

New light on white matter damage of the premature brain: a neonatologist's point of view

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The role of the clinical pathological dialogue in problem solving
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Abstract

Periventricular leucomalacia (PVL) is traditionally considered a multifactorial lesion related to three main mechanisms: ischemia, inflammation and excitotoxicity. For years it was believed that hypoperfusion, associated with the peculiar vascular anatomy of the premature brain (border zones), was the *conditio sine qua non* in the pathogenesis of PVL. More recently this theory has been questioned. Many studies have stressed the importance of the association between inflammation/infection and white matter injury and have supported the multi hit hypothesis according to which several (genetic, hormonal, immune and nutritional) factors may team up in a multi-hit fashion. The emerging concept is that the fetal white cell activation together with the interaction between the innate and adaptive immune system play a main role in white matter damage. Currently there are increasing evidence that PVL is a disease of connectivity. In this article we review the news in the basics of pathogenesis, the incidence, the definition and the diagnosis of PVL. Furthermore, recent follow-up studies and neuroprotective therapies are mentioned.

Keywords

Periventricular leucomalacia, white matter damage, cranial ultrasound, very low birth weight, outcome, neuroprotection.

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Background

For decades, white matter damage of the premature brain has been well defined by the term “periventricular leucomalacia” (PVL), coined in 1962 by Banker and Larroche [1], who first described white and softening spots in the periventricular white matter of brains of 51 preterms with a gestational age of 34 weeks or more. Currently, following the discovery of a more diffuse pattern of white matter damage in very immature preterm (23-28 weeks gestational age [GA]), some authors prefer speaking of “white matter damage” (WMD) while others continue to talk about PVL. According to the latter group of authors, the period of highest risk for PVL is between 23 and 32 weeks GA and it is possible to find two components in PVL: the focal (cystic PVL [cPVL]) and the diffuse (diffuse PVL [dPVL]). cPVL, more common described in the eighties, a period during which many very preterm did not survive, consists of localized necrosis of the periventricular white matter and it is generally cystic. The incidence of cPVL in very low birth weight (VLBW) in different series is significantly decreasing (from 5% to 1.3-0.5%) [2, 3].

dPVL, more frequent in the youngest premature babies (20% < 28 weeks old), affects the cerebral white matter widely and it is characterized by a decrease in premyelinating oligodendrocytes, subplate neurons and damaged connectivity among neurons. Thus, whereas in the nineties the paradigm was valid where an asphyxial insult of the immature brain caused WMD with relative sparing of the gray matter, nowadays it is well known that neuron loss and also cerebellar injury are part of WMD and this complex involvement of gray matter and white matter lesions (including white matter punctuate

lesions, loss of white matter volume, diffuse high signal changes throughout white matter) is known as “encephalopathy of prematurity”.

PVL can occur as an isolated lesion or in association with intra-periventricular hemorrhage (IVH-PVH). cPVL is the anatomical equivalent of the spastic cerebral palsy (which affect up to 10% of very preterm infants) [5, 6] while dPVL generally causes cognitive impairment and behavioural, attentional and socialisation deficits which develop in a further 25-50% of very preterm infants [7-9].

Physiopathology: the multi-hit hypothesis

During the last years the concept has been emerging that PVL is a form of a long lasting neuroinflammation without a single pathogenetic mediator but a process extended over a period of time. Although PVL is traditionally considered an ischemic lesion, it seems that hypoperfusion, despite being a common denominator of the PVL, may not be sufficient to cause WMD. Many studies have confirmed the association between PVL and infection/inflammation and, in particular, recently many authors have recognized the dominant role of inflammatory processes in the injury of the developing brain. In 1973 Leviton and Gilles [10] found at autopsy a correlation between gram-negative bacteremia and perinatal WMD. Subsequent studies described that Gram-negative bacteremia activates cells via Toll-like receptors, that are members of the pattern recognition receptor family involved in the regulation of neuronal proliferation in the developing brain and located on the surface of inflammatory cells, in the decidua and placental membranes with release of proinflammatory chemokines and cytokines [11-13].

More recent works have confirmed that infection and inflammation contribute to perinatal brain damage and that the fetal inflammatory response syndrome, with or without overt clinical chorioamnionitis, may cause an increase of proinflammatory cytokines and chemokines that are likely to be key mediators in the WMD. Thus, even a sterile chorioamnionitis that does not cause a clinical infection could result in a long lasting inflammation with permanent changes in the immature immune system of newborns. The release of intracellular content could also be related to the damage of the cells after an ischemic-reperfusion injury without

any micro-organism (sterile inflammation). Studies performed in pregnant sheep showed that small doses of lipopolysaccharide caused microglial activation in the fetal brain. In 2014, Strunk hypothesized that the passage of bacterial products and leucocytes across the blood brain barrier might cause the release of inflammatory mediators [14]. It is interesting to note that there is a strong inverse relation between GA, birth weight, and incidence of histologically diagnosed chorioamnionitis, which seems to be present in the 65% of placentas at 23-24 weeks GA, 30% of placentas at 29 weeks and 2-14% at term [14-19]. In addition, MRI studies show an association between infection and white matter lesions. Recent evidence suggests that genetic susceptibility could play a role in the development of WMD [20]; furthermore, this pattern is likely to be multifactorial. Therefore, a compromised intrauterine environment, including hormonal and nutritional deprivation, maternal-fetal in-

fections, maternal stress, pain, drugs, toxins, hypoxia, hypocarbia [21], multiple gestations [22], necrotizing enterocolitis [23], patent ductus arteriosus [24], monochorionic twinning, late-onset circulatory dysfunction of premature infants [25], ischemia and hyperoxia appear to be involved in such scenario. Inflammation might sensitize the developing brain to excitotoxic damage, amplify the risk of cerebral palsy and contribute to a spectrum of cognitive and behavioral disorders that manifest throughout life. Male preterms seem to be more vulnerable but the neurobiological cause of this prevalence is still unknown [26]. According to the multiple-hit hypothesis (**Fig. 1**), extreme prematurity plus such multiple hits appear to influence the ability of the immature brain to respond to subsequent stress (sensitization process). An hypoxic-ischemic insult insufficient *per se* to resulting in overt brain injury may cause a massive brain injury if it is preceded by the action of lipopolysaccharide.

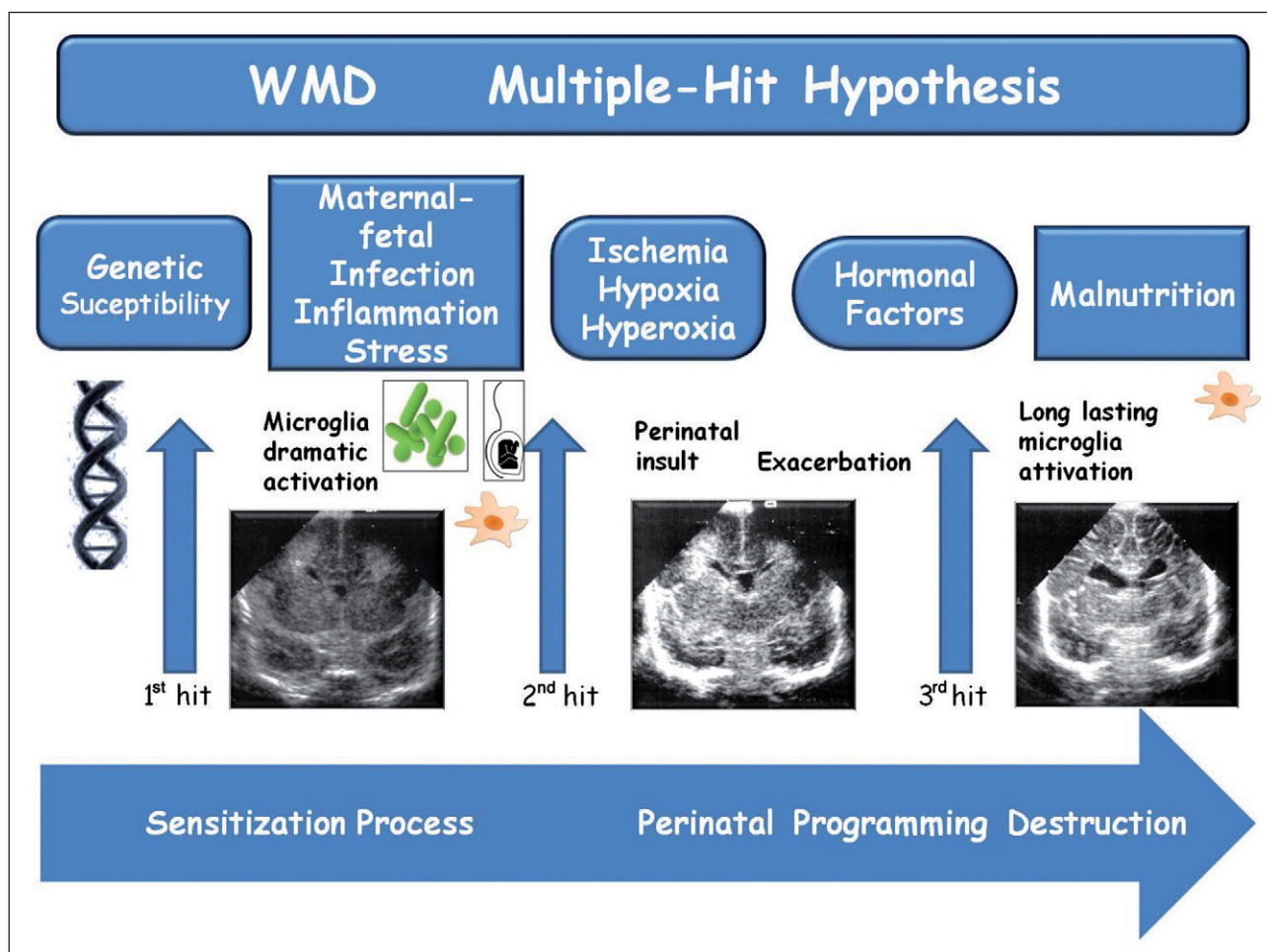


Figure 1. Schematic representation of the multiple-hit hypothesis in white matter damage (WMD): the premature brain is susceptible to various pre- and perinatal insults including genetic, infectious/inflammatory, ischemic/hypoxic/hyperoxic, hormonal and nutritional factors.

In addition a neuroinflammation hit could be not a single hit but an ongoing process extended over a period of time that could last long time [27-30]. The immature immune system of premature babies does not normally respond to inflammatory stimulus. It is likely that (similarly to other inflammatory diseases that affect very preterm babies such as necrotizing enterocolitis) after an initial trigger, the fetal white cell activation together with the abnormal interaction between the innate and adaptive immune system play a main role in WMD. Excitotoxic and free radical attack to active oligodendroglia is believed to be the third main pathogenetic mechanism of PVL. cPVL is characterized by necrosis of all cellular elements of the periventricular white matter adjacent to the outer corners of the lateral ventricles, with involvement of the central semiovale and the optic and acoustic radiation. The cell death that characterizes the classical form of PVL is attributed to excitatory amino acids and cytokines (death by murder). The main targets of the insult in dPVL are microglia and immature oligodendrocytes but also GABAergic and subplate neurons are affected. In the past it was believed that the brain was an immune privileged organ; actually, it has been discovered to present an innate immune response through microglia. Microglia are “non neural cells” originated from the bone marrow, and can be found in a “resting” or “activated” state. They are activated by the slightest central nervous system (CNS) damage [31-33]. Activated microglia, expressing Toll-like receptor 4, seem to play an important role in promoting immature oligodendrocytes death and excitotoxic, inflammatory and free radical injury to cells in the developing CNS. Pre-oligodendrocytes, which are the predominant oligodendrocytes type between 20 and 32 weeks GA (developmental window of vulnerability), are cells with high energy demand, which are very sensitive to ischemia and express NMDA receptors; they are very sensitive to glutamate and oxidative stress because they do not have a well developed antioxidant defense system. For this reason they can easily be damaged by free radicals produced by ischemia as well as by activated microglia. Subplate neurons are transitional cells which are essential for the maturation of the cortex. In the dPVL the insult is milder and cells death occur after some hours or days by apoptosis (death by neglect). In this case, the ischemia could result in a removal of neurotrophins that act as extracellular

survival signals and that normally antagonize the mechanism of programmed cell death.

Recently, several studies have emphasized the concept of the preterm connectome (map of all the connections among the neurons of the CNS system), suggesting that preterm birth with or without PVL could be defined a disease of connectivity. Functional MRI studies have enabled us to consider the immature brain as a dynamic structure organized in parallel, measurable and interacting areas. Thus this peculiar network organization may be negatively influenced by environmental insults [34, 35]. This theory is suggestive because other authors have demonstrated an overlapping between PVL topography and areas of altered connectivity in immature brains. In both cases, for example, posterior thalamic radiation and cortico-cortical connectivity may be affected. In addition the cognitive impairment typical of dPVL may be related to disruption of cortical and sub-cortical connectivity [36].

Variants of of periventricular leukomalacia

As previously said, PVL can be classified in two groups: cPVL and dPVL. The best way to screen cPVL is ultrasound while MRI exceeds ultrasonography in detecting dPVL. The cPVL is characterized by cystic lesions located in the periventricular area at a distance of about 1 cm from its lateral wall.

In 1992, de Vries [37] suggested dividing cPVL in four ultrasonographic grades (**Fig. 2**):

- grade I (transient periventricular densities);
- grade II (localized cysts adjacent to the external angle of the lateral ventricle);
- grade III (extensive cysts in frontoparietal and occipital periventricular white matter);
- grade IV (extensive cysts in subcortical white matter).

Many authors have reported different classification system for early clinical MR scoring [38-40].

Clinical aspects of periventricular leukomalacia

At birth signs and symptoms of PVL are not usually obvious. Motor impairment generally starts to appear at around 1 to 2 years old. cPVL may damage descending fibers of the corticospinal tracts and affected infants are at risk of cerebral palsy and namely spastic diplegia. Although

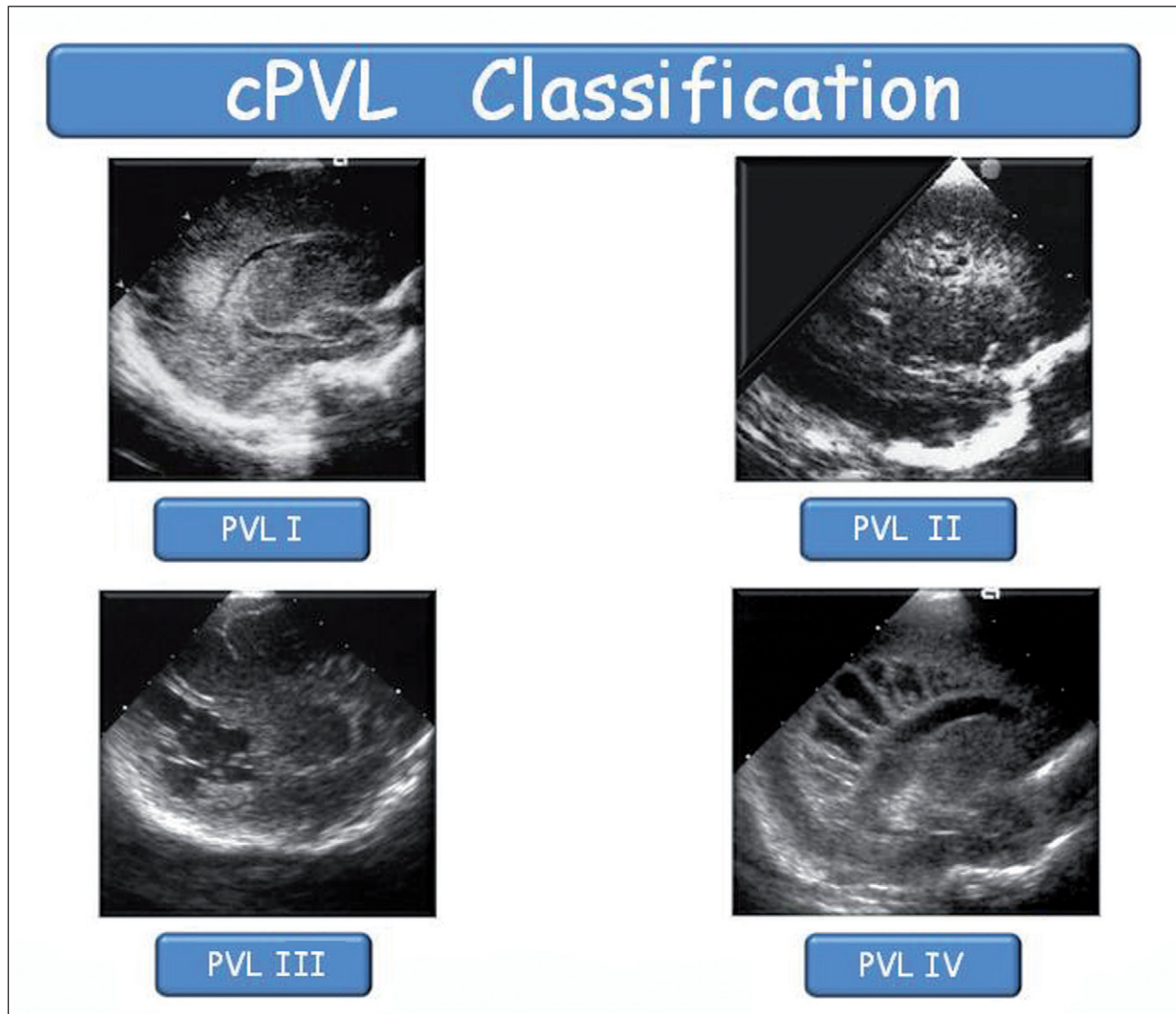


Figure 2. Ultrasound images of parasagittal sections illustrating the classification of cystic periventricular leukomalacia (cPVL) described by de Vries. PVL I: echoreflections nearly as echodense as the choroid plexus; PVL II: focal cPVL; PVL III: extensive cPVL; PVL IV: subcortical leukomalacia.

a motor dysfunction can occur without cysts, usually the prognosis of PVL is related to the site, the number and the size of the cysts. Thus frontal cysts have a better outcome than the parieto-occipital and a single cyst has a better outcome than bilateral parieto-occipital multiple cysts. Bilateral occipital cysts predict spastic diplegia and may result in visual impairment while subcortical leukomalacia predicts quadriplegia, severe learning disabilities and epilepsy. Whenever dPVL is prevalent in the peritrigonal zones visuoperceptual deficits occur, whereas if it is greater in the frontal zones it is possible to observe attention and executive functions impairment. Many preterm infants may exhibit deficient social cognition very similar to

autistic spectrum disorders, others exhibit way finding problems, tend to get lost very easily and may suffer from specific problems in calculation [41-46].

Differential diagnosis: periventricular leukomalacia versus venous infarction

The differentiation between PVL and venous infarction is generally easy. PVL is mainly bilateral with irregular border and shape and patchy appearance. Venous Infarction is usually unilateral, triangular with a sharply delineated inferior apex emerging from the original matrix lesion, and occurring together with ipsilateral intraventricular hemorrhage (**Fig. 3**).

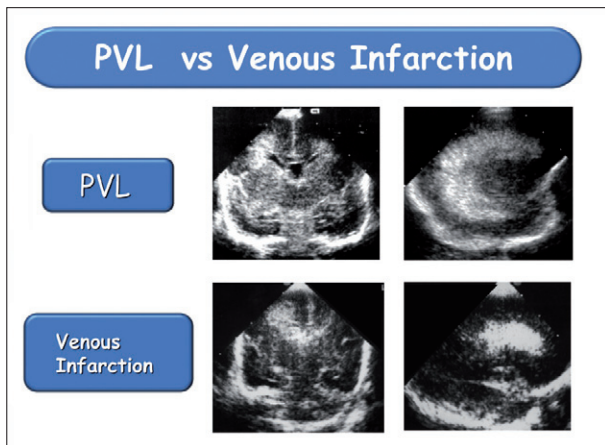


Figure 3. Ultrasound imaging showing the differentiation between periventricular leucomalacia (PVL) and venous infarction. PVL has patchy appearance, irregular border and shape, no intraventricular hemorrhage (IVH). Venous infarction: fan-shaped echodensity, sharply delineated together with ipsilateral, large IVH.

Differential diagnosis: periventricular leukomalacia versus prenatal frontal cysts

Prenatal frontal cysts are related to germinolysis originated during the mid-second to the early third trimester. These cysts are often oval, elongated, symmetrical, bilateral, located medially and lower than PVL (Fig. 4).

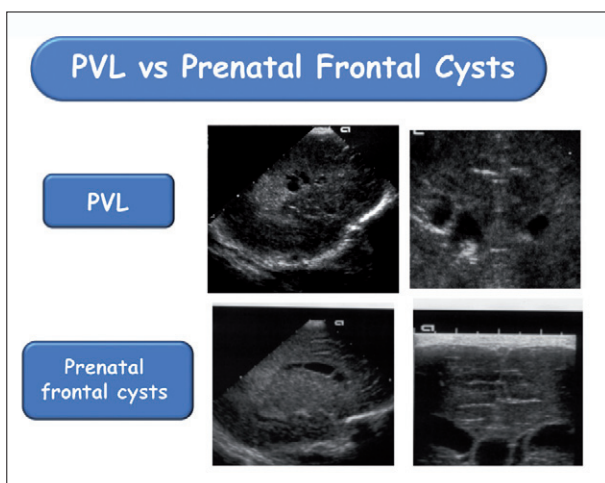


Figure 4. Ultrasound images showing the differentiation between periventricular leucomalacia (PVL) and prenatal frontal cysts. Cysts of PVL have irregular border with patchy appearance and are located at a distance of about 3-10 mm from the ependyma. Prenatal frontal cysts show regular border, are oval, elongated, symmetrical, located medially and lower than PVL.

Differential diagnosis: periventricular leukomalacia versus paraventricular porencephaly

Paraventricular porencephaly is a large cyst, widely communicating with the contralateral ventricle, that reproduces the shape of the venous infarction from which develops. Cysts of PVL have soft edges, are located at a distance of about 3-10 mm from the ependyma and are often multiple and bilateral (Fig. 5).

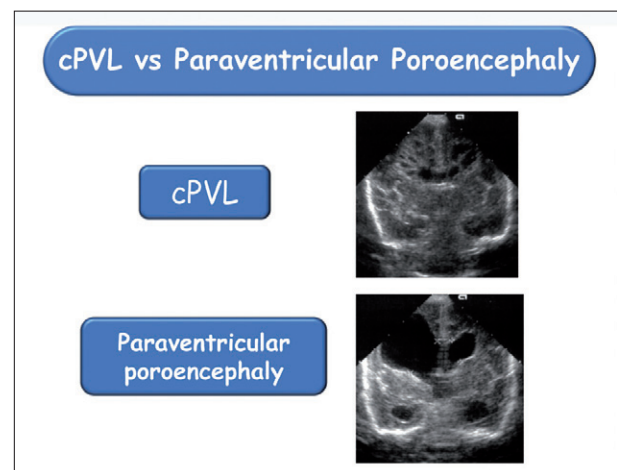


Figure 5. Ultrasound imaging showing the differentiation between periventricular leucomalacia (PVL) and paraventricular porencephaly. Cysts of PVL are multiple, often bilateral with soft edges and not communicating with the contralateral ventricle. Paraventricular porencephaly is a large cyst widely communicating with the ventricle.

Prevention and therapy of periventricular leukomalacia

While medical advances have made it possible to increase the survival of the tiniest premature babies, medical interventions to prevent and to treat the WMD have been elusive. PVL is influenced by a set of overlapping factors (genetic and environmental) occurring in combination or in succession, among which the current literature identifies the infection/inflammation as a major risk factor. Thus, protecting the brain from the infection/inflammation associated with chorioamnionitis, bacteremia, sepsis, and necrotizing enterocolitis during the fetal, peri-partum and neonatal periods is crucial. In the last years, treatment has been focused on inhibiting or treating infections. Efforts are currently focused on the identification of modulators

of inflammation, modulators of the immune system and neuroprotective drugs. Again, we must understand that the best timing for intervention and the appropriate route of administration (maternal intra-amniotic or fetal) to maximize effects and minimize adverse consequences are unknown. Guidelines targeted to maintain arterial PCO₂ levels above 35 mmHg are generally accepted [47, 48]. Cooling therapy has not yet been evaluated in premature infants [49, 50]. Tested drugs provided by neuroprotective characteristics are among the most various. Several studies have reported the beneficial properties of low doses of inhaled nitric oxide (iNO) in the developing brain of low birth weight infants. In animal studies iNO seems to prevent hyperoxia-induced WMD and promotes myelination [51, 52]. However, since there is not a consensus on the protective role of iNO, results of long term follow-up studies are requested [53]. In animal models endocannabinoid anandamide have shown to be protective against microglial cells inflammatory injury of the developing brain [54, 55]. Anticonvulsant drugs like the topiramate

attenuate glutamate-mediated excitotoxic injury to developing oligodendrocytes [56, 57] while minocycline is thought to reduce proinflammatory cytokine expression and microglial activation [58-61]. However, the use of tetracyclines in neonates and infants is not recommended because of concerns of disruption of normal formation of bone and tooth enamel. Erythropoietin (EPO), recognized only as a kidney-derived haemopoietic growth factor, seems to act as antioxidative, anti-apoptotic, and great anti-inflammatory factor. Its function consists in the prevention of the rise in IL-1b and in mitigating the leukocyte infiltration in the brain [62, 63]. N-acetylcysteine, a free radical scavenging antioxidant drug, has strong anti-inflammatory properties inhibiting neutrophilic inflammation, attenuating the lipopolysaccharide (LPS)-induced cytokine and free-radical generation [64-66]. However, N-acetylcysteine might impair fetal cardiovascular stability before delivery [67]. Estrogen also exhibit anti-inflammatory and immunomodulatory effects [68-70]. In animal models melatonin could prevent PVL and reduce

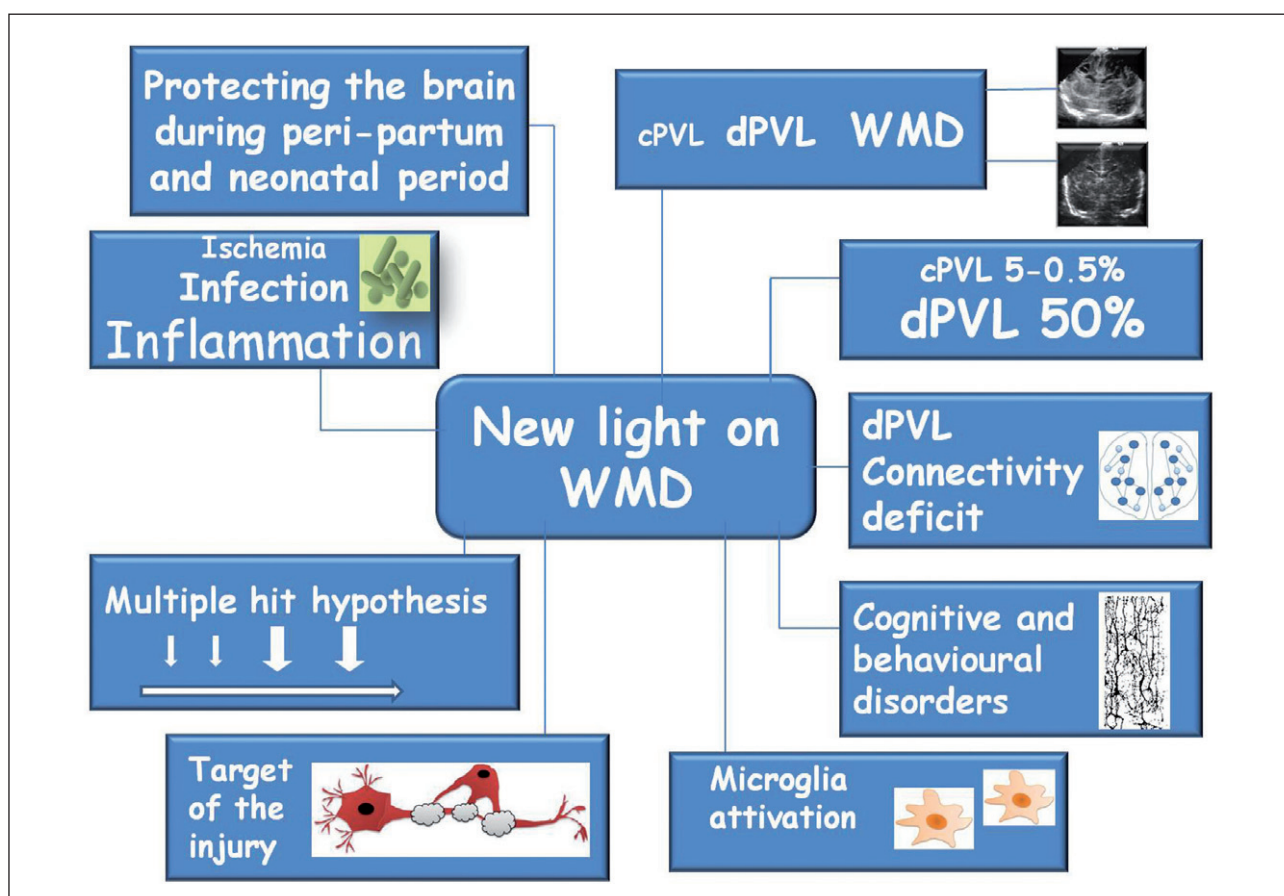


Figure 6. Mental map of “New light on white matter damage (WMD) of the premature brain: a neonatologist’s point of view”. cPVL: cystic periventricular leucomalacia; dPVL: diffuse periventricular leucomalacia.

microglial activation [71, 72]. Antidepressants like tianeptine seem to block the cytokines [73] while studies regarding the neuroprotective functions of granulocyte colony stimulating factor (G-CSF), magnesium sulfate, dexamethasone, indomethacin and ibuprofen are controversial [74-82]. Deoni et al. in 2013, using the mcDESPOT (multicomponent driven equilibrium single pulse observation of T1 and T2) white matter imaging technique, demonstrated the developmental advantages associated with breastfeeding, and supported the hypothesis that breast milk constituents promote healthy neural growth and white matter development [83].

The mind map shown in **Fig. 6** summarizes the different topics presented in the text.

Declaration of interest

The Authors declare that there is no conflict of interest.

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