal necrosis can be identified macroscopically as white or yellow areas spots, several millimeters in diameter, and sometimes centrally cavitated, in the deep WM. The most common areas of involvement are close to the frontal horn of the lateral ventricle and in the occipital WM. The deep WM elsewhere usually has a light brown discoloration and radial vessels appear very prominent. Histological appearance depends on the age of the lesions. In the early stages an irregular zone of coagulation necrosis is observed. Nuclear pyknosis, microglial reaction, decrease in premyelinating oligodendrocytes (pre-OLs) [32, 33] occur early, and then astrocytic proliferation, collections of macrophages and increase in oligodendroglial progenitors are observed [34]. Retraction balls may be prominent in adjacent tissue [35]. Capillary endothelial reactions around the zone of necrosis may be very striking. The endothelial nuclei become plump and rounded, and the endothelium thickens and may even be reduplicated. Nuclear karyorrhexis is common in capillary endothelium [36]. In older lesions a peripheral zone of deeply basophilic sticks or balls are observed, the remains of mineralized cells and axon stumps or capillaries. This accounts for the yellow or white discoloration of the macroscopic lesions.

Away from the focal necroses are more diffuse changes in the WM. Early on, the WM is edematous and may contain clusters of retraction balls. There is diffuse infiltration by macrophages and reactive astrocytes. Capillary endothelial thickening is prominent, and karyorrhectic nuclei are observed in capillary endothelium and in scattered cells throughout the WM. In the later stages the WM is diffusely gliotic.

Diffuse periventricular leukomalacia

Diffuse WM ischemic change has been reported up to 40% of infants postmortem [36, 37]. The macroscopic changes may be subtle and easily confused with postmortem artifact. The deep WM is gray or light brown, and radial vessels are prominent. Yellow streaks may be observed around or adjacent to blood vessels. The histological changes are much as those observed in the WM of infants with PVL away from the focal necroses. It is found throughout the cerebral WM, but most consistently in the occipital lobes. Examination of gray matter shows it to be largely spared, but scattered karyorrhectic nuclei may be found particularly in the hippocampus, brainstem, and cerebellar cortex.

Multicystic leukoencephalopathy

This term describes replacement of most of the cerebral WM by multiple large cysts with smooth, clean walls. In some cases the cysts are found predominantly in the subcortical WM, but in others they occupy almost all of the WM extending to the edge of the lateral ventricles, which are dilated due to tissue loss. The cortex is a thin band stretched over the cysts, and deep gray matter and brainstem are white and very firm to touch.

Histology shows extensive fibrillary gliosis in the walls of the cysts with very little myelination in the cerebral hemispheres. The overlying cortex is almost totally devoid of nerve cells and replaced by glial cells and small cystic cavities packed with macrophages that persist for many years.

Several cases of multicystic leukomalacia are documented in the literature. They all occurred between 30 and 44 weeks of gestation and usually resulted from an incident causing an abrupt interference in fetal blood supply including maternal hypotension due to anaphylaxis [38], maternal involvement in a motor vehicle accident, or maternal butane intoxication [39].

Ultrasound scans can differentiate between periventricular and subcortical cysts in surviving infants. There is a much poorer neurological outcome in infants with subcortical cysts or a mixed pattern than in those with purely periventricular cysts [40, 41].

Neuronal/axonal disease

Neuronal/axonal disease is a previously under-recognised accompaniment of PVL. The regions of involvement include the cerebral WM (axons and subplate neurons), thalamus, basal ganglia, cerebral cortex, brainstem, and cerebellum.

Cerebral white matter: axons

Cerebral WM axons (ie, projection, commissural, and association fibres) are in a phase of rapid growth during the premature period, the peak period of vulnerability for PVL. Earlier neuropathological evidence for axonal injury in PVL was derived from studies of the necrotic foci and included findings of axonal spheroids and positive immunocytochemical staining for beta-amyloid precursor protein, both indicators of overt axonal damage [33, 42-47]. A recent report used the apoptotic marker fractin to show that widespread axonal degeneration is present in the diffuse component of PVL, separate from the focal necroses [48]. Although the latter finding does not allow distinction of a primary destructive lesion from a secondary disturbance, the observation suggests that axonal abnormality with PVL is more pervasive than previously thought.

Cerebral white matter: subplate neurons

The major neuronal type in cerebral WM is the subplate neuron. This transient population of neurons reaches a maximum during the peak period for the occurrence of PVL in the premature infant and is central to both cortical and thalamic development. Subplate neurons contain excitatory amino acid receptors [49], and have been shown in a developing animal model to be selectively vulnerable to hypoxia-ischaemia [50].

Thalamus

Thalamic neurons are commonly affected in premature infants, especially those with PVL. In the early stages, macroscopic examination shows pink or brown discoloration of these nuclei. Histological

examination shows nuclear karyorrhexis, necrosis, glial and macrophage infiltration, and intense capillary proliferation from 5 days after injury. Some neuronal profiles become calcified. The chronic effects are either cyst formation or atrophy with dense gliosis and persistence of calcified neurones. The neuropathological findings are consistent with the finding of diminished volume of the thalamus (often measured with basal ganglia) by MRI studies of premature infants at term-equivalent age and later in childhood and adolescence [51-61]. In those studies that assessed the presence of PVL by MRI, the thalamic volumetric deficit was found to be particularly characteristic of (although not always confined to) infants with imaging features of WM injury [51-53]. The MRI abnormalities correlated with subsequent cognitive deficits [51, 53, 60].

Basal ganglia

Basal ganglia neurons are only slightly less commonly affected than thalamic neurons. Histological examination shows neuronal loss and gliosis in the caudate and putamen [62].

Cerebral cortex

Neurons of the cerebral cortex are less affected than those of the thalamus and basal ganglia. The cortical neuronal injury might accompany particularly severe forms of cystic PVL [43].

MRI studies of living infants with VLBW also indicate a disturbance of the cerebral cortex, especially in the presence of PVL. Decreased volume of the cerebral cortex in premature infants with non-cystic PVL has been documented as early as term-equivalent age [51, 63, 64]. Volumetric deficits occurred in multiple cortical regions, especially parieto-occipital cortex, which overlies the region of WM most susceptible to PVL. Premature infants studied later in childhood, adolescence, and adulthood show persisting cerebral cortical volumetric deficits [54-57, 65, 66]. The most pronounced decreases generally occur in parieto-occipital, sensorimotor, premotor, temporal, and hippocampal cortices [54, 55, 57, 60, 61, 67, 68]. These cortical neuronal deficits correlate with a wide variety of cognitive deficits observed at follow-up [54, 60, 61, 64-66, 68].

Cerebellum (and brainstem relay nuclei)

Cerebellar abnormality is particularly characteristic of premature VLBW infants. Histological

examination shows neuronal loss in the dentate nucleus, in the cerebellar cortex [69] and in the cerebellar relay nuclei. Gliosis is identified in the cerebellar cortex and in the dentate nucleus.

Development connectome in preterm brain and possible damage by hypoxic-ischemic injury

The macroscopic brain comprises a large number of anatomically and functionally distinct regions, linked by a complex web of structural WM pathways, known as the human connectome [70]. Adopting network science as a general mathematical framework to visualize and examine the connectome's complex network structure, recent structural and functional studies have reported several hallmark features of adult connectome organization, including the presence of a small-world modular organization with functionally coupled communities, short communication pathways, and the formation of central connectivity hubs facilitating efficient global communication [71-73]. In vivo analysis of WM connections typically requires a diffusion and high-resolution anatomic MRI, technique for noninvasively characterizing structural connectivity networks. The adult cerebral brain network is the result of a complex developmental trajectory. From the prenatal formation of the first neurons, throughout the first years of life and all the way into late adolescents, the brain undergoes an elaborate developmental trajectory [74].

During early development, the brain undergoes significant changes that are likely represented in the developing connectome, and preterm birth represents a significant environmental risk factor that impacts negatively on early cerebral development [75]. Tymofiyeva and coworkers [76] have developed an automated technique to map structural connectivity in the infant brain using diffusion MRI, and used this approach to characterize large-scale connectivity of the cortex in 17 six-month old babies with HIE at birth. In their work it was observed a trend to declining brain network integration and segregation with increasing neuromotor deficits.

Concluding remarks

A major challenge facing modern neonatology is the prevention of neurological sequelae in premature infants who survive the neonatal period. While the overall survival rate of all premature groups has sharply increased to over 90%, the

burden of neurological disability remains great: 25-50% of the VLBW infants born each year develop major cognitive deficits, 10% develop CP, and 2.1% of late preterm infants develop variable cognitive disabilities, CP, and/or seizures, a twofold increase compared to term infants. The immature brain should be more able to recover from injury than the more developed brain. Curiously, preterm infants exposed to hypoxic-ischemic insults of varying severity continue to have a high rate of debilitating neurodevelopmental handicaps despite a progressive improvement in structural damage to the brain, from acute necrotic injury of the periventricular WM, with axonal loss in historical cohorts, to diffuse gliosis with trivial axonal damage. Recent studies evidenced that disability after preterm birth is largely mediated by disturbed development of neuronal connections. These findings suggest that the insults during this critical phase alter the trajectory of brain development. To decrease the frequency and severity of neurological sequelae, the clinical research should investigate new therapies that facilitate the development of physiological connections.

Declaration of interest

The Authors declare that there is no conflict of interest.

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